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Studies on the Epidemiology and Control of Rabies in Bhutan

by

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Author's Declaration

Apart from the assistance stated in the acknowledgements and where reference is made in the text, this thesis represents the original work of the author. I certify that this thesis has not been submitted for any other degree or qualification at any other university or institution.

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Summary

Rabies, a fatal and neglected zoonotic disease, is reported mainly from the southern parts of Bhutan bordering India, but sporadic occurrences have been reported in other, previously free areas. Domestic dogs play a principal role in the transmission of rabies and no wildlife rabies cases have been reported so far in Bhutan. Although rabies has been endemic and causes substantial financial losses, no detailed studies have been conducted to understand the epidemiology of rabies in Bhutan. The overall objective of this research was to better understand the epidemiology of animal and human rabies and estimate the cost of various rabies intervention measures in humans and animals. This was the first epidemiologic research on rabies ever conducted in Bhutan. Rabies surveillance data (1996 to 2009) and field surveys were used for this epidemiologic research.

The spatial and temporal distribution of animal rabies cases was examined by using a Geographic Information System and time series analysis approaches. The study showed that 59 of the 205 sub-districts in Bhutan reported animal rabies from 1996 to 2009 with increased incidences in the four districts in southern parts of Bhutan. Significant ($P<0.05$) clusters of cases were observed in south central and south west Bhutan. More cases were reported in cattle (n=447) and domestic dogs (n=317) and a significant cross correlation between the number of reported cases in dogs and other domestic animals was demonstrated, wherein the report of cases in dogs predicted cases in other domestic animals. Rabies cases were reported throughout the year with more reports during spring and summer months, likely to be associated with the breeding season of dogs. The annual patterns of cases were relatively stable until 2005, but increased in 2006 and 2008. This increased incidence was associated with re-emergence of rabies in eastern and south west Bhutan between 2005 and 2008, areas that had been previously free from rabies. This major rabies outbreak in eastern Bhutan resulted in one human and 256 domestic animal deaths while the outbreak in south west Bhutan resulted in 97 animal deaths; both outbreaks caused serious financial losses to society. During these outbreaks, large numbers of people (~2000) were directly or indirectly exposed to either suspected rabid animals or animal products derived from rabid animals and were given post-exposure prophylaxis. The outbreak in eastern Bhutan was believed to have been due to an

incursion from across the border while local spread from the endemic areas or an incursion was hypothesized in the south-west Bhutan outbreak. The high densities and movements of stray dogs with inadequate control measures were responsible for the rapid spread and persistence of the infection for about two years (from May 2005 to November 2007) in eastern Bhutan. In contrast, the outbreak in south west Bhutan during 2008 was controlled within six months by culling of stray dogs, mass dog vaccination, and impounding of dogs. Anthropogenic factors − including human population characteristics and its movement, road network accessibility, and high dog density − played a major role in the spread of disease during both of these outbreaks.

The assessment of risk factors for the occurrence of rabies at the sub-district level identified the socio-demographic and anthropogenic factors significantly associated with reporting of rabies in domestic animals in Bhutan. Sharing a common border with India was found to be the most important individual predictor of the overall distribution of rabies occurrence in Bhutan (odds ratio 10.43; 95% CI: 4.42–24.64; $P<0.001$). Of the 59 sub-districts that reported rabies in Bhutan, 43 (73%) shared a border with India. The trans-border movement or translocation of stray dogs and an inadequate control program may be responsible for the maintenance of rabies endemicity and transmission among the stray dog population in these border areas.

Molecular and phylogenetic analyses further demonstrated that Bhutanese rabies virus isolates were found to be closely related to Indian rabies virus strain and belong to Arctic-like-1 viruses which are widely circulating in the Indian sub-continent. This study suggests that the rabies viruses spreading in southern parts of Bhutan have originated from a common ancestor. However, more sampling is needed from Bhutan-India border areas to understand the transmission dynamic of rabies virus in the region.

In humans, rabies cases were found to be sporadic, mainly reported in the canine rabies endemic areas of southern Bhutan. A total of 15 human rabies deaths was reported between January 2006 and July 2011 (with 5 deaths reported in 2011 alone), equivalent to a cumulative incidence of 2.14 per 100000 population (annual incidence of 0.28 per 100000 people). Although the number of human rabies deaths was sporadic, there were increased number of dog bite incidents and post-exposure prophylaxis (PEP) administration to the patients. In order to understand the use and distribution of rabies

PEP in humans, PEP data for the period from 2005 to 2008 were retrieved from the hospital medical database and analysed. The study showed that PEP was provided to the patients free of charge by the medical hospitals in Bhutan, and followed the 5-dose Essen intramuscular regimen. A significant ($P<0.001$) difference in gender and age groups receiving PEP was observed: males received more PEP than females across all age groups. Children − particularly 5–9 years of age − received more PEP than other age groups, indicating children and males are more at risk of rabies exposure in Bhutan. PEP was provided throughout the year with a higher number of doses administered during the winter and spring months, and was given to both animal bite and non-bite exposures. The study also identified a lack of patient compliance to complete the course of PEP: some 40% ($n = 3360$) of the patients received an incomplete course of vaccine (less than the required course of 5-doses). However, the results suggest that patients with animal bite injury were less likely to receive an incomplete vaccine course than non-bite recipients. Secondly, patients presented to hospitals in rabies endemic or outbreak areas were less likely to receive an incomplete course than in rabies free interior Bhutan, thus reducing the chances of vaccination failures. The study also showed that the PEP was provided to patients that have low or no risk of rabies exposure. Therefore, a thorough assessment of each individual case based on the WHO guidelines would reduce unnecessary use of PEP, and therefore costs in Bhutan. The main reason for providing PEP was found to be due to dog bites.

To better understand the dog bites incidents in humans, a hospital-based survey was conducted at the three hospitals in Western and Southern Bhutan (Thimphu, Phuentsholing and Gelephu) for a period of nine months. The study revealed that dog bites in human are common in the survey areas and showed significant ($P<0.001$) gender and age differences in bite incidents. Males were more at risk of dog bites than females, and the children aged 5–9 years were bitten more than other age groups, which substantiate our earlier findings of more use of PEP in males and children. The majority of victims were bitten by stray dogs, and the most common anatomical bite sites were on the legs.

Using data on the anatomical location of dog bites in humans and a probability of dying from rabies, a decision tree model was constructed to estimate human deaths from rabies in two rabies endemic areas of southern Bhutan. Based on the official reported cases of

rabies in two hospital areas (Gelephu and Phuentsholing) in southern Bhutan, the average number of human rabies death was 1.5 (95% CI: 0.75–3.00) per year, equivalent to an annual incidence of 3.14 (95% CI: 1.57–6.29) per 100,000 population. The decision tree model predicted 2.23 (95% CI: 1.20−3.59) human deaths from rabies per year, equivalent to an annual incidence of 4.67 (95% CI: 2.53–7.53) deaths per 100,000 populations. This indicated that no major underreporting of human rabies deaths has occurred, unlike in other rabies endemic countries, although some underreporting of dog bites is possible. In the absence of post-exposure prophylaxis, the model predicted 19.24 (95% CI: 13.69–25.14) deaths per year, equivalent to an annual incidence of 40.31 (95% CI: 28.70–52.68) per 100,000 population, suggesting post-exposure prophylaxis is important to prevent human rabies deaths**.**

Since both dog bite incidents and the use of PEP were high in Bhutan, a cross-sectional study was conducted at Gelephu (south central Bhutan), an area endemic for rabies, to understand people's level of knowledge and awareness about rabies. The study showed that a majority of the interviewed respondents had heard of rabies, and had a positive attitude towards the prevention and control of rabies. About 84 to 92% of the respondents also mentioned that they would report to the hospital for treatment if bitten by dogs and other animals, indicating good health seeking behaviours of the people. The respondents also had a positive attitude towards prevention and control of rabies in dogs by vaccination. However, these findings also indicated the existence of some knowledge gaps (knowledge about rabies and its transmission and importance of wound washing) which could be filled by creating awareness education programmes on: the danger of rabies and mode of transmission to humans and importance washing animal bite wound and visiting a hospital for post-exposure prophylaxis.

Since rabies causes substantial financial losses to society, understanding the cost-benefit or cost-effectiveness of the intervention programme is important. Quantification of the financial cost of rabies intervention in Bhutan suggested that the average direct medical cost of human PEP (using rabies vaccine only) was approximately Bhutanese Ngultrum (Nu) 1615 (US$ 35.65) per 5-dose Essen regimen per patient. The cost would increase to Nu. 2497 (US$ 55.13) and Nu. 19633 (US$ 433.41) per patient, if one dose of either equine rabies immunoglobulin (ERIG) or human rabies immunoglobulin (HRIG) was administered, respectively. The societal cost (public plus private cost) per patient was

estimated to be Nu. 2019 (US$ 45), Nu. 2901 (US$ 64), and Nu. 20037 (US$ 442) using vaccine alone, vaccine with ERIG and vaccine with HRIG, respectively. The average cost per dog vaccination was estimated to be Nu. 75 (US$ 1.66) and the cost per dog sterilization was estimated to be Nu. 288 (US$ 6.52). The total direct medical cost due to rabies (including surveillance and livestock loss cost, PEP in human and dog vaccination and sterilization) between 2001 and 2008 was estimated to be Nu. 48.54 million (US$ 1.07 million). The analysis also showed that mass dog vaccination would be more cost-effective than intensified post-exposure prophylaxis in human alone.

The above findings suggest that an area bordering India in the south were at higher risk of reporting rabies than the interior of Bhutan. More resources for rabies control programs and surveillance should be targeted and focussed in the highly endemic 'hot spot' areas of southern Bhutan. Mass vaccination of dogs in the border areas in the south would create an immune buffer (*cordon sanitaire*) and prevent incursion of rabies into interior Bhutan. A One-Health approach for rabies control in Bhutan should be implemented towards elimination of rabies through creation of effective partnership focussing on coordinating research, operational activities and pooling of resources between public health and veterinary services. Elimination of rabies through mass dog vaccination would reduce the recurrent cost of intensified PEP in humans and will produce economic savings in the long run by preventing human and livestock deaths and by discontinuing the intensified use of PEP in humans and rabies control programmes. Public awareness education is necessary and should include: the risk of rabies exposure; importance of preventing dog bites and wound washing and visiting health centres following dog bites and exposure to suspected rabid animals. Epidemiological surveillance of rabies should be improved by the laboratory confirmation of all suspected cases, including human, and the data so generated should be shared between the public health and veterinary sectors and also relevant international organizations. International collaboration is necessary for technical and financial support for sustaining rabies control in Bhutan.

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This thesis is also dedicated to all the victims of rabies in the world. May these thesis findings help in preventing this neglected and fatal zoonosis.

List of publications

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CHAPTER 1

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Rabies is a fatal zoonosis caused by rabies virus of the genus *Lyssavirus*, family *Rhabdoviridae* (Kaplin et al., 1986; Wunner, 2007). It is mainly transmitted through the bite of a rabid animal of which domestic dogs are responsible for the vast majority (99%) of human deaths from rabies worldwide (Knobel et al., 2005). Rabies remains a neglected zoonosis and poses a potential threat to more than 3.3 billion people in the world, despite development of the first vaccine against rabies by Louis Pasteur in 1885 (Knobel et al., 2005; WHO, 2010a). The most recent assessment estimates 70,000 human deaths from rabies each year in the world with most deaths occurring in Asia and Africa (Hampson et al., 2011a). The burden of rabies is influenced by age-related and socioeconomic factors: rabies is most commonly reported in children below 15 years of age and in poor and low income people that have no access to treatment facilities (Pancharoen et al., 2001; Cleaveland et al., 2002; Knobel et al., 2005; Sudarshan et al., 2007). Control of rabies in the animal reservoirs – domestic dogs – is the only means to prevent the transmission cycle of disease and eliminate both dog and human rabies cases in the world (Coleman and Dye, 1996; Cleaveland et al., 2006; Zinsstag et al., 2007; Zinsstag et al., 2009).

Bhutan is a small country (38,394 km^2) in South Asia, located between India in the south and the Tibetan province of the People's Republic of China in the north. While the border in the north with China has natural physical barriers (Himalayan Mountain ranges), it is porous with India and there is free movement of people for trade and other business between Bhutan and India. While there is no accurate record of the first occurrence of rabies in Bhutan, it was believed to be prevalent throughout Bhutan in the 1970s and 80s and caused deaths of both animals and humans (Joshi, 1991; Owoyele, 1992; Bhutan Observer, 2008). Currently, rabies is mainly reported in southern parts of Bhutan bordering India with sporadic occurrence in the previously free areas. Domestic dogs are responsible for transmission of rabies to both livestock and humans and no wildlife rabies cases have been reported in Bhutan. Although

human rabies cases are sporadic, dog bite incidents are common due to the presence of a large number of stray dogs in the streets.

Rabies in animals is a notifiable disease as per the Bhutan Animal Husbandry Act 1981 (amended in 2001). Any suspected cases have to be notified to the veterinary authorities for investigation and control programmes. The first national rabies control programme was initiated in 1985 under the United Nation Project (Joshi, 1991; Kinley Dorjee, Personal communication). The classical methods of canine rabies and stray dog population control constituted killing of stray dogs by shooting and oral poisoning. Later, mass dog vaccination and sterilization programmes were conducted annually but the coverage was variable due to lack of strategic control programmes. The issue on stray dog population was even discussed at the highest decision making body in the country – National Parliament – that passed a resolution to plan a strategic dog population control programmes (Kuensel, 2007). In 2007, a stray dog impounding programme was started to reduce stray dog population in urban areas (Kuensel, 2007; NCAH, 2007). Stray dogs were caught from the streets and kept in dog shelters constructed in each of the 20 districts in Bhutan. However, in late 2009 the impounding strategy was stopped due to logistic constraints and on animal welfare grounds. In September 2009, a nation-wide "catch-neuter-vaccinate-release (CNVR) project (at a cost of US$ 1 million) was started in Bhutan in collaboration with the Humane Society International organization to vaccinate and sterilize >70% of the estimated 50,000 dog population in Bhutan (MoA, 2009; HSI, 2010). The impact of this program is yet to be assessed in terms of the demography of the street dog population, dog bite incidents in humans, and prevalence of rabies in both human and animals.

Although rabies has been endemic and caused economic losses to society, no detailed studies have been conducted to understand the spatial and temporal patterns of occurrence, rabies virus strains circulating in the dog population, and the risk factors associated with rabies occurrence in Bhutan. Furthermore, no studies have been conducted to understand dog bite incidents in humans, its risk factors and the use of rabies post-exposure prophylaxis in humans. The true public health burden of rabies and its economic implications to the government and to society is also unknown despite there being increased public expenditure on rabies prevention and the implementation of control programmes. Understanding these issues is vital for designing better rabies prevention and control programmes in Bhutan.

Therefore, the main objectives of this study were to:

1. Describe the distribution (patterns, trends, seasonal, spatial and temporal) of rabies occurrence in animals in Bhutan.
2. Identify risk factors associated with endemic rabies occurrence and re-emergence of rabies in Bhutan.
3. Identify rabies virus strains circulating in the dog population through molecular and phylogenetic studies.
4. Understand the prevalence and risk factors associated with dog bites in humans, and to estimate human rabies mortality in Bhutan.
5. Understand the use of rabies post-exposure prophylaxis in humans.
6. Better understand the level of community knowledge, attitude and perception of rabies and the rabies control program.
7. Estimate the cost of various rabies interventions measures in humans and animals compare the cost-benefit of dog rabies elimination on human post-exposure prophylaxis cost, and to estimate the overall economic burden of rabies in Bhutan.

The above research components were implemented through epidemiologic data analysis using rabies surveillance data and the conduct of epidemiologic surveys as described in the chapters of this thesis.

Thesis outline

Chapter 1 of this thesis is a literature review and provides an account of the epidemiology and control of rabies. In Chapter 2, a review of the past and present rabies situation and control programmes in each country of South, South East and East Asia are described. Chapters 3 describe the spatio-temporal patterns of rabies occurrence in animals in Bhutan between 1996 and 2009. Chapters 4 and 5 describes the re-emergence of rabies in domestic dogs and its spill-over infection to other domestic farm animals in eastern and south west Bhutan between 2005 and 2008. The possible risk factors for the spread of disease, the role of various control measures implemented in these two separate outbreak areas and the use of human post-exposure prophylaxis are described in these chapters. Chapter 6 describes the overall spatial distribution of rabies in dogs and cattle in Bhutan, analyzed using Geographic Information System and spatial statistics. Chapter 7 describes the anthropogenic and environmental risk factors of rabies occurrence at the sub-district level in Bhutan. Chapter 8 documents the

rabies virus strains circulating in Bhutan and their potential origins. In Chapter 9, the trend and use of rabies post-exposure prophylaxis in human is described. Chapter 10 describes the incidence and risk factors associated with dog bites in humans in the three hospital regions of Bhutan. This chapter also presents the predicted human rabies mortality in the two rabies endemic areas of southern Bhutan which was estimated using a decision tree model. Chapter 11 presents a community-based study on people's level of knowledge, attitude and perception of rabies and rabies control measures in one of the rabies endemic sub-districts in southern Bhutan. Chapter 12 addresses the cost of rabies prevention and control and the cost−benefit of dog rabies elimination versus human post-exposure prophylaxis cost. Finally, the thesis concludes with a "General Discussion" of the main results from each of the chapters and with some specific recommendations for rabies control measures in Bhutan (Chapter 13). Since all chapters were written in a published journal format, there is some overlapping information among the chapters.

1.2.Historical perspective of rabies

Rabies has been recognized as a disease of dog and man since ancient times. The Latin word 'rabies' is derived from an old Sanskrit word *'rabhas'* which means 'to do violence' (Steele, 1975; Wilkinson, 2002). The first references to mad dogs had appeared in legal documents in Mesopotamia as early as 2300 B.C. where the owners of mad dogs were held responsible for any deaths resulting from their bites (Tierkel, 1975a; Wilkinson, 2002; Baer, 2007). It was apparent to them that the disease was transmissible from dog to man via bites and caused death of the bitten person (Baer, 2007). Isolation and control of mad dogs was used as an effective public health protective measure even during that time. Several types of remedies were described for dog bites and rabies in humans since 500 B.C. Cutting of the frenulum linguae (a mucous membrane) that attached the dog's tongue and removing a fold of mucus membrane was believed to have removed the worm that caused rabies and prevent rabies. Cauterization of the wound with hot iron, and keeping the wound open and application of ordinary blistering chemicals on it were some of the other remedies practised. In addition, cupping, sucking and application of salts to the bite wound and giving a hot baths to hydrophobic patients were other remedies practiced in olden days (Steele, 1975).

In 1804, German physician, Zinke demonstrated that the disease was infectious by inoculating normal dogs with saliva from a rabid dog – the inoculated dog became sick by the

seventh day and showed symptoms of rabies on the tenth day (Baer, 2007). Later, in 1879, Victor Galtier, professor at the Veterinary School in Lyon, transmitted rabies from dog to rabbit and from rabbit to rabbit by injection and bite (Baer, 2007). In 1880, Louis Pasteur (a chemist) had followed the rabies work on rabbits that Victor Galtier had initiated. After five years of research together with his collaborators (Emile Roux – a medical physician, Charles Chamberland – a physiological chemist, Edmong Nocard – a veterinarian and Louis Thullier), Pasteur immunized dogs by injecting suspensions of brain tissues, obtained from rabies infected rabbits, that were dried for several days to reduce the virulence of the virus. The 50 dogs immunized in this group resisted rabies infection when challenged by injecting virulent rabies virus intracerebrally (Baer, 2007). At about the same time a nine year boy, Joseph Meister, who had been bitten severely by a rabid dog, arrived in Paris, and on July 5, 1885, Pasteur inoculated rabies vaccine into Joseph Meister and he never developed rabies (Wilkinson, 2002; Baer, 2007). This became a milestone in medicine for treatment of rabies in humans. In 1888, the Pasteur Institute of Paris was founded and within a decade Pasteur Institutes were established throughout the world, with a primary focus on rabies control (Wilkinson, 2002).

In 1908, Fermi modified Pasteur's methods of vaccine preparation. He treated the infected nerve tissue with phenol to inactivate rabies virus which reduced the chance of infecting a patient with a live virus from the vaccine (Baer, 2007; Briggs, 2007). In 1911, Sir David Semple, an Englishman working at the Central Research Institute in Kasauli, India, also modified Pasteur vaccine (Baer, 2007; Briggs, 2007). He developed nerve tissue vaccine from the brain tissue of young sheep and goats (called 'Semple vaccine') by inactivating the infected nerve tissue materials with phenol or beta propiolactone (Baer, 2007; Briggs, 2007). Since then, the Semple vaccine production facilities have been established in Asia and the rest of the world. In 1955, Fuenzalida and Palacios made an improvement to nerve tissue vaccine and produced suckling-mouse brain tissue vaccine by inactivating the infected brain tissues of the suckling mouse with ultraviolet light or beta-propiolactone which reduced the sensitivity reaction to myelin tissue (Briggs, 2007). However, since the nerve tissue vaccine contain myelin components of brain tissue and had resulted in neuroparalytic adverse reactions, the WHO recommended complete replacement of nerve tissue rabies vaccines with modern cell culture rabies vaccines (Hemachudha et al., 1987a; Hemachudha et al., 1987b). Subsequently, the nerve tissue vaccine production and use has been stopped in most countries

and switched over to cell culture vaccines, although it is still produced and used in Pakistan and Bangladesh (Salahuddin, 2009; Hossain et al., 2011), and some of the Latin American countries (Suzuki et al., 2008).

Although rabies vaccine was developed by Pasteur in the 1880s, the first national program to vaccinate dog against rabies began only 35 years after Pasteur's studies. The first phenol inactivated dog vaccine was developed in Japan in 1918 by Umeno and Doi (Umeno and Doi, 1921), and in 1921 Japan started a national rabies control campaign by vaccinating dogs in Nagasaki and Tokyo. However, Japan only became free from rabies in 1957 (Tamashiro et al., 2007; Takahashi-Omoe et al., 2008). Hungary conducted the first successful field trials to demonstrate elimination of rabies through mass dog vaccination and killing of stray and rabid dogs during 1935–1944 (WHO, 1987; Knobel et al., 2007). Later in the 1950s several other countries carried out rabies elimination programs through mass dog vaccination and killing of dogs with canine rabies being eliminated from Malaysia in 1954 (Wells, 1954), Hong Kong in 1955 (SPCA, 1980), Taiwan and Portugal in 1961 (CDC, 2007; Weng et al., 2010), the USA in the 1960's (Krebs et al., 2005), and South Korea in 1985 (Hyun et al., 2005).

1.3.Rabies virus characteristics and disease

1.3.1. Aetiology

Rabies is caused by rabies virus, the prototype species of the genus *Lyssavirus*, family *Rhabdoviridae* and order *Mononegavirales* (ICTV 2011; Wunner, 2007). According to the International Committee on Taxonomy of Viruses, 12 species are classified under the *Lyssavirus* genus: Rabies virus (RABV); Lagos bat virus (LBV); Mokola virus (MOKV); Duvenhage virus (DUVV); European bat Lyssavirus 1 (EBLV-1); European bat Lyssavirus 2 (EBLV-2); Australian bat Lyssavirus (ABLV); Aravan virus (ARAV); Khujand virus (KHUV) and West Caucasian bat virus (WCBV) (see Table 1.1) (ICTV, 2011). A new virus, Shimoni bat virus (SHIBV), which was identified in 2009 from a bat in Kenya (Kuzmin et al., 2010), is now classified and accepted by ICTV as the twelfth species of Lyssavirus genus (ICTV, 2011). In addition, two newly identified lyssaviruses − Bokeloh bat lyssavirus (BBLV) isolated from a Natterer's bat (*Myotis nattererii*) in Germany in 2010 (Freuling et al., 2011) and Ikoma lyssavirus (IKOV) isolated from an African civet (*Civettictis civetta*) on May 11, 2009 (Marston et al., 2012) may represent new members of the genus Lyssavirus,

but have not been classified yet. More lyssaviruses in bat species are expected to be detected and identified in future as the increasing scientific research efforts in the bat population continues.

Rabies virus (RABV) is the most widespread and recovered from terrestrial mammals globally and from *Chipropteran* bats in the Americas. Other rabies-related lyssaviruses are more restricted in their host range and geographical distribution (Banyard et al., 2011) (see Table 1.1).

Table 1.1: Classification of the genus *Lyssavirus*, familiy *Rhabdoviridae*, their potential reservoir and geographic distribution

Virus name and abbrevation[1]	Geographic origin and distribution	Potential reservoirs	Reference source
Rabies virus (RABV)	Worldwide distribution (except Australia, Antartica, island nation and designated rabies-free countries)	Canivora: domestic dogs and wild carnivores species (worldwide); mongoose, raccoons, shunks; and different bats species (in Americas only).	(Shope, 1982)
Lagos bat virus (LBV)	First isolated in 1956 from fruit bats (*Eidolon helvum)* at Lagos Island in Nigeria, then in 1974 from fruit bats (*Micropeterus pusillus*) in the Central African Republic, and in 1980 from a fruit bat (*Epomophorus wahlbergi*) in South Africa, domestic cat from South Africa in 1982 and Zimbabwe in 1986; from domestic dog in Ethopia in 1989/90, insectivorous and fruit bats in Senegal in 1985	Bats Megachiroptera: (*Eidolon helvum; Micropeterus pusillus ;Epomophorus wahlbergi)*	(Boulger and Porterfield, 1958; Kuzmin and Rupprecht, 2007)
Mokola virus (MOKV)	First isolated in 1968 from shrews at Mokola forest in Nigeria, then in 1969 and 1971 from humans in Nigeria. It has also been isolated from domestic cats in South Africa, Zimbabwe, and Ethopia; from domestic dogs in Zimbabwe, screw in Cameroon and rodent in Central African Republic.	Shrew-Insectivora; Crocidura spp; Rodent species: Rodentia (*Lopyhromys sikapusi*)	(Shope et al., 1970; Kuzmin and Rupprecht, 2007)
Duvenhage virus (DUVV)	First isolated in 1970 from human bitten by insectivorous bats and then in 1981 directly from insectivorous bats in South Africa. Also isolated from fruit bats in Zimbabwe in 1986 and in Guinea	Bats: Microchiroptera; (*Miniopterus schreibersii; Nycteris gambiensis; N. thebaica)*	(Meredith et al., 1971; Kuzmin and Rupprecht, 2007)
European bat Lyssavirus 1 (EBLV-1)	First isolated in 1985 from insectivorous bats *(Eptisecus serotinus)* in Europe. Distributed in the Netherlands, Denmark, Germany, Poland, Hungary, Russian Federation, France, Spain	Bats: Microchiroptera *(Eptisecus serotinus)*	(Warrell and Warrell, 2004)
European bat Lyssavirus 2 (EBLV-2)	First isolated in 1985 from insectivorous bats *(Myotis sp.)* in Europe. Distributed in the Netherlands, UK, Germany,Ukraine, Switzerland	Bats: Microchiroptera *(Myotis dasycneme; M. daubentonii)*	(Warrell and Warrell, 2004)
Australian bat Lyssavirus (ABLV)	First isolated in 1996 from humans. Insectivorous and frugivorous bats in eastern Australia. Possibly prevalent in SE Asia.	Bats:Megachiroptera.*(Pteropus alecto, P. scapulatus)* and also in Microchiroptera sp.	(Speare et al., 1997; Gould et al., 1998)
Aravan virus (ARAV)	First isolated in 1991 from insectivorous bats *(Myotis blythi)* in Kyrghyzstan	Bats: Microchiroptera *(Myotis blythi)*	(Arai et al., 2003)
Khujand virus (KHUV)	First isolated in 2001 from insectivorous bats *(Myotis mystacinus)* in Tajikistan	Bats: Microchiroptera *(Myotis mystacinus)*	(Kuzmin et al., 2003)
Irkut virus (IRKV)	First isolated in 2002 from insectivorous bats *(Murina leucogaster)* in Eastern Siberia near Lake Baikal	Bats: Microchiroptera *(Murina leucogaster)*	(Botvinkin et al., 2003)
West Caucasian bat virus (WCBV)	First isolated in 2003 from insectivorous bats *(Miniopterus schreibersi)*, from Western Caucasus Mountains	Bats: Microchiroptera *(Miniopterus schreibersi)*	(Botvinkin et al., 2003)
Shimoni bat virus (SHIBV)	First isolated in 2009 from the brain of a dead Commeron's leaf-nosed bat (*Hipposideros commersoni*), found in a cave in the coastal region of Kenya, Africa	Bat: *(Hipposideros commersoni)*	(Kuzmin et al., 2010; ICTV 2011)
Bokeloh bat lyssavirus (BBLV) (not classified yet)	First isolated from a Natterer's bat (*Myotis nattererii*) in Germany in 2010	Bats: (*Myotis nattererii*)	(Freuling et al., 2011)
Ikoma lyssavirus (IKOV) (not classified yet)	First isolated from an African civet (*Civettictis civetta*) on May 11, 2009	African civet (*Civettictis civetta)*	(Marston et al., 2012)

[1]International Committee on Taxonomy of Virus (ICTV). Data extracted from (Warrell and Warrell, 2004; Childs and Real, 2007; Kuzmin and Rupprecht, 2007; Nadin-Davis, 2007) and other listed articles. Table adapted from (Childs and Real, 2007).

1.3.2. Rabies virus structure

Rabies virus has a negative-sense, non-segmented, single stranded RNA (ribonucleic acid) genome, and a distinctive bullet shape (with one rounded end and the other a planar end). The virion is 11−15 kb in size, 70 nm in diameter and 100−300nm in length (Tordo, 1996; Murphy et al., 1999). The virus is composed of a single molecule of genomic RNA and five structural proteins: the nucleoprotein (N protein), phosphoprotein (P protein), matrix protein (M protein), glycoprotein (G protein), and the RNA-dependent RNA polymerase (L protein) (Tordo, 1996; Wunner, 2007). The structure of virion is composed of two structural and functional sub-units. The internal helically packaged ribonucleocapsid complex (RNP) is formed by the N, P, and L proteins, associated with genome transcription and replication in the cytoplasm, and potentially plays a role in the establishment of immunologic memory and long-lasting immunity (Wunner, 2007). The RNP is surrounded by a lipid bi-layer associated with the G and M proteins. The outer envelope is covered with spike-like projections (10 nm in length and 5 nm apart) corresponding to G-protein which recognize specific viral receptors on susceptible cell membranes. The envelope glycoprotein G of rabies virus induces the production of rabies virus-neutralizing antibodies, which are important in protection against rabies (Grassi et al., 1989; Tordo, 1996; Wunner, 2007; Murphy et al., 1999). The protein M occupies an intermediate position between the envelopes and the RNP core, and is associated with both the RNP and the G protein and responsible for virus budding and the bullet-shaped morphology (Wunner, 2007) (see Figure 1.1).

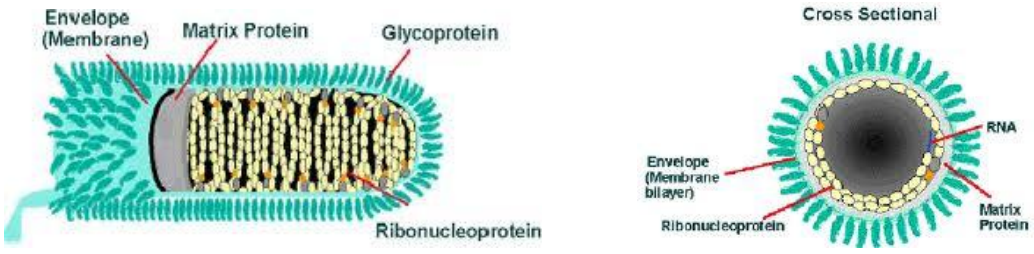

Figure 1.1: Rabies virus virion (Source: http://www.cdc.gov/rabies/transmission/virus.html).

1.3.3. Physical properties of the virus

Rabies virus is quite fragile and can be destroyed by various concentrations of formalin, ether, phenol, halogens, mercurials, mineral acids, soap solution and other detergents. The virus can remain stable at pH values between 5 and 10 and becomes unstable on exposure to acid and alkaline condition (below pH 4 or above pH 10) (Turner and Kaplan, 1967). The virus is also rapidly inactivated by desiccation, ultraviolet radiation, sunlight, and does not survive for long periods in the environment. In experiments the virus was stable at 37°C for up to 2 hr, lost 90% of its infectivity after 4 to 6 hr and > 99.9% after 24 hr when partially purified rabies virus suspensions were exposed to different temperatures for different periods (Turner and Kaplan, 1967). However, the virus can remain stable for a few days at 0 to 4°C in the internal organs; in saliva in temperate climate for about 24 hours and survives indefinitely when freeze dried or kept at -70°C (Kaplin et al., 1986). Glycerol preserves the virus and therefore, brain and other tissues for laboratory examination are preserved in 50% glycerol in phosphate buffered saline solution (Barrat, 1996; OIE, 2011a).

1.4. Transmission

Various possible routes of rabies transmission are described below:

1.4.1. Direct contact: animal bites and licks

Rabies is mainly transmitted by the bite of a rabid animal that contain rabies virus in the saliva (Kaplin et al., 1986; Jackson, 2007a). The virus can also be transmitted via direct contact of fresh wound or intact mucosal surface (eyes, nose) with infectious saliva or by licks of infected animals, and transdermal scratches contaminated with infectious material. The virus cannot penetrate the intact skin (Kaplin et al., 1986; Jackson, 2007a).

1.4.2. Ingestion and oral transmission

Ingestion of raw meat or other tissues from animals infected with rabies is not a known source of human infection (WHO, 2010a). However, transmission of rabies in humans through handling and skinning of infected carcases and subsequent consumption of raw meat has been reported in Iran (Tariq et al., 1991). Similarly, transmission of rabies through

ingestion of raw dog meat has also been reported in South East Asia (Kureishi et al., 1992; Wallerstein, 1999; Hu et al., 2009; Wertheim et al., 2009; Dimaano et al., 2011; Hanh, 2011). The skinning and handling of carcases with bare hands and touching eyes or lips with hands while they are contaminated by traces of the dog's fluids have been suggested as the main cause of contracting rabies (Kureishi et al., 1992). Pasteurization and cooking will inactive rabies virus (Turner and Kaplan, 1967); therefore, drinking pasteurized or boiled milk or eating thoroughly cooked animal products do not constitute rabies exposures (WHO, 2010a; CDC, 2011).

1.4.3. Aerosol transmission

Transmission of rabies by inhalation of virus-containing aerosol is rare but has been reported and can be a potential hazard for laboratory workers. Inhalation of an aerosolized rabies virus during homogenization of fixed virus in the laboratory had resulted in two human rabies cases (Winkler et al., 1973; Tillotson et al., 1977). Similarly, aerosolised virus from bat urine has been suspected as an exposure pathway for wildlife investigators (Constantine, 1962; Winkler, 1975). In 2002, a Scottish bat conservationist was diagnosed with rabies infection following exposures to bats, but the possibility of a bite could not be excluded completely in this incident (Fooks et al., 2003; Johnson et al., 2006a).

1.4.4. Human-to-human transmission

Eight cases of human-to-human transmission of rabies through corneal transplant from undiagnosed organ donors (patient died of neurological symptoms, but later was confirmed to have died of rabies) to the recipients has been documented in the United States (Houff et al., 1979; Arjun et al., 2005), France (CDC, 1980) Thailand (Thongcharoen et al., 1981), India (Gode and Bhide, 1988) and Iran (WHO, 1994a; Javadi et al., 1996). In addition, seven human-to-human transmission of rabies through other solid organ tissue transplants (e.g. liver, kidney, lung, pancreas, iliac artery) has also been reported and documented in the USA (Srinivasan et al., 2005) and in Germany (Johnson et al., 2005). Although rabies virus has been isolated from a variety of tissues and body fluids, including cerebrospinal fluid, saliva, tears and urine sediment (Helmick et al., 1987; Hemachudha and Wacharapluesadee, 2004; Madhusudana and Sukumaran, 2008), no well documented evidence of transmission of virus

from rabies victim's saliva and secretions to other humans including the close attendant, relatives, friends and medical staff exists. However, some anecdotal reports of suspected transmission of rabies from human-to-human have been inadequately documented in rabies endemic areas, and did not exclude a possible animal exposure (Helmick et al., 1987; Gibbons, 2002). In Ethiopia, human-to-human transmission of rabies between mothers and children involving bites and kisses have been reported (Fekadu et al., 1996; Jackson, 2007a). This evidence, although rare, suggest that the person handling rabid patients (hospital nurses and family members of patients) should take necessary precautionary measures (Helmick et al., 1987; Jackson, 2007a).

1.4.5. Transplacental transmission

Rabies in pregnancy is very rare. There is only one recorded case of transplacental transmission of human rabies reported in Turkey. A nine-month pregnant woman was bitten by dog 34 days before she gave birth to a baby boy by induction. The baby died after 40 hours and 30 minutes, and the laboratory examinations confirmed rabies in both the mother and the baby (Sipahioglu and Alpaut, 1985). In contrast, in Thailand, infants have survived delivery from mothers infected with rabies, when the child was given a series of post-exposure rabies vaccination (Lumbiganon and Wasi, 1990).

1.5. Pathogenesis

The lyssavirus is a highly neurotrophic virus that causes an acute encephalomyelitis of the central nervous system (CNS) (Jackson, 2007b). After entry of the virus, commonly through infiltration of virus-contaminated saliva from a rabid animal into a bite wound, the virus replicates in the muscle cells and in the neuromuscular spindles at the site of the bite. The virus then enters the peripheral nerves, and is transported by retrograde axoplasmic flow via peripherial nerves to the CNS. This occurs via sensory and motor nerves at the initial site of infection (Hemachudha, 1989; Jackson, 2007b). The speed of virus retrograde transport has been estimated between 50 to 100 mm per day but depends on the amount of virus inoculated at the site of bite (Tsiang et al., 1991). The exposed individual will not show any symptoms during this time. Once the virus reaches the brain, it further replicates (due to the large numbers of neuronal cell bodies in the brain) and disseminates within the CNS. The patient

or animals will show first signs of rabies after the virus has multiplied in the brain. Finally, the virus travels centrifugally from the CNS through peripheral nerves to various tissues, most notably the salivary glands, and the transmission cycle is repeated. Salivary gland infection and shedding of virus in saliva is essential for the transmission of virus to its natural susceptible hosts, again usually through a bite wound or contamination of mucous membranes by virus-contaminated saliva, and the maintenance of the epidemiologic cycle (Hemachudha, 1989; Jackson, 2007b). In experiments, rabies virus is excreted in the saliva of infected dogs before signs of disease were observed and during the course of disease (Fekadu et al., 1982; Vaughn et al., 1963; Vaughn et al., 1965). No specific gross pathognomonic lesions in brain have been observed due to rabies, other than infiltration of cells (Negri bodies) (Hemachudha, 1989).

1.6. Clinical signs

1.6.1. Humans

The incubation period of rabies in humans is usually about 1 to 3 months after exposure, but can range from less than 7 days to over 1 year (Hemachudha, 1989; Hemachudha and Phuapradit, 1997; Jackson, 2007a; Johnson et al., 2008). The longest incubation period of 27 years was reported in the Philippines (Dimaano et al., 2011). However, in rabies endemic areas, there is always a possibility of recurrent unrecognized natural infection (Hemachudha, 1989; Jackson, 2007a).

The incubation period depends on various factors including location and severity of bite wounds, amount of virus inoculated into the bite wound, the degree of innervations at the bite site, and host factors. The bites on the face, neck, hand (nearer to the brain) have higher risk and have shorter incubation period than bites on the extremities (Hemachudha, 1989; Jackson, 2007a).

Rabies is manifested as encephalitic or classical (also called furious) and paralytic rabies. About 80% of the patients develop a furious form of rabies, and about 20% have a paralytic form of rabies (Jackson, 2007a). The clinical course of rabies in humans can be divided into

three phases: the prodomal phase, the excitative phase, and paralysis, coma and death (Hemachudha, 1989; Jackson, 2007a).

During the prodromal period of the first 2−10 days, the onset of the disease can be exhibited as fever, chills, insomnia, headache, loss of appetite, tiredness, weakness and anxiety. About 30−70% of the patients may develop local paraesthesia, numbness, burning pain, tingling and itching at or close to the bite site or the whole limb (Hemachudha, 1989; Hemachudha and Phuapradit, 1997; Jackson, 2007a). The recognition of these prodromal signs is crucial if early post-exposure prophylaxis is to alter the fatal course of disease (Hemachudha, 1989).

The prodromal phase is followed by 2−7 days of acute neurologic excitative symptoms (encephalitic/furious forms) of rabies with anxiety, confusion and hallucinations. Patients may experience pain in the throat or difficulty in swallowing due to painful spasms of larynx muscles leading to fear of water called hydrophobia (about 50−80% of patients develop hydrophobia) (Jackson, 2007a). Hydrophobic attacks and external stimuli such as sound, air and touch may be associated with episodes of excitement, agitation, and aggression. There will be frothing of thick saliva from the mouth (hypersalivation), lacrimation, sweating, piloerection (gooseflesh), and dilated pupil (Hemachudha, 1989; Hemachudha and Phuapradit, 1997; Jackson, 2007a). The excitative phase progresses to severe flaccid paralysis, coma, multiple organ failure and terminates in death during 1 to 2 weeks of illness (Jackson, 2007a).

Paralytic rabies (also called dumb rabies) is associated with flaccid muscle weakness that develops early in the course of the disease (Jackson, 2007a). The weakness often begins in the bitten extremity and then spreads to involve other extremities. The muscle fasciculation and bilateral facial muscle weakness have been observed in paralytic rabies (Phuapradit et al., 1985). Paralysis of the respiratory muscles ultimately leads to death due to cardio-pulmonary arrest (Hemachudha, 1989; Hemachudha and Phuapradit, 1997; Jackson, 2007a). Paralytic rabies may be confused with Guillain-Barré syndrome and related disorders of peripheral nerves (Hemachudha et al., 2005). Hydrophobia is more unusual in the paralytic form of rabies, but mild inspiratory spasm is commonly observed (Hemachudha et al., 1988). It has been observed that survival in the paralytic form is usually longer (up to 30 days) than

in the furious form of rabies, but the mechanisms responsible for the weakness and longer survival periods are unclear (Hemachudha et al., 2005).

1.6.2. Animals

The incubation period of rabies in dogs is 3−8 weeks on average, but may vary from 10 days to as long as 6 months, but is rarely more than 4 months (Tierkel, 1975a). In general, rabid animals of all species commonly exhibit typical signs of central nervous system disturbances with behavioural changes (Niezgoda et al., 2002).

A rabid dog may show either the furious or the paralytic (dumb) form of rabies. The major clinical signs in dogs are aggression, abnormal barking, biting unusual things like sticks and stones, roaming, laryngeal paralysis, and excessive salivation, tremors, ataxia, and generalized seizures (Tierkel, 1975a; Bowen-Davies and Lowings, 2000; Niezgoda et al., 2002).

In experimental studies of rabies infection in cattle (n=20), the average incubation period was 15 days and the average morbidity period was 4 days (Hudson et al., 1996a). The major clinical signs in cattle included excessive salivation (100%), behavioural changes (100%), muzzle tremors (80%), vocalization (bellowing; 70%), aggression, hyperesthesia and/or hyperexcitability (70%), and pharyngeal paresis/paralysis (60%) (Hudson et al., 1996a). The clinical signs in sheep included muzzle and/or head tremors, aggressiveness, hyperexcitability, and/or hyperaesthesia, trismus, salivation, dropping ears, vocalization, and recumbency (Hudson et al., 1996a; Bowen-Davies and Lowings, 2000). The furious form of rabies was seen in 70% of the cattle and 80% in sheep (Hudson et al., 1996a).

In horses (of the 21 experimental infections), the average incubation period was 12 days and the average morbidity period was 6 days with 43% of horses developing furious rabies (Hudson et al., 1996b). Muzzle tremors were the most frequently observed and most common initial sign (80%). Other common signs observed were pharyngeal spasm or pharyngeal paresis (71%), ataxia or paresis (71%), lethargy or somnolence (71%) (Hudson et al., 1996b).

The paralytic form (dumb form) of rabies is characterized by the inability to swallow, hanging of jaw due to paralysis leading to a typical sign of foaming saliva around the mouth. There will be ascending paralysis which begins at the hind extremities and eventually complete paralysis is followed by death (Kaplin et al., 1986).

1.7.Diagnosis of rabies

In developing countries, rabies diagnoses are mostly made based on clinical signs, history of exposure and epidemiological information due to lack of facilities (Hemachudha, 1989; Fooks et al., 2009). Although primary diagnosis may depend on clinical signs, rapid and accurate diagnosis by laboratory tests is important for deciding on post-exposure prophylaxis in humans and initiating rabies control in animals (Meslin and Kaplan, 1996; Trimarchi and Smith, 2002).

The detection of Negri bodies in brain samples using Sellers Stain techniques was the first method used to diagnose rabies, but has now been replaced with highly sensitive and specific modern techniques briefly discussed below (Tierkel and Atanasiu, 1996; Trimarchi and Smith, 2002; Fooks et al., 2009). The standardised techniques of rabies diagnosis are available in the fourth edition of the *WHO's Laboratory Techniques in Rabies* (WHO, 1996) and the *OIE Terrestrial Manual 2011* (OIE, 2011a). The emerging technologies for the detection of rabies virus have been described by Fooks et al. (2009) and Dacheux et al. (2010). The main principles of rabies diagnosis are based on antigen and antibody detection (Fooks et al., 2009).

1.7.1. Fluorescent Antibody Test (FAT)

FAT is the 'gold-standard' and most widely used test for rabies diagnosis, recommended by both the World Health Organization and the World Organization for Animal Health (OIE). The test is sensitive, specific, and cheap and can be completed in less than two hours to confirm the presence of rabies antigen (Dean et al., 1996; Meslin and Kaplan, 1996; Fooks et al., 2009; OIE, 2011a). This technique involves the preparation of impression smears from brain tissues, tissue fixation in cold acetone, and then staining with fluorescent isothiocyanate-labeled polyclonal or monoclonal anti-rabies antibodies and visualization

under florescent microscope (Dean et al., 1996; OIE, 2011a). The test can be directly used on fresh brain tissues or glycerol preserved tissues, and can also be used to confirm the presence of rabies antigen in cell culture or in brain tissues of mice that have been inoculated for diagnosis. However, the sensitivity of the test depends on the degree of autolysis (autolyzed samples can reduce the sensitivity and specificity of the test), sampling of the brain, the competencies of the technicians, and the quality of the reagents (conjugates) used (Fooks et al., 2009; OIE, 2011a).

1.7.2. Cell culture and Mouse inoculation test

These tests are based on the principles of detecting the infectivity of a rabies virus tissue suspension in cell cultures or in laboratory animals (mouse) after inoculation. These tests should be used if FAT gives an uncertain result or when FAT is negative in the case of known human exposure (OIE, 2011a). Virus isolation on cell culture is preferable to the mouse inoculation test because cell culture tests give more rapid results, and help avoid the use of animals. Both tests require longer turnaround times compared to FAT (4-days for cell culture test and 28-days for mouse inoculation test) (King, 1996; Fooks et al., 2009; OIE, 2011a).

1.7.3. Direct Rapid Immunohistochemical Test (dRIT)

A direct Rapid Immunohistochemical Test employs anti-rabies monoclonal antibodies specific for the nucleoprotein (a viral protein produced in abundance during productive infection), and can detect rabies antigen by direct staining of fresh brain impressions within 1 hour (Fooks et al., 2009). The dRIT uses a cocktail of highly concentrated and purified biotinylated anti-nucleocapsid monoclonal antibodies (Lembo et al., 2006). A field study in Tanzania has shown that the dRIT was 100% sensitive and specific as compared to standard FAT, and the viral antigen could be detected on samples preserved in glycerol solution for 15 months or frozen for 24 months and in variable conditions of preservation (Lembo et al., 2006). In addition, a field trial in Chad has revealed that the results between the dRIT and FAT were 100% in agreement (Durr et al., 2008). The dRIT will enable developing countries to perform routine rabies surveillance under field conditions at greatly reduced cost (Lembo et al., 2006; Fooks et al., 2009).

1.7.4. Rapid Immunodiagnostic test (RIDT)

Rabies virus antigen can also be detected from brain samples using RIDT which works based on the principles of immunochromatography (Fooks et al., 2009). The immunochromatographic lateral flow strip test is a one-step test that facilitates low-cost, rapid diagnosis of rabies virus without the need for laboratory equipment, and can be conducted in the field (Fooks et al., 2009). The test evaluation of this technique had revealed an overall specificity of 100% and a sensitivity of more than 88% when compared to FAT and Rapid Tissue Culture Infection Test (Servat et al., 2012).

1.7.5. Molecular techniques

Various molecular diagnostic tests, e.g. detection of viral RNA by reverse transcription polymerase chain reaction (RT-PCR), PCR-ELISA, real-time PCR, hemi-nested PCR, and nested PCR are used as rapid and sensitive tests for rabies diagnosis (Fooks et al., 2009; OIE, 2011a). The RT-PCR with subsequent nucleotide sequencing permits the diagnosis of rabies, typing and molecular epidemiological studies, but is not used for routine diagnosis due to cost and the facilities required (Fooks et al., 2009; OIE, 2011a).

1.7.6. Serological tests

Serological tests are used to measure the level of virus neutralizing antibody in vaccinated individuals and to detect host response to rabies infection by measuring antibodies in cerebrospinal fluid/serum in suspected rabid cases (Fooks et al., 2009). Rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973) and fluorescent virus neutralization test (FVNT) are currently recommended by the WHO for detecting the virus neutralization (Cliquet et al., 1998). However, these tests require a specialized laboratory and facilities to handle tissue culture and the virulent rabies virus, and also are too complex for large scale screening of field sera (Cliquet et al., 2000; Zhang et al., 2009; OIE, 2011a). Several enzyme-linked immunosorbent assays (ELISA) based methods using either the whole virus or purified G glycoprotein as the detection antigen are available to effectively detect and quantify rabies antibody in the sera of vaccinated animals or humans, and are applicable for testing large numbers of field sera (Sugiyama et al., 1997; Cliquet et al., 2000; Servat and Cliquet, 2006; Servat et al., 2007; Zhang et al., 2009). However, the main concern when

using assays that employ whole virus as the target antigen is the possibility of cross-reactivity with other antigens that may lead to false positives or inaccurate estimations of the levels of neutralizing-related antibodies present in the sample (Servat et al., 2007; Zhang et al., 2009).

1.7.7. Ante-mortem diagnoses of rabies

Humans

Rabies virus or RNA can be detected from biological fluids such as saliva, urine, cerebrospinal fluid, and corneal impressions by using FAT or RT-PCR during the clinical course of disease (Dacheux et al., 2010). The saliva has the highest rate of positivity, of approximately 75% and can reach 100% sensitivity when three successive samples are tested (Nagaraj et al., 2006; Dacheux et al., 2008; Dacheux et al., 2010; Wacharapluesadee and Hemachudha, 2010). The diagnostic value of urine is limited and the sensitivity was less than 10% using RT-PCR (Dacheux et al., 2008; Dacheux et al., 2010). The sensitivity of CSF was found to range between 8 to 43% (Crepin et al., 1998; Dacheux et al., 2010).

Ante mortem diagnosis can also be performed by examining the nuchal skin biopsy. After the infection of the brain, the virus becomes widely distributed in the nerve fibres surrounding the base of hair follicles. Examination of skin biopsy material has been shown to be a valuable technique for ante-mortem diagnosis of rabies in humans. For the test, a section of skin (5−6 mm in diameter containing a minimum of 10 hair follicles) needs to be taken from the posterior region of the neck along the hairline in order to include the cutaneous nerves at the base of the hair follicle. The tissue is fixed in acetone and subjected to routine FAT or RT-PCR test (Blenden et al., 1986; Bingham and Mlambo, 1995; Elmgren et al., 2002; Hemachudha and Wacharapluesadee, 2004; Macedo et al., 2006; Perez and Werchniak, 2007; Madhusudana and Sukumaran, 2008). The sensitivity of the nuchal skin biopsy test depends on the stage of the disease. The test becomes more sensitive in the terminal stage of disease. A recent study showed that RT-PCR on skin biopsies reached about 98% sensitivity and 100% specificity, which correlates well with the gold standard of the immunofluorescence test on brain biopsies (Dacheux et al., 2008; Dacheux et al., 2010). The examination of skin biopsies may be useful for post mortem diagnosis of rabies in humans, in

cases where the opening of the skull of the dead person is not accepted by relatives on cultural grounds (Meslin and Kaplan, 1996).

The presence of rabies RNA on the ends of the hair follicles (bulbar hair follicles) has also been demonstrated by using nucleic acid sequence-based amplification techniques (NASBA) which gives 50% sensitivity, indicating that the hair follicles can also be used for ante-mortem diagnosis of rabies (Hemachudha and Wacharapluesadee, 2004).

Dogs

In dogs, latex agglutination (LA) test for rabies virus antigen detection in dog saliva was developed and used in Thailand (Kasempimolporn et al., 2000). Rabies virus antigen could be detected by agglutination on a glass slide using latex particles coated with gamma globulin. When the paired saliva-brain specimens from 238 dogs were evaluated, the LA test using saliva was 99% specific and 95% sensitive compared to the FAT on brain smears (Kasempimolporn et al., 2000). The LA test is comparatively simple and there is no need to kill the animal for examination (Kasempimolporn et al., 2000).

The dog saliva samples can also be tested for rabies virus by a rapid immunodiagnostic test (RIDT) method using an immunochromatographic test strip (Kang et al., 2007; Nishizono et al., 2008; Fooks et al., 2009; Kasempimolporn et al., 2011). A test evaluation of a rapid immunodiagnostic test strip carried out in South Korea has revealed that RIDT had a sensitivity of 91.7% and specificity of 100% compared to standard FAT (Kang et al., 2007). The assay did not produce any cross-reaction with non-rabies virus microbes causing symptoms similar to rabies. A similar performance evaluation in Thailand showed that the saliva strip test was found to be 94.4% specific and 93% sensitive when compared to the gold standard FAT on brain smears (Kasempimolporn et al., 2011). The immunochromatographic strip test can be used as a screening tool for epidemiological surveys and disease control since it can be used outside the laboratory as an on-site testing assay and the results will be available within 10 minutes (Kasempimolporn et al., 2011).

1.8.Human rabies prevention

Rabies is an invariably fatal disease and there is no cure once clinical symptoms are present in the patient, but can be prevented by pre-exposure prophylaxis and immediate post-exposure prophylaxis following exposure to rabid animals (WHO, 2010a).

1.8.1. Pre-exposure prophylaxis

Pre-exposure prophylaxis consists of intramuscular (IM) or intradermal (ID) injection of tissue culture rabies vaccine (potency of at least 2.5 IU per dose), and is given as one dose (1ml as IM or 0.1ml as ID) each on day 0, 7, and 21 or 28 (WHO, 2010a). Pre-exposure prophylaxis is recommended for persons at continual high risk of exposure to rabies such as laboratory staff working with rabies virus, veterinarians, animal handlers and wildlife officials (WHO, 2010a).

There are a number of important reasons to utilize pre-exposure prophylaxis (Bernard et al., 1985; CDC, 1999; Briggs and Mahendra, 2007).

- Pre-exposure prophylaxis will provide protection to persons with in-apparent or unrecognized exposures to rabies virus that occur due to their vocation, hobby, or due to endemicity of rabies in the region in which they live.

- It also simplifies post-exposure prophylaxis in the event of subsequent exposure to rabies by reducing the amount of vaccine required from 5 to 2 doses and by eliminating the need to administer rabies immunoglobulin, which is expensive and often unavailable.

- Pre-exposure prophylaxis will prime the immune system so that an immediate anamnestic response will occur in a previously vaccinated individual

- It may protect persons whose post-exposure prophylaxis might be delayed.

The necessity of including rabies vaccine along with other expanded immunization programs in children in rabies high risk areas have been discussed (Dodet, 2010; WHO, 2010b; RIACON, 2011). Clinical trials in Vietnam concluded that the 2 pre-exposure rabies

vaccinations given to children at 2 and 4 months of age, followed by a booster at 1 year along with a combined diphtheria, tetanus, pertusis and inactivated poliomyelities vaccine series, were both safe and immunogenic, and resulted in long term persistence of sero-protective anti-rabies antibody concentrations in the majority of vaccinated children without interfering with other vaccine series responses (Lang et al., 1997; Lang et al., 1999; Lang et al., 2007). In the Philippines, pre-exposure prophylaxis are given to children under 15 years of age in high rabies endemic areas (Deray, 2009). However, the comparative studies between pre- and post-exposure prophylaxis have found that pre-exposure prophylaxis is not cost-effective unless the probability of exposure to rabies is about 18 to 20% or higher than this (Mann, 1984; Bernard and Fishbein, 1991; LeGuerrier et al., 1996).

1.8.2. Post-exposure prophylaxis

The recommended post-exposure prophylaxis (PEP) includes thorough cleaning of the bite wound with soap and water or detergent, administration of rabies vaccine to stimulate an active immune response, and administration of rabies immunoglobulin to provide immediate passive immunity (WHO, 2010a). However, the indication for PEP depends on the type of contact with the suspected rabid animal and should follow the WHO guidelines (see Table 1.2).

Table 1.2: Categories of exposures and recommendation of post-exposure prophylaxis for rabies source (adapted from WHO 2010a)

Category of exposure to suspect rabid animal	Treatment/recommendation
Category I – touching or feeding animals, licks on intact skin (i.e. no exposure)	None (not required if there is clear history)
Category II – nibbling of uncovered skin, minor scratches or abrasions without bleeding	Immediate vaccination and local treatment of the wound
Category III – single or multiple transdermal bites or scratches, licks on broken skin; contamination of mucous membrane with saliva from licks; exposures to bats.	Immediate vaccination and administration of rabies immunoglobulin; local treatment of the wound

1.8.2.1. Treatment of bite wounds

The WHO recommends that all animal bite wounds and scratches should be thoroughly washed and flushed (for about 15 minutes) with soap and water, detergent or water alone immediately after the bite. Where available, any viricidal agents such as 40−70% alcohol, povidone iodine or 0.1% quaternary ammonium compounds can be applied to the wound (WHO, 2010a). The proper cleaning of the wound would remove most of the virus but this simple and cheap treatment procedures are often omitted in most cases (Warrell and Warrell, 2004). It is recommended to postpone immediate suturing of the bite wound and if suturing is necessary, it should be done after careful infiltration of antiserum into and around the wound (WHO, 1973; Dean, 1975).

1.8.2.2. Administration of rabies vaccine

There are two WHO approved methods of rabies post exposure vaccination: (1) intramuscular and (2) intra dermal schedule of administration using tissue culture vaccines. Table 1.3 summarizes the rabies vaccine schedules in humans.

Table 1.3: Rabies post-exposure prophylaxis regimens in humans

Name of regimen	No. of clinic visits required	Days of injection	No. of injections per visit	Doses of vaccine per injection (ml)	Route of administration	Approved status
Essen 5-dose	5	0, 3, 7, 14, 28	1, 1, 1, 1, 1	1	IM	WHO 1992
Zagreb	3	0, 7, 21	2, 1, 1	1	IM	WHO 1992
Essen 4-dose	4	0, 3, 7, 14	1, 1, 1, 1	1	IM	ACIP 2009
Updated TRC-ID	4	0, 3, 7, 28	2, 2, 2, 2	0.1	ID	WHO 2005
4-site ID	4	0, 7, 28, 90	4, 2, 1, 1	0.1	ID	Not yet approved
1-week ID	1	0, 3, 7	4, 4, 4	0.1	ID	Not yet approved

ACIP: Advisory Committee on Immunization Practice, USA; IM: intramuscular; ID: intradermal. Table adapted from (Hampson et al., 2011b).

Intramuscular administration of post exposure prophylaxis

The intramuscular vaccination schedule includes 1-dose (1ml) injection on days 0, 3, 7, 14, and 28 (Essen-5 dose regimen), 1-dose (1ml) on day 0, 3, 7 and 14 (Essen-4 dose regimen), and 2-doses injection on day 0 and then 1-dose injection on day 7 and 21 (Zagreb schedule)

(WHO, 1992a; Rupprecht et al., 2010; WHO, 2010a). The vaccine should be administered in the deltoid area of the arm in adults and children aged ≥2 years, to the anterolateral area of the thigh in young children (<2 years old), and should not be administered in the gluteal area as it would affect the induction of an adequate immune response (WHO, 2010a).

Intradermal administration of post exposure prophylaxis

The updated Thai Red Cross 2-site intradermal regimen prescribes injection of 0.1 ml at 2 sites (deltoid and thigh) on days 0, 3, 7, and 28 (WHO, 2010a). The intradermal regimen is immunogenic and as effective as that of the intramuscular regimen, and is used in many Asian countries because of its cost-effectiveness (WHO, 1992a; Khawplod et al., 2002a; WHO, 2010a). The ID procedure can reduce the cost of PEP up to 60 to 70% compared to the standard Essen regimen, and make the PEP affordable to the people in canine rabies endemic areas (WHO, 1992a; Khawplod et al., 2002a).

The main drawback of the ID regimen is that the reconstituted vial cannot be stored for more than 6 to 8 hours and results in wastage of the vaccine if there are no other patients on the day of injection (WHO, 1992a). However, studies in Thailand have found that the individuals that were injected with the reconstituted and stored vaccine for up to 7 days (on day 0, 3, 7 days) produced protective neutralizing antibodies (>0.5IU/ml) (Kamoltham et al., 2002; Khawplod et al., 2002a; Khawplod et al., 2002b). However, use of the reconstituted vaccine after the lapse of 6 to 8 hours is not yet approved by the WHO. In addition, one study in Thailand has shown that the 2-site ID Purified Vero-cell rabies vaccine schedule that was deliberately injected into subcutaneous tissue also produced high neutralizing antibodies in the serum, comparable to the ID regimen injection (Phanuphak et al., 1990). The other point to be considered with ID regimen is that immuno-compromized patients and people taking chloroquine for malaria treatment will have a reduced response to ID rabies vaccination and they should be given vaccine intramuscularly (Pappaioanou et al., 1986; WHO, 2010a).

Two additional ID regimens of PEP have been developed and are under clinical trial. One is the ‘4-site ID’ (4-2-1-1) regimen in which 4-doses (0.1ml each) of vaccine is given at 4 sites on day 0; 2-doses on day 3 and then 1-dose each on day 28 and 90 (Warrell et al., 2008). The

second is ‘one-week ID’ (4-4-4) regimen in which 4 doses (0.1ml per dose) are given at 4 sites on day 0, 3 and 7. Studies have shown that these two regimens are also immunogenic and as effective as the Essen regimen (Shantavasinkul et al., 2010a; Sudarshan et al., 2011). However, these two regimens have not yet been approved by the WHO. Once they are approved, ‘one-week ID’ regimen is expected to increase the patients’ compliance to complete the course since it takes only one week to complete the course, reducing the loss of income and travel cost.

1.8.2.3. Administration of rabies immunoglobulin

The rabies immunoglobulin (RIG) should be infiltrated into and around bite wounds at a dose rate of 20 IU/kg body weight for human rabies immunoglobulin (HRIG) and 40 IU/kg body weight for equine rabies immunoglobulin (ERIG) (WHO, 2010a). The entire calculated dose should be injected directly into and around the bite wound if feasible (but avoiding possible compartment syndrome) with any remainder of the dose injected intramuscularly at the site distant from the site of vaccine administration (WHO, 2010a). RIG is recommended to all people with category III exposure (confirmed rabid exposure) and to those with category II exposure who are immunodeficient (WHO, 2010a). RIG can neutralize rabies virus at the inoculation sites (e.g., bite site) and thus closes the gap until endogenous antibodies elicited by active immunization appear in about two weeks time (Quiambao et al., 2008). It is recommended that RIG be administered ideally at the time of the first vaccine dose and can be safely administered up to seven days after vaccine administration, but should not be administered after the elapse of 7 days since it would interfere with the endogenous antibodies production elicited by active immunization (Khawplod et al., 1996). However, only a negligible number of patients receive RIG in rabies endemic countries due to limited supply and high cost (Sudarshan et al., 2007; Hossain et al., 2011). For example, only about 1.3 % of the patients have received RIG in India that is highly endemic for rabies (Sudarshan et al., 2007). The production of human rabies virus neutralizing monoclonal antibodies cocktail and mouse monoclonal antibody (MoMAb) cocktail could be an alternative to RIG as PEP in future (de Kruif et al., 2007; Bakker et al., 2008; Muller et al., 2009).

1.8.2.4. Post-exposure prophylaxis for previously vaccinated person

The WHO recommends that persons who have previously received full pre- or post-exposure prophylaxis with a potent cell-culture vaccine should be given only two booster doses, either intramuscularly (1ml dose) or intradermally (0.1ml dose), on days 0 and 3, and no RIG administration is necessary (WHO, 2010a). This has been shown to induce an anamnestic (accelerated due to immunologic memory) immune response (WHO, 1992a; Vodopija et al., 1997). However, complete post-exposure course (including RIG) is indicated if the person has been vaccinated with a vaccine of unproven potency, and do not demonstrate acceptable rabies neutralizing antibody titers (>0.5 IU/ml) (WHO, 1992a).

In Thailand, a 4-site 1-day ID PEP for previously vaccinated individuals is being routinely followed at the Queen Saovabha Memorial Institute since 1998 (WHO, 2010b). Clinical studies have shown that patients receiving four ID injections (two on deltoids and two on thighs) of 0.1 ml of tissue culture rabies vaccine during a single clinic visit developed satisfactory antibody titres, and these rabies virus neutralizing antibody titres were significantly higher than those achieved with the standard two-booster dose on days 0 and 3 (Tantawichien et al., 1999; Khawplod et al., 2002c). For instance, between 1998 and 2008, 5116 patients received four-site ID booster injections in Thailand of which > 65% had severe potential rabies exposures classified as category III, and 253 patients (4.9%) were bitten by laboratory-confirmed rabid animals. They have not reported any serious adverse reactions to the 4-site 1 day ID booster regimen and no human rabies deaths were reported (Tantawichien et al., 1999; Khawplod et al., 2002c). The use of multiple-site intradermal inoculation for booster vaccination may reduce the patient non-compliance rate and speed up the immune response to rabies virus infection (Tantawichien et al., 1999; Khawplod et al., 2002c). This single-visit 4-site intradermal regimen consisting of 4 injections of 0.1 ml equally distributed over left and right deltoids or thighs is also recommended by the WHO for booster vaccination (WHO, 2010a).

1.8.2.5. Failures of rabies post-exposure prophylaxis

Rabies post-exposure prophylaxis is 100% effective if administered following WHO guidelines and has saved millions of human lives. However, a few cases of vaccination failures have been reported, most of which occurred in developing countries and involved deviations from the WHO-recommended PEP protocol (Shill et al., 1987; Wilde et al., 1989; Wilde et al., 1996; Gacouin et al., 1999; Sriaroon et al., 2003; Matha and Salunke, 2005; Wilde, 2007; Rupprecht et al., 2009). In addition, a few cases of true failures of PEP have also been reported even when correct PEP protocol was followed. The reasons for failure are unclear (Hemachudha et al., 1999; Wilde, 2007; Shantavasinkul et al., 2010b).

1.8.2.6. Human survival from rabies

Although rabies in humans almost inevitably ends in death, six survivors of rabies have been documented between 1970 and 2004 in USA (Hattwick et al., 1972; Tillotson et al., 1977; Willoughby et al., 2005), Argentina (Porras et al., 1976) Mexico (Alvarez et al., 1994), and India (Madhusudana et al., 2002). Five of the six patients had received post-exposure rabies vaccine series before the onset of clinical symptoms. All showed nervous signs, but recovered gradually with some neurological disorder (Warrell and Warrell, 2004; Jackson, 2007a). The sixth patient who survived rabies was reported in 2004 in Wisconsin, USA (Willoughby et al., 2005). This 15 year old girl was bitten by a bat on her left index finger, but did not receive any post-exposure prophylaxis at that time. She developed numbness and tingling of her left hand, and then developed neurologic symptoms of rabies. She was treated with a combination of supportive care; a drug induced coma and ventilator support system, and antiviral and sedative treatment with ketamine, midazolam, ribavirin and amantadine (called the 'Milwaukee' protocol) (Willoughby et al., 2005). She gradually recovered from disease and is the first documented survivor who had not received rabies vaccine before the onset of clinical rabies. However, several attempts with this protocol in other patients have been unsuccessful, and all patients died (Hemachudha et al., 2006; McDermid et al., 2008; van Thiel et al., 2008; Wilde et al., 2008; Jackson, 2009; Rubin et al., 2009; Aramburo et al., 2011). In recent years (between 2008 and 2009) three more survival from rabies have been reported following bat bites in Brazil, Colombia and the USA (ProMED-mail, 2008; CDC, 2010). It should be noted that the four survivor cases using the Milwaukee protocol (since

2004) involved rabies virus strain of bat origin while none survived with canine rabies virus strains, indicating that canine strains are more virulent than bat strains (Rubin et al., 2009). However, rabies cannot be considered as a curable disease on the basis of these few successful recovery cases.

1.9. Prevention and control of rabies in dogs

Domestic dogs are the main reservoir of rabies in many developing countries, and account for about 99% of all human rabies cases in Asia and Africa (Knobel et al., 2005). The principles of canine rabies control programme is described elsewhere (WHO, 1987) and should consist of: awareness education on rabies, movement restriction of dogs through registration and legislation, international collaboration and cooperation, epidemiological surveillance, mass dog vaccination and dog population control and management. Only dog vaccination and animal birth control is discussed here.

1.9.1. Dog vaccination

Vaccination of dogs using tissue culture vaccine is the best way to eliminate rabies in the animal reservoir and to prevent infection in humans (Cleaveland et al., 2006).

1.9.1.1. Vaccine coverage

Coleman and Dye have calculated that between 39 to 57% vaccination coverage is required to eliminate rabies from a dog population (Coleman and Dye, 1996). However, the WHO recommends that a vaccination coverage of minimum of 70% of the dog population is necessary to eliminate dog rabies (WHO, 1987). It is recommended that mass dog vaccination should be carried out intensively within the shortest possible time (within a 2 to 3 week period) each year depending on the immune responses of the vaccinated population (Tierkel, 1975b; CDC, 2011). There are some field examples in which vaccination coverage (ranging from 60 to 87%) resulted in significant decrease in incidence of dog rabies and human exposures, such as in Memphis, Tennessee in the USA (Steele and Tierkel, 1949), in the Serengeti district of Tanzania (Cleaveland et al., 2003); Petchabun province in Thailand (Kamoltham et al., 2003); Jaipur in India (Reece and Chawla, 2006) and in Latin America

(Belotto et al., 2005; Schneider et al., 2007) among others. In contrast, the recommended 70% coverage is hardly ever achieved in many developing countries where canine rabies is endemic, resulting in continuous transmission and maintenance of endemicity of the virus.

1.9.1.2. Vaccination centre

It is recommended that the vaccination points should be located in strategic locations chosen on the basis of dog population concentration within the community (Bogel and Hoyte, 1990). Central-point vaccination was found to be cost-effective in carrying out mass vaccination campaigns in some of the African nations and resulted in good vaccination coverage (Cleaveland et al., 2003; Kayali et al., 2003; Kaare et al., 2009).

1.9.1.3. Age of dog at first vaccination

Although it is recommend not to vaccinate puppies (<3 months age) born of immunized bitches, so that vaccine induced active immunity is not affected by the presence of maternally-derived antibodies, field studies have demonstrated that puppies (<3 months age) have responded well to rabies vaccination and produced protective antibodies without any significant interference by maternally-derived antibodies (Chappuis, 1998; Seghaier et al., 1999). Given the high birth rate of stray dog populations in most developing countries, it is important to include all categories of dogs irrespective of age, including puppies, in vaccination programme to maintain sufficient herd immunity for rabies control (Kaare et al., 2009). Moreover, puppies are the most easily accessible segment of the dog population for vaccination. One study in Thailand revealed that 14% of rabid dogs were puppies < 3 months old and 42% were dogs between 3 and 6 months old, indicating that puppies are an important source of human rabies cases and pose a risk to humans, especially children, if left unvaccinated (Mitmoonpitak et al., 1998).

1.9.1.4. Identification marks

It is recommended that vaccinated dogs be given identification marks by the use of coloured tags, coloured plastic collars, or paint on the body to allow for verification of vaccination status (Bogel and Hoyte, 1990; Kayali et al., 2003; Reece and Chawla, 2006). This is more

applicable in the stray dog population. Permanent marking such as ear notching has to be done under general anesthesia and can best be performed along with an animal birth control programme (see Figure 1.2) (WHO, 1987; Bogel and Hoyte, 1990).

Figure 1.2: Identification marks of vaccinated and sterilized stray dogs in Bhutan (ear notching and yellow nylon collaring).

1.9.1.5. Oral vaccination of dogs

One of the major obstacles for establishing satisfactory vaccination coverage in the dog population through parenteral vaccination is the accessibility of dogs. After a successful elimination of fox rabies in western Europe through the use of oral bait vaccine, the WHO has encouraged several studies on the development of safer and more effective vaccines for oral vaccination of dogs (OVD) (WHO, 1988, 1989, 1990, 1992b, 1993, 1994b, 1995, 1998; Estrada et al., 2001; Rupprecht et al., 2005; WHO, 2005; Cliquet et al., 2007; WHO, 2007). The OVD promises a substantial increase in vaccination coverage both when applied exclusively and when used in combination with parenteral vaccination, as demonstrated in field trails in Tunisia, Turkey and Sri Lanka (Matter and Fico, 1993; WHO, 1998; Harischandra, 2001). However, OVD safety for non target species, especially humans and the cost of the bait vaccine has remained the main barrier for adoption and has still not become an operational component of large dog rabies control and elimination programmes.

1.9.1.6. Serosurveillance of vaccinated dogs

Rabies vaccinated dogs should have an effective antibody titre for protection against rabies infection. A serum titre of ≥0.5 IU/ml of rabies virus-specific antibodies is considered adequate protection against rabies, and a titre below this level is less likely to be protected for rabies (Fooks et al., 2002; Kennedy et al., 2007). It has been demonstrated that not all dogs respond equally to vaccination; some proportion of dogs fails to develop a protective immune response, leaving them susceptible to disease (Mansfield et al., 2004). Studies have shown that larger breed dogs and younger aged dog elicited a lower antibody response to rabies vaccination than smaller sized and adult dogs (Mansfield et al., 2004; Kennedy et al., 2007; Berndtsson et al., 2011; Gazi and Seyyal, 2011).

Field studies in Turkey have shown that only 13% (65/500) of the sampled dogs had a protective antibody titre of ≥ 0.5 IU/ml after vaccination (Gazi and Seyyal, 2011) while only 1% of the street dogs and 16% of the pet dogs had a protective antirabies antibody titre ≥ 0.5IU/ml in Chandigarh city in India (Singh et al., 2011), indicating serious risk of rabies transmission. Examination of serum samples from 3314 stray dogs in Bangkok, Thailand have demonstrated an antibody seroprevalence of 62%, suggesting that the seroprevalence achieved from previous vaccination campaigns is low to protect the dog and human populations (Kasempimolporn et al., 2007). In Peru about 270,000 dogs (65% of the estimated dog population) were vaccinated over the course of one month during mass vaccination campaign in 1985 with an inactivated tissue culture vaccine. The immune response among randomly selected vaccinated dogs showed that 97% of the sampled dogs had a rabies neutralizing antibody titer of >0.5 IU/ml, and 87% had a titer of >1.0 IU/ml after one year of mass vaccination program in the field condition (Chomel et al., 1988).

1.9.2. Dog population control

Rabies transmission is dependent on dog density and is likely to be maintained endemically in areas where dog density exceeds the threshold for persistence, considered to be about 4.5 dogs/km^2 (Brooks, 1990; Cleaveland and Dye, 1995; Kitala et al., 2002; Lembo et al., 2008). In most developing countries the dog population density, particularly that of the stray or free-

roaming populations, is expected to be much greater than such a threshold required for disease persistence. Comprehensive dog population control programme guidelines (including habitat control through proper waste management, movement restriction, responsible dog ownership) are available elsewhere (WHO, 1987; Bogel and Hoyte, 1990; ICAM, 2007), and only animal birth control (ABC) will be discussed here.

1.9.2.1. Surgical sterilization

Surgical removal of reproductive organs (castration, hysterectomy, ovariohysterectomy) is an effective permanent method for dog birth control, but is costly (although it may be more cost efficient in the long term) (Bogel and Hoyte, 1990; Reece and Chawla, 2006; ICAM, 2007). The cost per dog sterilization may vary from approximately US$ 6 to 25 (WHO, 2010b). Sterilization may be required for many years and is difficult to sustain in a resource limited country. In addition, the sterilization of dogs in one or more areas may divert resources away from the priority of mass vaccination, which is required for elimination of rabies in dogs (WHO, 2010b). Nevertheless, ABC programs have been launched in a number of countries with encouraging results (Reece and Chawla, 2006; MoA, 2009; Totton et al., 2010). For example, in an organized pilot campaign in India (Jaipur city), about 24,986 neighbourhood dogs (about 65% coverage) were captured, sterilized, vaccinated and released between November 1994 and December 2002 (Reece and Chawla, 2006). As a result, the dog population declined by 28%, and human cases of rabies declined to zero in the programme area but increased in other areas where the campaign was not organized (Reece and Chawla, 2006). Similarly in another intensive field study in Jodhpur (India), population size and demographics of stray dogs were measured before and after implementation of an ABC program between 2005 and 2007 (Totton et al., 2010). About 62–87% of the free-roaming dog population was surgically sterilized and vaccinated for rabies in the five survey areas of Jodhpur by 2007, which resulted in a drastic decline of the dog population (Totton et al., 2010). A population demographic model predicted that at the current level of sterilization, the dog population would decrease by 69% and reach stability after 13–18 years, suggesting it would require long term planning and can be very expensive (Totton et al., 2010).

1.9.2.2. Non-surgical contraception

The immunocontraception method (or immunosterilization/immuncastration) involves injection of exogenous synthetic reproductive proteins (antigens) which then trigger the animal to produce antibodies, neutralising their activity and inhibiting the normal reproductive processes (WSPA, 2007). Hormonal contraception using progestins, androgens, or gonadotropin releasing hormone (GnRH) analogs act to either directly block reproductive hormone receptor-mediated events, or indirectly block conception via negative feedback mechanisms (Kutzler and Wood, 2006; WSPA, 2007). However, these methods are still limited by the cost and are not suitable for large scale use with free-roaming dog populations. Other concerns are unquantified side effects, and the risk beyond the target population (especially human) when used as oral bait since there is no species-specific products available in the market (WSPA, 2007).

1.9.2.3. Chemical sterilization

Chemical sterilization (chemical castration) method includes intratesticular injections of chemical sterilants in male dog and cats that result in testicular necrosis and replacement by fibrous tissue, and impair spermatogenesis inducing azoospermia (no measurable level of sperm in the semen) (Kutzler and Wood, 2006; WSPA, 2007). In clinical trials chemical sterilants such as zinc gluconate (Tepsumethanon et al., 2005), calcium chloride (Jana and Samanta, 2007; Jana and Samanta, 2011), and zinc-based solution (Oliveira et al., 2007) have been injected intratesticularly into dog and cats and have effectively impaired spermatogenesis. Although some swelling of the testicular organ was observed after injection, no significant adverse effects and changes in animal behaviour were evident (Tepsumethanon et al., 2005). Effective mass dog reproductive control using immunocontraception and chemical sterilization are expected in the future through an active research and development of effective and suitable agents (ICAM, 2007; WSPA, 2007).

1.10. Epidemiology

1.10.1. Reservoir hosts of rabies

Rabies is maintained in two inter-related epidemiological cycles, urban and sylvatic. Urban rabies, affecting mainly dogs accounts for an estimated 99% of all recorded human cases and for 92% of all human PEP (King and Turner, 1993; Knobel et al., 2005). Urban rabies transmitted by domestic dogs is predominant in the developing countries of Asia, Africa and South America.

The sylvatic cycle is maintained by wildlife of *Canidae* (including dogs, jackals, wolves and foxes), *Mephitidae* (skunks), *Herpestidae* (mongoose spp.), *Procyonidae* (raccoons), and the order *Chiroptera* (bats spp.) in different ecosystems of the world (Hanlon et al., 2007; Kuzmin and Rupprecht, 2007). In western Europe, the red fox (*Vulpes vulpes*) had been the main reservoir of rabies while the Arctic fox (*Alopex lagopus*) and introduced raccoon dogs (*Nyctereutes procyonides*) have been sustaining the chain of infection in northern and eastern Europe (King and Turner, 1993; Wandeler, 2004). The *Microcheropteran* bats species are responsible for maintaining European bat lyssaviruses 1 and 2 (see Table 1.1). In Canada, the most important reservoir is the red fox (*Vulpes vulpes*). In the USA, the major reservoir hosts of rabies are raccoons, striped skunks, bats and foxes (Blanton et al., 2009; Blanton et al., 2010). The Indian mongoose (*Herpestes auropunctatus*) is the reservoir of rabies in the Caribbean islands while the vampire bat (*Desmodus rotundus)* is a reservoir in many Latin American countries (Belotto et al., 2005; Hanlon et al., 2007). Arctic foxes are the reservoir of rabies in the arctic region. In Africa, fruit bats are a reservoir of lyssavirus in different African countries (see Table 1.1). In addition, the black backed jackal and the yellow mongoose in South Africa, jackal and hyena in Zimbabwe, and wild cats in Botswana are also reported to be involved in the transmission of rabies (Cleaveland and Dye, 1995; Sabeta et al., 2003; Hampson et al., 2007; Rosatte et al., 2007a). In the Middle East foxes, wolves and jackals are the reservoir of wildlife rabies. In Australia, *Megachiptera* and *Michrochiroptera* bats are reservoir for Australian bat lyssavirus (Samaratunga et al., 1998; Hanna et al., 2000). In Asia, wildlife plays a very minimal role in the epidemiology of rabies (Wilde et al., 2005), but mongoose (*Herpestes* spp.), jackals (*Canis aureus*), foxes (*Vulpes*

bengalensis) and wolves (*Canis lupus*) have been incriminated as wildlife reservoirs of rabies in Bangladesh, India, and Nepal (Gongal and Wright, 2011), raccoon dogs in South Korea (Kim et al., 2006), foxes and wolves in Mongolia (Odontsetseg et al., 2009), and ferret, badgers and wolves in China (Hu et al., 2009).

1.10.2. Epidemiology and control of rabies in the world

Rabies is a widespread zoonosis causing a significant social and economic burden in many countries worldwide. In accordance with the provisions of the OIE *Terrestrial Code*, only 32 of the 178 OIE member countries would be eligible to qualify for historical freedom or have successfully eliminated rabies in domestic animals (OIE, 2011b). At least 110 member countries are considered endemically infected with rabies. Of the 178 OIE member countries, rabies is a notifiable disease in dogs in only 161 member countries (OIE, 2011b).

1.10.2.1. Europe

Canine rabies was widely prevalent in Europe during the 17th century but was eliminated in the early 20th century from most countries in western Europe (King et al., 2004). The United Kingdom eradicated canine rabies in 1902 and again in 1918 (after its re-introduction) by strict implementation of muzzling of dogs, movement restriction and destruction of stray dogs (Pastoret and Brochier, 1999; Fooks et al., 2004). However, the fox rabies epizootic in Europe spread progressively from Russian-Polish border in the beginning of 1940s and had spread to most countries of East, Central and Western Europe by the middle of the 1970s (Steck and Wandeler, 1980; Pastoret and Brochier, 1998). The epizootic spread in a wavelike fashion with a speed of approximately 25-60 km per year (Pastoret and Brochier, 1999; Wandeler, 2004; Rosatte et al., 2007a). Different methods such as hunting, gassing of fox dens, digging up the cubs, and poisoning of fox were done in Europe in an effort to reduce fox density and control rabies, but this resulted in only a transient decrease of the prevalence of rabies (Pastoret and Brochier, 1999; Vitasek, 2004; Rosatte et al., 2007a). Then a parental vaccination of foxes was initiated in Switzerland and Germany, but was found to be impractical since too few foxes were captured in a limited area (Vitasek, 2004; Rosatte et al., 2007a). In 1977, the first field trials of oral rabies bait vaccine were successfully conducted in Switzerland and used extensively in an attempt to

stop an advancing fox rabies epizootic in the Rhone Valley area of Switzerland (Vitasek, 2004; Rosatte et al., 2007b). This resulted in the control of rabies in Switzerland by 1985. In 1983, Germany also started oral bait vaccination of foxes which resulted in elimination of fox rabies in large areas of West Germany by 1987. Between the 1980s and the 1990s, oral vaccination of foxes and raccoons was also started in other European countries (Rosatte et al., 2007a), but rabies is still prevalent in south-eastern Europe, and remains endemic in both canine and wildlife (Bourhy et al., 2005; Freuling and Müller, 2010, 2011). Both dog and wildlife mediated rabies is prevalent in Turkey, a country that occupies a cross-road between Europe and Asia (Johnson et al., 2006b; Johnson et al., 2010). In 2010, as many as 2207 rabies cases were reported in Europe, 1373 of which occurred in wild animals, 820 in domestic animals, 1 in bat and 3 cases in humans (Freuling and Müller, 2010). In 2011, 1734 rabies cases were reported in Europe (1038 in wild animals, 694 in domestic animals, and 2 cases in humans) (Freuling and Müller, 2011). The epidemiological situation of rabies in Europe is updated in Rabies Bulletin Europe and is published quarterly (www.rbe.fli.bund.de/Journal). In addition, a detailed description of rabies in Europe has been reviewed in the 'Historical perspective of rabies in Europe and the Mediterranean Basin' (King et al., 2004). Although most parts of Europe is free from canine rabies and is progressively becoming free of sylvatic rabies, reintroduction remains a threat from imported rabies (Johnson et al., 2011a).

1.10.2.2. North America

Canine rabies was endemic throughout the United States during the first half of the 20th century (Held et al., 1967). An extensive dog vaccination campaign and stray dog control program was implemented during the 1940s and 1950s, which resulted in elimination of canine rabies in the 1960s (Smith et al., 1995; Krebs et al., 2005), but wildlife rabies has steadily increased over the years (Smith et al., 1995; Velasco-Villa et al., 2008). Raccoons, skunks, bats and foxes are the wildlife most affected with rabies in the USA. Wild animals accounted for 93% of the 6,841 reported cases of rabies in 2008 and 92% of 6690 reported cases in 2009 (Blanton et al., 2009; Blanton et al., 2010). Wildlife has also been responsible for transmission of rabies to domestic animals (dogs, cats, cattle) and humans. Two human cases were reported in 2008 and four in 2009 (Blanton et al., 2010). The epidemiological

situation regarding rabies in the USA is updated in the form of annual rabies surveillance reports and publications (Blanton et al., 2009; Blanton et al., 2010). In Canada, dog rabies was eliminated, but red fox rabies is still a public health problem and causes spill over infection in domestic animals, but no human cases of rabies had been reported during 2009 (Blanton et al., 2010). Application of oral bait vaccine has successfully eliminated rabies in red foxes in the eastern and southern Ontario and Toronto in Canada (MacInnes et al., 2001; Slate et al., 2005; Rosatte et al., 2007b).

1.10.2.3. South America

Canine rabies has been prevalent in South America (Latin America and Caribbean countries) for many years (Schneider et al., 2007). In 1983, the countries of the Americas, with support from the Pan American Health Organization (PAHO), developed the 'Plan of Action for the Elimination of Urban Rabies from the principal cities of Latin America' with an aim to eliminate human rabies transmitted by dogs in major cities (Belotto et al., 2005; Schneider et al., 2007). The main control strategies within the regional and national rabies control programmes included mass dog vaccination (80% of the estimated dog population), treatment of people bitten by dogs, and epidemiological surveillance in the main city areas (Belotto et al., 2005). In 1991, the scope was expanded to include neglected areas and small villages and set the year 2005 as the target date for the control of rabies in the region (Belotto et al., 2005; Schneider et al., 2007). In addition, the epidemiological surveillance of wildlife rabies, especially rabies transmitted by bats (various species) was incorporated into the action plan in 1992 (Belotto et al., 2005). Since the start of the nationally and regionally coordinated rabies control programmes, the countries in the region have achieved a remarkable success: human and canine rabies cases dropped by nearly 90% (Belotto et al., 2005; Schneider et al., 2007). In 2001, only two of the 22 capital cities reported cases of human rabies (Belotto et al., 2005). Epidemiological surveillance activities − in which the periodic data on human and animal rabies cases are submitted from every country in the Americas Region to "The Regional Information System for Epidemiological Surveillance of Rabies in the Americas (SIRVERA)" administrated by Pan American Health Organization (PAHO) − have also been strengthened in the region (Belotto et al., 2005). During the period from 1990 to 2003, approximately one million people potentially exposed to rabies received treatment, and about

42 million dogs were vaccinated annually. Most of the vaccinated dogs (75%) were from Brazil (17 million) and Mexico (16 million), which have the largest canine populations in Latin America (Belotto et al., 2005; Schneider et al., 2007). However, in some areas, the coverage was < 40% while some countries or geopolitical units within countries no longer carry out mass vaccination campaigns because they have been free from rabies for >10 years (Schneider et al., 2007). Analysis of surveillance data between 1982 and 2003 reveals a decline in the number of human cases from 355 to 35 (91% decrease) and rabies in dogs has decreased from 15,686 cases to 1,131 (93% decrease) in the same period (Schneider et al., 2005). Canine rabies transmission is under control in many parts of Latin America and human rabies transmitted by dogs is currently localized to low income, outlying areas of large cities in Latin America (Schneider et al., 2007; Suzuki et al., 2007). A large portion of the Southern Cone, including Chile and Uruguay, vast areas of Argentina, and all of southern Brazil, is already free of canine rabies, in addition to Panama, Costa Rica and some Departments in Peru (Schneider et al., 2007). However, the rabies transmitted by vampire bats represents and important economic and public health problem in most of continental Latin America and Trinidad and Tobago and Grenada in the Caribbean (WHO, 1987; Belloto, 2004). In the Caribbean Islands such as Puerto Rico, Grenada and Cuba, the small Indian mongoose, which was introduced from South Asia in the second half of the 19th century for rodent control, is recognized as an important rabies vector and a growing public health problem (Belloto, 2004; WHO, 2005).

1.10.2.4. Asia

The rabies situation in Asia is characterised by a high rate of human mortalities (about 31,000 deaths per year), contributing to 56% of the total human rabies related deaths in the world (Knobel et al., 2005). Approximately 20,000 people die of rabies each year in India alone (APCRI, 2004; Sudarshan et al., 2007). Only a few countries in Asia (Japan, Singapore, Malaysia, Taiwan, Hong Kong, South Korea) have eradicated canine rabies, applying mass dog vaccination and dog elimination programmes (Wells, 1954; Takahashi-Omoe et al., 2008; Weng et al., 2010), but no other Asian country has been able to eliminate rabies during the past four to five decades. However, Thailand, Sri Lanka and Vietnam have reported a substantial reduction in human rabies deaths following mass vaccination of dogs

and PEP in humans (Kamoltham, 2007; Xuyen, 2008; Kumarapeli and Awerbuch-Friedlander, 2009). In contrast, there is a resurgence of rabies in the northern area of South Korea bordering the demilitarized zone with North Korea due to wild raccoon dog mediated rabies transmission to domestic animals (Kim et al., 2006; Yang, 2011). South Korea eradicated canine rabies in 1986. In China, rabies re-emerged and resulted in 3303 human rabies deaths in 2007, after it was under control with only 159 cases reported in 1996 (Si et al., 2008; Wu et al., 2009a; Tu, 2011). The latest emergence of rabies in Asia is on the Indonesian Island of Bali, where rabies was first reported in November 2008 within the southernmost peninsula of Bali, and then spread throughout the province by June 2010 (Putra et al., 2011). About 137 humans have died of rabies in Bali since it was first introduced in 2008 (ProMED-mail, 2011). The initial response to the outbreak was mass killing of dogs (about 108,000 dogs were killed between 2008 and October 2010), and then an Island-wide mass vaccination of dogs (Putra et al., 2011). The detail review of rabies situation and its control programmes in each Asian country is described in Chapter 2.

1.10.2.5. Africa

After Asia, Africa is the second most affected continent with an estimated 23,000 human rabies deaths reported every year (Knobel et al., 2005). Rabies is endemic throughout the African continent – Northern, Southern, Eastern, Western and Central Africa (Hampson et al., 2007; Dodet et al., 2008; Talbi et al., 2009; Harrak, 2011; Nel, 2011; Hayman et al., 2011). The cosmopolitan rabies lineage is thought to have been introduced into sub-Saharan Africa from North Africa and Eurasia following patterns of human colonization (Bourhy et al., 2008; Talbi et al., 2009). Rabies deaths in Africa are linked to poverty, lack of awareness among people and medical practitioners, and lack of infrastructure for the management of rabies exposure (Dodet et al., 2008; Harrak, 2011). However, studies by Lembo et al (2010) have confirmed that there are no insurmountable problems to canine rabies control in most of Africa and that rabies elimination is epidemiologically and practically feasible through mass dog vaccination (Lembo et al., 2010). In 1992, a group of independent scientists and public health officers created a network called Southern and Eastern African Rabies Group (SEARG) (consisting of south and east African countries) to strengthen surveillance systems and coordinate rabies control programmes in the continent (www.searg.info). Similarly, in

2008 another network of rabies experts from 14 African countries (Algeria, Benin, Burkina Faso, Cameroun, Congo, Côte d'Ivoire, Gabon, Madagascar, Mali, Morocco, Nigeria, Central African Republic, Senegal, Togo), called the Africa Rabies Expert Bureau (AfroREB) was established to improve surveillance and coordinate effective rabies control (Dodet et al., 2008). Meetings or conferences are held annually among the member countries of the organizations to share information and experience of rabies control programmes. A pilot rabies control program with the support of the Bill and Melinda Gates Foundation has been implemented in the Kwa-Zulu Natal area in South Africa and in Northwestern Tanzania (Cleaveland et al., 2003; Lembo et al., 2011; Nel, 2011).

1.10.2.6. Middle East

In the Middle East, rabies is endemic in Syria, Lebanon, Israel, Jordan, Oman, Saudi Arabia, Yemen, Egypt, Iran and Iraq (Stanley, 1990; David et al., 2000; Yakobson et al., 2004). Although domestic dogs are the main reservoirs, wildlife such as jackals, foxes and wolves have also been implicated as a source of rabies transmission.

1.10.2.7. Island nations

Antarctica, New Zealand and smaller Island nations of the Pacific Ocean are historically free from rabies. Although Australia is free from dog rabies, bat rabies has been detected (Australian bat lyssavirus) and has caused 2 human deaths in 1996 and 1998 (Samaratunga et al., 1998; Hanna et al., 2000).

1.10.3. Molecular epidemiology

Molecular and phylogenetic studies on rabies have been performed since the early 1990s to investigate geographical virus diversity at the global, national and regional level (Smith et al., 1992; Kuzmin et al., 2004; Bourhy et al., 2008; Gong et al., 2010). Different genome regions of the rabies virus have been used in molecular epidemiological studies, but the N gene has been most extensively targeted for epidemiological and evolutionary studies because the gene is highly conserved (Smith et al., 1992; David et al., 2000; Brookes et al., 2004; Bourhy et al., 2008). For instance, a recent global molecular and phylogenetic study of rabies viruses using 192 isolates sampled from 55 countries in five continents over a time period of 37

years revealed the existence of seven distinct lineages (Bourhy et al., 2008). Bat and dog-associated rabies viruses form distinct phylogenetic groups, with the dog associated viruses comprising six major clusters (Africa 2, Africa 3, Arctic-related, Asian, Cosmopolitan and Indian subcontinent clades), each of which has a distinct geographical distribution, suggesting that major physical barriers might have prevented the gene flow (Bourhy et al., 2008). The emergence and wide distribution of the cosmopolitan lineage around the world (North America, South America, Africa, Asia, Middle East) indicates that it had originated from a common progenitor virus, and the colonisation by the Europeans is suggested to have spread the virus around the world (Nadin-Davis and Bingham, 2004; Bourhy et al., 2008). The Africa clade 2 has a wide geographical distribution in West Africa, including Mauritania, Guinea, Ivory Coast, Burkina Faso, Cameroon, Benin, Nigeria and Chad (Kissi et al., 1995; Bourhy et al., 2008). The Africa 3 group of viruses was associated with carnivores of the family *Herpestidae* (yellow mongoose), which is the main vector of rabies in the central plateau of southern Africa (Nadin-Davis and Bingham, 2004; Davis et al., 2007; Bourhy et al., 2008). The Arctic-related clade is circulating in a large area across the Northern hemisphere, ranging from North America and Greenland to central, southern and eastern Asia (Russia, Iran, Pakistan, Afghanistan, Nepal, India, Korea, Inner part of Mongolia in China) (Kissi et al., 1995; Kuzmin et al., 2004; Hyun et al., 2005; Mansfield et al., 2006; Nadin-Davis et al., 2007; Kuzmin et al., 2008; Shao et al., 2011; Nadin-Davis et al., 2012). The extensive distribution of the rabies virus isolates in India have been described to be Arctic-like viruses and thought to be spillover transmission from dogs to wildlife rather than the converse (Nadin-Davis et al., 2007; Nadin-Davis et al., 2012). The Asian clade is distributed in South-east Asian countries – Myanmar, Thailand, Laos, Cambodia, and Vietnam (Yamagata et al., 2007), Indonesia (Susetya et al., 2008), the Philippines (Nishizono et al., 2002) and China (Zhang et al., 2006; Meng et al., 2010). The Indian subcontinent clade of rabies virus is distributed only within southern India and Sri Lanka (Arai et al., 2001; Nanayakkara et al., 2003) and do not cluster with isolates from northern India. In addition, several molecular and phylogenetic studies have been conducted at the national and regional levels in Asia (Denduangboripant et al., 2005; Nagarajan et al., 2006; Tao et al., 2009; Ming et al., 2010), Africa (Sabeta et al., 2003; Davis et al., 2007; Talbi et al., 2009), Middle East (David et al., 2000; David et al., 2007), Latin America (deMattos et al., 1996; Kobayshi et

al., 2007; Paez et al., 2007) and have identified the distribution of different groups and lineages, providing valuable information on the diversity of the viruses. In South East Asia, the movement of humans and their dogs between the countries or between regions within a country as migrant workers or for trade has been hypothesized as the cause of the spread of rabies in the region (Denduangboripant et al., 2005; Meng et al., 2007; Susetya et al., 2008; Meng et al., 2010).

1.11. Socio-economics and public health burden of rabies

Rabies kills around 55,000 people in Asia and Africa each year (Knobel et al., 2005). The recent assessment suggests that an estimated 70,000 people die of rabies each year in the world, but mostly in Asia and Africa (Hampson et al., 2011b). Deaths due to rabies are responsible for an estimated 1.74 million disability adjusted life-years lost per year in Asia and Africa (Knobel et al., 2005). The burden of rabies is influenced by age-related and socioeconomic factors: rabies is most commonly reported in children below 15 years of age and in poor and low income people that have no access to treatment facilities (Pancharoen et al., 2001; Cleaveland et al., 2002; Knobel et al., 2005; Knobel et al., 2007).

Rabies also represents a significant economic burden to society in rabies endemic countries (Fishbein et al., 1991; Knobel et al., 2005). It has been estimated that globally ≥15 million people receive rabies prophylaxis annually, the majority of whom live in China and India (WHO, 2010a). The estimated expenditure for rabies prevention exceeded US$ 1 billion in 2005 (WHO, 2010a). In Africa and Asia, the estimated annual cost of rabies is US$ 583.5 million, where the patient-borne costs for PEP form the bulk of expenditure, accounting for nearly half the total costs of rabies (Knobel et al., 2005). The total cost of PEP (direct and indirect expenses) has been estimated to be equivalent to 5.8% of the annual per capita gross national income in Africa (US$ 40 per human treatment) and 3.9% (US$ 49 per human treatment) in Asia (Knobel et al., 2005). In addition, the total cost of dog rabies control has been estimated to be US$ 86.7 million per year (US$ 9.7 million in Africa and US$ 77.0 million in Asia) while the annual cost of surveillance and livestock loss has been estimated at US$ 0.12 million and US$ 12.3 million, respectively (Knobel et al., 2005).

The cost-effectiveness studies of rabies control have demonstrated that dog rabies elimination is more economical than the intensified use of PEP in humans (Bogel and Meslin, 1990; Fishbein et al., 1991; Zinsstag et al., 2009). The World Animal Health Organization has stated that just 10% of costs currently used to treat people bitten by potentially rabid dogs would be sufficient to eradicate dog rabies in the world and thereby prevent almost all human rabies cases (Vallat, 2011). Therefore, human public health agencies should support or provide funds to the veterinary services for mass dog vaccination programmes since the elimination of rabies in the reservoirs species is the only strategy that will ultimately prevent human rabies deaths and also reduce the recurring cost of human PEP.

1.12. Rabies surveillance system

1.12.1. Diagnosis and reporting system

Animal disease surveillance systems comprise passive and active surveillance. In the passive surveillance system, data are collected when the owner reports to the relevant authorities or the animals showing clinical signs of a disease present themselves in some manner, while the animals are sought, caught and tested in active surveillance. Active surveillance systems, although they provide better data, are more expensive (Dorrell, 2007). The risk-based surveillance concept is gaining importance and can provide a higher benefit-cost ratio with existing or reduced resources (Doherr et al., 2001; Stärk et al., 2006). Like any other diseases, it is expensive and logistically impractical to conduct nation-wide active rabies surveillance. But there is a wide difference in surveillance systems used in developed and developing countries. For example, in the USA, animal rabies surveillance is done based on laboratory diagnosis using standard direct FAT by the 126 state health, agriculture and the university laboratories (Blanton et al., 2010). A targeted enhanced rabies surveillance system is also carried out by the wildlife biologists engaged by the United States Department of Agriculture Wildlife Services to work with oral rabies vaccination programs, and focuses on the following types of samples: strange acting (extremely aggressive or docile) animals

where no human or domestic animal exposure has been reported, road kills, animals found dead in addition to road kills, animals with injuries or lesions indicative of highly aggressive behaviour, and euthanized animals from focal trapping at sites where rabid animals were recently confirmed (Slate et al., 2009). The laboratory-confirmed cases of animal rabies are reported to either the health or agricultural department in all states, which then notify the CDC on a regular (weekly) basis (Blanton et al., 2010). These rabies surveillance data are analysed and published in a summary report each year (Krebs et al., 2005; Blanton et al., 2006a; Blanton et al., 2007; Blanton et al., 2009; Blanton et al., 2010). However, it has been stated that the current surveillance systems are inadequate for the efficient management and evaluation of the large scale wildlife rabies vaccine baiting programs in the US (Blanton et al., 2006b). In order to improve the surveillance system, a GIS-based rabies surveillance database and internet mapping application had been created (RabID) which provides a new resource for the rapid mapping and dissemination of data on animal rabies cases in relation to unaffected, enzootic and baited areas where current rabies interventions are ongoing (Blanton et al., 2006b).

In Europe, rabies cases are confirmed by laboratory tests and the rabies surveillance data are collected electronically in Rabies Bulletin Europe (www.rbe.fli.bund.de) and is published quarterly every year as a summary report (Freuling and Müller, 2010, 2011).

In South America, the Pan American Health Organization (PAHO), established a rabies surveillance information system called 'The Regional Information System for Epidemiological Surveillance of Rabies in the Americas (SIRVERA)'. The member countries were required to submit the periodic data on human and animal rabies cases to this information system so as to monitor the rabies situation in the region (Belloto, 2004; Belotto et al., 2005).

Canine rabies free countries also have a strict quarantine and regulatory system for the import of dogs and cats from rabies endemic countries. Despite these regulations, imported cases of dog rabies are occasionally recorded in those countries through failure of border controls or ignorance of importation rules, presenting risk of introducing rabies and posing a potential

public health risk (Mailles et al., 2004; Castrodale et al., 2008; Fooks et al., 2008; Mangieri et al., 2008; McQuiston et al., 2008; Johnson et al., 2011a; Johnson et al., 2011b; Mailles et al., 2011). In addition to imported dog rabies, cases of human imported rabies are also reported. A total of 42 human deaths from rabies were reported in Europe, the United States and Japan between 1990 and 2010 (based on clinical literature); all of these victims were assumed to have contracted the rabies infection abroad (Malerczyk et al., 2011). The most common continent of rabies origin was Asia (n=16) and Africa (n=14) and at the country level, the most cases were contracted in India (n = 6), the Philippines (n = 6), and Mexico (n = 5) (Malerczyk et al., 2011).

In contrast, systematic and effective rabies surveillance systems are lacking in the developing countries of Asia and Africa (Cleaveland et al., 2002; Knobel et al., 2005; Ly et al., 2009; Wu et al., 2009b; Sudarshan et al., 2007; Hossain et al., 2011). Most rabies diagnoses in both humans and animals are made based on clinical signs, with the exception of few countries (Kitala et al., 2000; Cleaveland et al., 2002; Knobel et al., 2005; Sudarshan et al., 2007; Ly et al., 2009; Wu et al., 2009b; Hossain et al., 2011). In developing countries patients may not present to medical facilities for treatment of clinical disease because of the grave prognosis of rabies, and clinical cases are often not reported by local authorities to central authorities (Cleaveland et al., 2002; Knobel et al., 2005; Sudarshan et al., 2007).

At the global level, the WHO collects rabies data through World Survey of Rabies, which obtains rabies data electronically (called RABNET) and has become accessible through the internet for data consultation and online data entry (www.who.int/rabies/rabnet/en). However, the information available in this database depends on the accurate and regular data uploading from the WHO member counties. For instance, the WHO's 1999 World Survey of Rabies (WHO, 1999) reported 1722 human rabies deaths in Asia and Africa (147 in Africa and 1575 in Asia), and the decision tree modelling based approach predicted 55000 deaths (24,000 in Africa and 31,000 in Asia) suggesting that only 3% of human rabies deaths are recorded by central health authorities, which translates into a rate of underreporting of 20 times in Asia and 160 times in Africa (Knobel et al., 2005). In addition, the active rabies surveillance studies in Kenya and Tanzania have revealed that the passive surveillance

programme and the officially recorded figures grossly underestimates the true incidence of human rabies by about 75 to 100 times (Kitala et al., 2000; Cleaveland et al., 2002; Mallawa et al., 2006)

The World Animal Health Organization (OIE) also collects animal rabies data electronically through the World Animal Health Information System (WAHIS) (http://web.oie.int/wahis/public.php?page=home). Similarly, the information available in this data also depends on the accurate and regular data uploading from the OIE member counties and may grossly underestimate the true figures of animal rabies cases (Kitala et al., 2000; Knobel et al., 2005).

1.12.2. Surveillance of healthy-dog carriers of rabies virus

The intermittent excretion of rabies virus in the saliva of apparently healthy dogs and dogs that have recovered from rabies has been observed in Ethiopia (Fekadu, 1975; Fekadu and Baer, 1980; Fekadu et al., 1981; Fekadu et al., 1982). Rabies virus was also isolated from clinically healthy and previously unvaccinated dogs in Nigeria (Aghomo and Rupprecht, 1990) and in Thailand (Kasempimolporn et al., 2007). The reports of excretion of rabies virus in the saliva of apparently healthy dogs lead to the belief that a "carrier state" for rabies might exist (Warner et al., 1996). Healthy dog-carriers were later described in China, with virus being isolated from the brains of apparently healthy domestic dogs (Tang et al., 2005; Lu et al., 2006; Zhang et al., 2006; Dong et al., 2007; Tao et al., 2009; Wu et al., 2009b). For example, Lu et al (2006) found that the brain tissue specimens from 5 of 283 (1.76%) healthy looking dogs collected from rural areas of 13 cities in Guangxi province tested positive for rabies virus by RT-PCR and virus isolation (Tang et al., 2005; Lu et al., 2006). Similarly, an infection rate varying from 2.3% to 20% were reported in apparently healthy dogs sampled from regions of high incidence of rabies (Luo et al., 1995; Ge et al., 2002; Dong et al., 2007; Song et al., 2009; Tao et al., 2009). Further investigation to understand the role of dogs as potential healthy carriers of rabies virus was conducted in China (Zhang et al., 2008a). In their experiment (Zhang et al., 2008a), 153 domestic dogs were collected from a rabies enzootic area and monitored for 6 months. Fifteen dogs tested positive to rabies virus antigen in the saliva by ELISA test, but none of the dogs showed any clinical signs of rabies

during the 6 months observation period. Also none of the saliva samples collected either at the time of acquisition or during the observation period was found to be positive for rabies virus RNA by RT-PCR. The viral antigen or viral RNA were not detected in the brain samples collected at the time of euthanasia (Zhang et al., 2008a). These phenomenon and contention may be explained by the fact that rabies virus has reached the CNS before clinical signs appear in those dogs, rather than the existence of carrier or asymptomatic rabies (Song et al., 2009; Wu et al., 2009b).

1.13. Rabies data analysis

1.13.1. Analysing public health burden of rabies

Lack of accurate data on the true incidence of rabies mortality and its public health impacts has been the main reason for the low level of political commitment to rabies control in developing countries (Cleaveland et al., 2002; Knobel et al., 2005). The officially reported deaths is believed to be greatly underestimates the true incidence (Knobel et al., 2005; Mallawa et al., 2006). Therefore, a probability decision tree modelling based predictive approach has been developed and used to estimate human deaths from rabies using dog bites data (Cleaveland et al., 2002; Fevre et al., 2005; Knobel et al., 2005; Zinsstag et al., 2009). The model uses the distribution of injuries on the body together with the likelihood of the patient receiving successful treatment to predict the outcomes of bites from rabid dogs (Cleaveland et al., 2002; Knobel et al., 2005). One of the principal factors influencing the outcome of a bite from a rabid dog is the location of the bite on the body: a bite nearer to the brain (e.g. on head) is considered more at risk of developing rabies than a bite on the extremities (Cleaveland et al., 2002; Knobel et al., 2005). The model is also based on the principles that not all bites from rabid dogs result in infection and that not every infection leads to clinical signs and death (Cleaveland et al., 2002). Using this decision tree modelling approach, human rabies mortalities in Asia and Africa were estimated at 55000 per year (Knobel et al., 2005). Reassessment of the global burden of rabies is ongoing. Preliminary estimates indicate 70,000 human deaths occurring worldwide from rabies each year, with the majority of deaths estimated in Asia and Africa (Hampson et al., 2011b). The predictive

approach using dog bite data is a useful tool to quantify human rabies mortality to enhance rabies surveillance in human and animals in a poor surveillance system but can be influenced by the accuracy of rabies recognition probability of the biting dogs (rabies suspected dogs may not be available for examination and confirmatory diagnosis), and the level of post-exposure treatment received by the patients (Cleaveland et al., 2002).

1.13.2. Analysing economic impacts of rabies

The economic impacts of rabies in Asia and Africa has been analysed by estimating direct and indirect cost of PEP in humans, rabies control cost in dog, disease surveillance cost and the livestock losses using various input cost parameters (Knobel et al., 2005). The economic cost estimates of rabies in Asia and Africa by Knobel et (2005) have included both direct medical cost and the indirect patient cost (income loss and the transport cost), and have revealed that the private patient cost for PEP represents the bulk of expenditure (Knobel et al., 2005). Bogel and Meslin (1990) have developed guidelines for rabies control program and compared the cost of intensified human PEP versus mass dog vaccination, and demonstrated that mass dog vaccination in combination with PEP in humans is more cost-effective than intensified PEP in human alone (Bogel and Meslin, 1990). Similarly, the benefits and costs of eliminating animal and human rabies in the Philippines was estimated by Fishbein et al. (1991) using benefit-cost ratio: their study showed that dog rabies elimination would benefit from the abolition of expenses associated with rabies prevention such as animal vaccination, PEP in human, animal rabies examination costs, and the benefit would also accrue from an additional earnings of humans whose death due to rabies would be prevented. The costs of an elimination program have been calculated to be recouped by 4.1 to 11 years after the initiation of a one-year elimination campaign (Fishbein et al., 1991). Shim et al. (2009) have calculated the cost-effectiveness ratio for intramuscular Essen regimen of PEP in humans in Tanzania and have demonstrated a cost-effectiveness ratio for PEP of US$ 27/quality-adjusted life year (QALY) from a health care perspective and US$ 32/QALY from a societal perspective, and showed that it is ‘very cost-effective’ to administer PEP to patients bitten by an animal suspected to be rabid from both the health care and societal perspective (Shim et al., 2009). In a rabies transmission dynamic study in Chad, Zinsstag et al. (2009) compared the cost-effectiveness of mass dog vaccination to PEP in humans, and

estimated US$ 46 per disability adjusted life-years averted. Their study also demonstrated that the cost-effectiveness of PEP, together with a dog-vaccination campaign, breaks even with cost-effectiveness of PEP alone after almost 5 years, and they found it to be more cost-effective to combine parenteral dog vaccination campaigns with human PEP compared to human PEP alone after a time-frame of 7 years (Zinsstag et al., 2009). The cost description of rabies PEP in humans has also been calculated by other authors (Bogel and Meslin, 1990; Wilde et al., 1999; Goswami et al., 2005).

However, in animals, there are only few studies that have calculated the real cost of intervention through mass vaccination of dogs or stray dog control programs, but they have used the same principles of calculating the public and societal cost. For example, Kayali et al. (2006) have estimated the public and private sector cost of a pilot mass vaccination campaign in N'Djaména (in Chad) in Africa that vaccinated 3000 dogs using parenteral methods of vaccine administration within 2 weeks and then extrapolated results to the whole city level and estimated the cost of vaccinating 23,600 dogs in N'Djaména (Kayali et al., 2006). Their study included detailed description of costs and calculated €1.69 per dog vaccinated to the public cost and €2.45 to the full societal cost during pilot vaccination of 3000 dogs. When this cost was extrapolated to vaccinate all 23600 dogs in N'Djaména, the average cost was found to fall to €1.16 to the public and €1.93 to society (Kayali et al., 2006). In other studies, cost per dog vaccinated range from US$ 0.52 in Thailand to US$ 0.95 in Tunisia (Bogel and Meslin, 1990), US$ 1.19 in the Philippines (Fishbein et al., 1991) to US$ 2.70 in Malawi (Edelsten, 1995), but none of these estimates include the private cost..

1.13.3. GIS based spatial analysis of rabies data

Geographic Information System based analysis has gained importance and is increasingly used as a tool to manage and analyse data in epidemiologic research both in public health (Booman et al., 2000; Tiwar et al., 2006; Zhang et al., 2008b; Wu et al., 2009b) and veterinary medicine (Norstrøm, 2001; Ward, 2006; Ward, 2007a, b; Frössling et al., 2008; Ward et al., 2008). The use of GIS technology has provided important information about the distribution and risk areas of diseases for control programs. The GIS techniques have also been used to understand the epidemiology of rabies but more publication are available with

respect to wildlife rabies epizootiology than canine rabies epidemiology, and only a few publications will be discussed here. Recuenco et al. (2007) have applied the spatial scan statistic to identify spatial and temporal patterns of enzootic raccoon variant rabies and to search for significant terrestrial rabies clusters by year in New York State, 1997–2003 (Recuenco et al., 2007). Their study identified statistically significant clusters of rabies in some areas and also identified high risk raccoon rabies areas, which is useful for planning control programmes. Directional test (mean center and deviational ellipse) and time series analysis was used by Guerra et al. (2003) to describe the epizootiology of skunk rabies in the eastern United States and to determine if skunk and raccoon rabies epizootics are associated spatially and temporally (Guerra et al., 2003). The mean direction and distance travelled by the skunk and raccoon epizootics were found to be similar (south westerly direction) from 1990 to 2000, and the time series analysis showed that the number of rabid raccoons predicted the number of rabid skunks through time, with a 1-month lag (Guerra et al., 2003). Similarly, Jones et al. (2003) have used a GIS and time series analysis approach by using land use and demographic data as predictors discriminating between counties experiencing large or small first epizootics of rabies among raccoons in Maryland, Pennsylvania and Virginia in the USA (Jones et al., 2003). Their study revealed that a high percentage of agricultural land use, high water coverage in combination with low human population density, and low water coverage with high human population density were positively associated with large rabies epizootics, while the counties with >15% of mixed forest were less likely to experience large epizootics than were counties with ≤15% of mixed forest (Jones et al., 2003). Several other studies have used GIS methods to either map wildlife rabies epizootics and surveillance data to produce risk maps (Carey et al., 1978; Tinline and MacInnes, 2004; Krebs et al., 2005; Blanton et al., 2006a; Blanton et al., 2007; Dorrell, 2007; Blanton et al., 2009; Blanton et al., 2010) or to find spatio-temporal associations of rabies occurrence between species (Childs et al., 1997; Gordon et al., 2004).

A Gaussian kernel smoothing method and the space-time scan statistic was used by Suzuki et al (2007) to assess the spatio-temporal distribution of the endemic canine rabies in Santa Cruz dela Sierra, in Bolivia. They identified significant spatio-temporal clustering of canine

rabies cases and found a trend towards clusters with high incidence rates in deprived areas and traditional markets harbouring a large number of stray dogs (Suzuki et al., 2007).

Although GIS is a powerful tool for designing, assessing and implementing surveillance, the data collected in the surveillance system needs to be spatially accurate and timely (Ward, 2007a). The details of GIS techniques and its application in veterinary epidemiology are discussed elsewhere (Pfeiffer, 1996; Ward and Carpenter, 2000; Carpenter, 2001; Durr and Gatrell, 2004; Pfeiffer et al., 2008).

1.14. Conclusions

This review has outlined the historical points of rabies, rabies virus characteristics and disease, diagnostic techniques, principles of prevention and control of rabies in humans and animals, rabies epidemiology, socio-economic and public burden of rabies, surveillance system and some analytical tools used for analysing rabies epidemiologic and economic data.

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CHAPTER 2

2. REVIEW OF RABIES EPIDEMIOLOGY AND CONTROL IN SOUTH, SOUTH EAST AND EAST ASIA: PAST, PRESENT AND PROSPECTS FOR ELIMINATION

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CHAPTER 2

Review of Rabies Epidemiology and Control in South, South East and East Asia: Past, Present and Prospects for Elimination

Abstract

Rabies is a serious public health problem in Asia. It causes substantial animal welfare, economic and human health impacts, with ~39,000 human deaths each year. Domestic dogs are the main reservoir and source of rabies in Asia. Common constraints for the control of rabies in the countries of Asia include inadequate resources; lack of political commitment to control programs; lack of consensus on strategy; weak intersectoral coordination and inadequate management structure; insensitive surveillance systems; limited accessibility to modern rabies vaccine and supply problems; lack of public awareness and public cooperation; and the existence of myths and religious issues. In this review, we summarise the epidemiology of rabies in both human and animals in each South and South East Asian country, the past and current approaches to control, and the prospect for rabies elimination. We conclude that defining the cost of rabies to society and communicating this to decision-makers might be the key to achieving such an advance.

Keywords: Rabies, Human and animals, Epidemiology, control programs, south Asia, South East Asia

Impacts

- Rabies is a serious public health problem in Asia characterized by a high rate of human mortalities with the estimated death of 39,000 persons each year.
- The main constraints for the control of rabies identified are a lack of political commitment to control programs, weak intersectoral coordination, insensitive surveillance systems, limited accessibility to modern rabies vaccine and lack of public awareness and cooperation.
- There is a prospect for reducing the human rabies mortalities and rabies elimination through a regionally coordinated control programs by improving surveillance system, awareness education, mass dog vaccination and post exposure prophylaxis. Defining the cost of rabies to society and communicating this to decision-makers might be the key to achieving such an advance.

2.1. Introduction

The rabies situation in Asia is characterised by a high rate of human mortalities: there is an estimated death of 39,000 persons each year, contributing to 56% of the total human rabies deaths in the world (Hampson et al., 2011). Only a few countries in Asia (Japan, Singapore, Malaysia, Taiwan, Hong Kong, South Korea) have eradicated canine rabies through mass dog vaccination and stray dog population control programs (Wells, 1954; Cleaveland et al., 2006; Takahashi-Omoe et al., 2008; Weng et al., 2010), but no new Asian country has been able to eliminate rabies during the past four to five decades (Wilde et al., 2005; Cleaveland et al., 2006). However, Thailand, Vietnam and Sri Lanka have reported a substantial reduction of human rabies deaths through dog mass vaccination, intensified post-exposure prophylaxis in humans and awareness education (Kamoltham et al., 2003; Kamoltham, 2007; Xuyen, 2008; Hien, 2009; Kumarapeli and Awerbuch-Friedlander, 2009; Harischandra, 2011; Wimalaratne, 2011; Wiriyakitja, 2011).

There are several constraints that some countries in South and Southeast Asia share for the control of rabies: inadequate resources; lack of political commitment to control programs; lack of consensus on strategy; weak intersectoral coordination and inadequate management

structure; insensitive surveillance systems; limited accessibility to modern rabies vaccine and supply problem; lack of public awareness and public cooperation; and prevalence of myths and religious factors (WHO, 2001, 2005). In addition, emphasis on emerging infectious disease such as avian influenza A H5N1 or other major disease problems might have lead to the relative neglect of lower profile disease like rabies in Asia or elsewhere despite rabies being number one zoonotic disease that has high case fatality rate than any other infectious disease and kills more people than yellow fever, dengue fever and Japanese encephalitis (Cleaveland et al., 2006; Lembo et al., 2010; Coker et al., 2011). However, rabies has gained international recognition in recent times and progress has been made towards uniting global expertise to promote global campaign against rabies (Lembo et al., 2011). For example, in Asia, a network of experts from twelve Asian countries (Bangladesh, Cambodia, China, India, Indonesia, Laos, Myanmar, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam) was established in 2004 and called 'The Asian Rabies Expert Bureau (AREB)' (http://www.areb.info/website). AREB members meet annually to review the regional rabies situation; to define the best clinical management practices in local situations; and to discuss how to increase awareness for the elimination of rabies in Asia (Dodet, 2011). Another organization – 'Rabies in Asia Foundation' (RIA) – was also established in 2006 under the Indian Health Trust and currently has chapters in nine Asian countries (Afghanistan, China, Bangladesh, Thailand, India, Pakistan, Philippines, Sri Lanka and Vietnam) (Sudarshan, 2011). The RIA Foundation's main objective is to liaise, coordinate, network and to influence the Governments of member countries for the control and elimination of rabies in Asia and the world (http://www.rabiesinasia.org).

In this article, we review the rabies situation of South, South East and East Asian countries from a range of perspectives, including human and animal cases, epidemiology, and past and present control strategies to promote a better understanding of rabies in this region and the prospects for elimination.

2.2.Rabies situation in South Asia

South Asia comprises eight countries: Afghanistan, Bangladesh, Bhutan, Maldives, Nepal, Pakistan, India, and Sri Lanka. All countries are endemically infected with rabies, except the Maldives which has been historically free (Gongal and Wright, 2011). South Asia contributes about 40% of the total human rabies mortality in the world, and about 71% in Asia (Hampson et al., 2011).

India

Rabies is endemic in India and has been reported from almost all regions of the country (Nagarajan et al., 2006; Nadin-Davis et al., 2007; Nagaraja et al., 2008; Nagarajan et al., 2009; Babu et al., 2011; Reddy et al., 2011). Only the islands of Andaman, Nicobar and Lakshadweep are historically free of rabies (APCRI, 2004). Dog bites are the principal source of human rabies in India (97%), followed by cats (2%) and wildlife – mongoose and jackals (1%) – with an estimated 17 million animal bites reported per year (Sudarshan et al., 2006). A multicentric epidemiological study conducted in 2003 estimated 20,000 human rabies deaths (2.86/100,000 population) annually in India, accounting for about 29% of all global human rabies deaths (APCRI, 2004; Sudarshan et al., 2007). The detail of rabies situation is described in a national multicentric survey progress report (APCRI, 2004). Unfortunately, rabies is not a notifiable disease in India and disease surveillance is poor despite the high human rabies mortality (Sudarshan et al., 2007; Sudarshan, 2011).

India had been manufacturing and using Semple vaccine (nerve tissue vaccine) since its first introduction in 1911 by Sir David Semple at the Central Research Institute in Kasauli (Briggs et al., 2002). However, production was discontinued in January 2005 and currently use modern cell culture rabies vaccines only (Sudarshan, 2009). India manufactures BHK 21 Cell line vaccines for use in dogs and cats, and Purified Chick Embryo Cell Vaccine, Purified Vero Cell rabies vaccine, and Purified Duck Embryo Vaccine for human use, and also produces purified equine rabies immunoglobulin (Sudarshan, 2009; RIACON, 2011). India is the only country in the region producing various types of quality rabies tissue-culture vaccines and has a capacity to produce 15 million doses of rabies vaccine annually

(Sudarshan, 2009; Gongal and Wright, 2011). However, the level of coverage of cell culture vaccine in animal bite victims is only about 50% whereas the use of rabies immunoglobulin is 2%, leading to a large number of preventable rabies deaths in India (APCRI, 2004; Sudarshan et al., 2007). Following the shortage of cell culture vaccines, in February 2006 the Government of India approved the use of intradermal rabies vaccination. Currently, most States in India have started intradermal vaccination due to its cost-effectiveness (Sudarshan, 2009; Mathew, 2011).

Although India accounts for nearly 29% of the global rabies mortality burden, there is no organised national rabies control program. Rabies control programs that have been initiated by some organizations are generally confined to small urban areas, with minimal intersectoral co-ordination (Mahendra, 2007; Sudarshan et al., 2007; Sudarshan, 2009; Abbas et al., 2011). However, a significant decrease in human rabies deaths have been observed in areas where a rabies control program had been implemented (for example, in the city of Jaipur and Jhodpur) (Reece and Chawla, 2006; Totton et al., 2010). Tamil Nadu state in south India also implemented a successful state-wide, multi-sectoral rabies control initiative under a 'One Health' program, which reduced human mortality because of rabies (Abbas et al., 2011). A non government organization called Blue Cross India (http://www.bluecrossofindia.org) in Tamil Nadu state and Sikkim Anti-rabies & Animal Health Programme (SARAH) in Sikkim (http://www.vetsbeyondborders.org/our-projects/sikkim-anti-rabies-and-animal-health-programme-sarah) has been carrying out successful mass dog vaccination and sterilization program in their respective States.

Bangladesh

Rabies is widely distributed and a major public health problem in Bangladesh with an estimated 2000–2500 human deaths annually (Rahman et al., 2007; Rahman, 2009; Gongal and Wright, 2011; Hossain et al., 2011). Rabies is not a notifiable disease in Bangladesh and there is no organized surveillance system for rabies. Therefore, reliable data are scarce and the mortality might be higher than what is reported (Hossain et al., 2011). Stray dogs are the principal animal responsible for bites and the transmission of rabies, but wildlife rabies

(jackals, mongoose) has also been reported (Rahman, 2009; Gongal and Wright, 2011; Hossain et al., 2011).

The nerve tissue vaccine derived from sheep brain is still produced by the Infectious Disease Hospital (IDH) in Dhaka and used for human post exposure treatment (Rahman et al., 2007; Rahman, 2009; Hossain et al., 2011; Ziauddin, 2011). The IDH in Dhaka is the main referral centre for rabies patients and most of the animal bites cases from different areas of the country report to this hospital to receive free vaccine and treatment. From January 2004 to December 2008, 150,068 patients with animal bites visited the IDH hospital and 86.2% and 13.8% of them received nerve tissue and tissue culture vaccine, respectively (Hossain et al., 2011). Only a negligible number of patients receive rabies immunoglobulin, but rabies cases are reported even in vaccinated individuals due to the use of the less effective nerve tissue vaccine (Hossain et al., 2011).

The Veterinary Public Health section of the Department of Livestock (since its establishment in 1985), has initiated a rabies control program which include elimination of stray dogs, registration and vaccination of pet dogs and publicity campaigns (WHO, 1996). However, its success was very limited because of shortage of manpower, diagnostic facilities, coordination among different organizations and financial support for conducting the control program (WHO, 1996). A stray dog elimination program has been implemented in urban areas but is non−existent in rural areas (Hossain et al., 2011).

Pakistan

Rabies is endemic in Pakistan (Parviz et al., 2004) with an estimated 2000–5000 human rabies deaths each year (Salahuddin, 2009). The nerve tissue vaccine derived from sheep brain is still produced at the National Institute of Health and used in government hospitals in Pakistan (WHO, 1996; Parviz et al., 1998; Parviz et al., 2004; Salahuddin, 2009), but an increasing number of rabies centres are reported to be using modern cell-culture vaccines (Salahuddin, 2009; Dodet, 2010). Rabies immunoglobulin is used infrequently because of the high cost of the product (Salahuddin, 2009).

Only pet animals under the care of private vets are vaccinated, whereas stray dogs roam freely and are unvaccinated (Salahuddin, 2009). The Pakistan Chapter of Rabies in Asia

Foundation was established in May 2006 (Salahuddin, 2007). The main objectives of this organization is to address the issues of rabies control programs both at federal and at city district levels, conduct public awareness campaigns, teach correct post-exposure prophylaxis and advocate against the use of nerve tissue vaccine (Salahuddin, 2007). There is no organized rabies control programs for either animals or humans in Pakistan, and access to medical care for dog bites is poor (RIACON, 2011)

Sri Lanka

Sri Lanka has been endemic for canine rabies and no wildlife rabies has been detected (Kumarapeli and Awerbuch-Friedlander, 2009; Harischandra, 2011; Matsumoto et al., 2011). Rabies was declared a notifiable disease in 1971, and after that 377 human rabies deaths were reported in 1973. In 1975, Sri Lanka launched an island-wide 5 year rabies control program (1976-1980). During 1985-1991 an AGFUND/RB/WHO assisted rabies control project was launched in 7 districts (Harischandra, 2011). The main control program included mass dog vaccination and elimination of stray dogs. In 1989, the nationally conducted rabies control program was decentralized to provinces, and gradually the herd immunity against rabies within the dog population increased (Harischandra, 2011).

Since the establishment of the rabies control program in 1975 there has been a remarkable decline in the national incidence of human rabies deaths from 310 (22.2 per 1,000,000) in 1977 to 56 (2.8 per 1,000,000) in 2007 (Kumarapeli and Awerbuch-Friedlander, 2009; Harischandra, 2011). This reduction was mainly because of mass dog vaccination, dog population control and increased availability of modern PEP. The rate of mass dog vaccination increased from 3.2% in 1975 to 49.3% in 2007 whereas the rate of dog elimination was less than 10% throughout the period 1975–2005. A significant negative correlation between the human rabies death rate and dog vaccination rate (−0.836; $P<0.01$) and between the human rabies death rate and dog elimination rate (−0.589; $P<0.01$) for the period 1975–2005 was observed after the implementation of the rabies control program (Kumarapeli and Awerbuch-Friedlander, 2009). However, the dog elimination was replaced by an animal birth control program in 2005. During 2010, 972,541 dogs were vaccinated against rabies, and 130,900 dogs were sterilized. An island-wide prompt garbage removal

program was also launched in mid-2010. These activities led to further reduction of human rabies deaths from 58 in 2009 to 49 (0.2 per 100,000 population) in 2010. Similarly, rabies cases in dogs have also been reduced from 709 in 2009 to 579 in 2010 (Harischandra, 2011; Wimalaratne, 2011).

In Sri Lanka, nerve tissue vaccine production started in 1900 at the Medical Research Institute and ceased in 1995. Human diploid cell culture rabies vaccine was introduced in 1986 and purified chick embryo cell vaccine and vero cell vaccine were introduced in 1990. Intradermal administration of rabies vaccine was initiated in 1997, and currently >95% of patients are given rabies vaccine by the intradermal method (Wimalaratne, 2011). Anti-rabies treatment units have been established in major hospitals in Sri Lanka. In addition, a new diagnostic tests – the polymerase chain reaction and immunochromatography tests were introduced for rabies diagnosis in Sri Lanka (Wimalaratne, 2011).

Nepal

In Nepal, rabies occurs throughout the country with high numbers of cases reported from the densely populated districts in the south Terai and Mid hills region that border India (WHO, 1996; Sharma, 2005; Joshi, 2007, 2009; Karki and Thakuri, 2010). In addition to domestic dog rabies, wildlife mediated rabies are also reported – involving wolves, jackals, mongooses, and foxes (Karki and Thakuri, 2010; Gongal and Wright, 2011; Pant et al., 2011). An average of 200 domestic animals (mainly cattle) are reported to die of rabies each year in Nepal through dog and fox bite rabies infection (Karki and Thakuri, 2010). Approximately 30,000 people receive PEP and about 200 persons die because of rabies every year (Joshi, 2007, 2009; Pant et al., 2011). There is only one diagnostic laboratory in the country, located in Kathmandu (capital city) and administered by the Department of Livestock Services.

Nepal stopped production of the nerve tissue vaccine in 2005 and now uses cell-culture vaccine. Tissue culture vaccine is produced and used for vaccination of dogs whereas it is imported for human use (Joshi, 2009). The alliance group for rabies control in Nepal comprises the Department of Livestock Services, Veterinary Public Health Division; Kathmandu Metropolitan City (KMC), the Department of Public Health and Social Welfare;

Kathmandu Animal Treatment (KAT) Centre and the National Zoonoses and Food Hygiene Research Centre (Joshi, 2009). Rabies control strategies included mass dog vaccination and elimination of stray dogs. However, the elimination of stray dogs has been reduced because of cultural sentiments of teh people.

Afghanistan

Rabies is endemic in Afghanistan, and is a reportable disease for the Ministry of Agriculture, but not for the Ministry of Public Health (Safi, 2011). However, there is lack of accurate epidemiological information about the rabies situation in Afghanistan (Safi, 2011). There are no specific rabies prevention and control measures either in the Ministry of Public health or in the Ministry of Agriculture.

Afghanistan became a member of the Rabies in Asia Foundation in early 2011, establishing the Afghanistan Chapter. In 2011, six vaccination campaigns were conducted in various districts, and 19,500 house dogs were vaccinated (Safi, 2011). World Rabies Day 2011 was celebrated in major provinces and districts and house dogs were vaccinated free of cost. Public awareness education on rabies was done through radio broadcast and public posters/banner displays (Safi, 2011).

Bhutan

There are no proper records of the first reports of rabies in Bhutan but the disease was widespread in the country in the 1970s and 1980s (and up until the early 1990's) (Joshi, 1991; Bhutan Observer, 2008; Kinley Dorjee, personal communication). Rabies is a notifiable disease as per the Bhutan Animal Husbandry Act 1981 (amended in 2001). In 1987, 15 human and 150 animal rabies cases were reported in Bhutan (Joshi, 1991). In 1991, clinical cases in 37 dogs, 24 cattle, 2 pigs and 2 cats were reported in areas of the capital city, Thimphu (Owoyele, 1992). Currently, rabies is reported mainly from southern parts of Bhutan that share a border with India (Tenzin et al., 2011a). However, occasional re-emergence has been reported in previously free areas. For example, there was an introduction of rabies in Paro district (west Bhutan) in 1998 through migratory livestock (which had

probably been bitten by rabid dogs in southern Bhutan). Between 2005 and 2007, a major outbreak of rabies occurred in animals (dogs and cattle) in eastern Bhutan, an area which had been free of rabies for at least 18 years (Tenzin et al., 2010).

The first rabies control programs in Bhutan was initiated in 1985 under United Nation Project (Joshi, 1991; Kinley Dorjee, personal communication). Rabies control strategies included culling of stray dogs by shooting. Later, oral strychnine poisoning of stray dogs was adopted to control the dog population, followed by a mass vaccination and sterilization programs. During 2007 and 2008, mass impounding of stray dogs was implemented in urban areas of Bhutan to control the stray dog population, but was discontinued in late 2009 on animal welfare grounds and because of logistical problems. From September 2009, a nation-wide "catch-neuter-vaccinate-release" (CNVR) program was implemented in Bhutan in collaboration with the Human Society International organization. This project is expected to cover > 70% of the total dog population within the project period, 2009–2015 (MoA, 2009; HSI, 2010).

Rabies cases in animals are diagnosed using rapid antigen detection kits in the field and later confirmed by the fluorescent antibody test (FAT) at the two veterinary diagnostic laboratories in Bhutan whereas human cases are diagnosed based on clinical signs because of lack of diagnostic facilities. Moreover, the families of rabid patients would not allow sample collection on cultural grounds. Although human rabies cases are sporadic (approximately 0.28 deaths/100,000 people per year), dog bite incidents are common and result in a large usage of post-exposure prophylaxis (Tenzin et al., 2011b; Tenzin et al., 2011c). Human diploid cell vaccines are imported and provided free of charge to patients by the government medical hospitals in Bhutan (Tenzin et al., 2011b). Bhutan is initiating the use of low cost intradermal rabies post-exposure prophylaxis in humans.

2.3.Rabies situation in South East Asia

Rabies is endemic in all Southeast Asian countries, except Timor-Leste and Brunei Darussalam which has been historically free from rabies (Gongal and Wright, 2011). An estimated 5000 people die of rabies in South East Asia each year (Hampson et al., 2011).

Thailand

Rabies in Thailand was first recorded in 1912 as the cause of death of the Princess Bunlusirisarn known as Princess Pao, following bite by a rabid dog (Mitmoonpitak et al., 1998; Panichabhongse, 2001). At that time there was no post-exposure treatment facilities in Thailand and she missed the ship to Saigon (the Pasteur Institute in Saigon), Vietnam for rabies PEP and then died of rabies (Panichabhongse, 2001). In memory of her death, the Pasteur Institute in Thailand called as "Paturasapha" was established on April 26, 1913 which is now known as "Queen Sauvabha Memorial Institute" (QSMI) (Wilde et al., 1991; Panichabhongse, 2001). This institute became the principal centre for vaccine production, rabies diagnosis and human rabies PEP in Thailand (Mitmoonpitak et al., 1998; Panichabhongse, 2001).

In 1980, rabies became a notifiable disease in Thailand (Wiriyakitja, 2011). Rabies control activity in animals (vaccination and elimination of dogs) was started in 1955 via the Rabies Act B.E. 2498 (1955) under the responsibility of the Ministry of Public Heath (Panichabhongse, 2001). More than 300 human rabies deaths (0.78/100,000 population) were reported annually before 1982 and Thailand ranked third in Asia in numbers of human deaths (Panichabhongse, 2001). This was mainly because of low coverage of dog vaccination. This led to the replacement of the Rabies Act 1955 (B.E.2498) by the Rabies Act 1992 (B.E. 2535) (Panichabhongse, 2001). The two main regulations for rabies control in the Rabies Act B.E. 2535 (1992) are: all dogs greater than two months of age must be vaccinated and tagged and dogs found in public without any valid vaccination tag to be caught and destroyed after five days unless contact is received from the owner (Wasi et al., 1997; Panichabhongse, 2001). Later, the National Board of Rabies Committee was formed and implemented the National Rabies Control Programme. Since then, there has been a substantial reduction of human rabies deaths from 370 reported deaths in 1980 (0.78/100000) to 75 in 1996 (0.12/100000) and to only 9 deaths (0.027/100,000 population) in 2008 and 15 human deaths in 2010 (Wasi et al., 1997; Mitmoonpitak et al., 1998; Wiriyakitja, 2011).

For example, in Phetchabun province in northern Thailand, a well coordinated 5-year rabies control project was initiated in 1993 with an aim of eliminating rabies by year 2000 (Kamoltham et al., 2003). The program strategies included: increasing accessibility to PEP

using intradermal rabies vaccination, increasing educational awareness programs; mass dog vaccination and sterilization programs; increasing the cooperation between the Ministries of Public Health, Agriculture, and Education on a provincial level; and assessing the impact of the program through intensified follow-up of patients exposed to suspected and laboratory-confirmed rabid animals (Kamoltham et al., 2003). This control program resulted in decrease of human rabies deaths from 25 deaths reported each year between 1989 and 1996 to only two human deaths in the first 2 years of the program implementation, and no deaths occurred during the last 3 years of the program (Kamoltham et al., 2003).

In Thailand, nerve tissue vaccine was replaced with tissue culture vaccine in 1993 and then the Thai Red Cross intradermal vaccination was introduced in 1995 (Wasi et al., 1997; Kamoltham, 2007). Thailand has been instrumental in introducing ID rabies vaccination, which has been approved by the World Health Organization. The keys to the success of rabies control in Thailand have been due to improved PEP and public education, mass dog vaccination and dog population control (Wasi et al., 1997). The Ministry of Public Health is responsible for human rabies prevention and control wheareas the Department of Livestock Development, under the Ministry of Agriculture and Cooperatives, is responsible for animal rabies prevention and control (Panichabhongse, 2001; Wiriyakitja, 2011). Thailand is targeting eradication of the disease from the country by the year 2020 with a declaration of a rabies-free zone at the provincial, district, and local administrative levels through improved surveillance system (Wiriyakitja, 2011).

Philippines

Rabies is endemic and considered a major public health problem in the Philippines (Fishbein et al., 1991; Camba, 1997; Salva, 1997; Dodet, 2010; Dimaano et al., 2011). The annual average number of human rabies cases was 45 during 1902−1910, 210 cases during 1946−1951 and 253 cases during 1958−1968. The annual rabies death rate was 0.5−0.6 per million people during 1973−1977 and 5−8 per million (400 cases per year) between 1983 and 1993 (WHO, 1996; Salva, 1997). Between 1987 and 2006, 1839 human rabies deaths (average: 92 (57−119) per year) were recorded at the San Lazaro Hospital, the national referral centre for infectious disease in Manila, the Philippines (Dimaano et al., 2011). In

2008, 190,000 human animal bites were reported to the National Center for Disease Prevention and Control, and 250 humans died of rabies (Deray, 2009; Dodet, 2010). The main reason for human rabies death were because of consultation with local non-physicians (*'tandoks' or faith healer*) instead of seeking PEP from the hospitals (Dimaano et al., 2011).
A rabies control program had been carried out in the Philippines since the 1950s, but on a limited scale with limited governmental agency involvement (WHO, 1996). In 1989, the Department of Health (DOH) and Department of Agriculture (DOA) initiated a national control program, and then implemented in May 1991. A multisectoral national rabies program through a Memorandum of Agreement between the DOH, DOA, Department of Education, Culture & Sports, Department of Interiors and Local Government, and non-government agencies was established (WHO, 1996; Camba, 1997; Salva, 1997). The main strategies included public awareness education on responsible pet ownership, provision of free PEP in humans, mass dog vaccination, enforcement of ordinance on stray dog control, and strengthening of rabies surveillance, with an objective to reduce human rabies incidence by 10% each year and declare Philippines rabies free by 2020 (WHO, 1996).

In 2006, the government of the Philippines enacted Republic Act 9482 (known as the Anti-Rabies Act 2007) to provide legal backing for the National Rabies Prevention and Control Program with an aim of eliminating rabies throughout the Philippines by 2020 (Deray, 2009; Dodet, 2010). Rabies awareness education materials were incorporated into the school curriculum and implemented in the regions that reported highest number of human rabies cases including pre-exposure rabies immunization to children aged 5−14 years (Quiambao, 2007; Dodet, 2010). In 1997, the more cost effective intradermal regimen of PEP in humans was introduced and is given in specialized centres termed 'animal bite treatment center' in existing hospitals or health centres in the Philippines (Quiambao, 2007, 2009). The Philippines has more than 250 government and about 100 private bite centers, providing PEP to animal bite victims (Quiambao, 2009).

Rabies-free projects funded by Bill and Melinda Gates Foundation and Japan International Cooperation Project on Rabies Elimination for Small Islands were launched and implemented in some of the island groups in the Philippines (Dodet, 2010; Atienza, 2011;

Lembo et al., 2011). The main strategies adopted were ensuring active community participation, increasing mass dog vaccination coverage and also management of humans exposed to rabies. In 2008, the island province of Siquijor was declared as the first rabies free Island in the Philippines (Deray, 2009). In 2010, Batanes Province and Apo Island in Negros Oriental were declared as rabies free zones. In 2011, Municipality of Daan Bantayan, and the four municipalities in Camotes Island were declared as rabies free zones (Atienza, 2011). In Bohol, the 4-year (2007−2010) 'Rabies Prevention and Eradication Program' was implemented with funding support from the National government and the Bohol Provincial government, the Alliance for Rabies Control and a private Swiss foundation. No human rabies cases have been reported from the island of Bohol following project implementation compared with approximately 10 human rabies deaths reported annually before the rabies prevention program was initiated (Dodet, 2010). These are some of the examples of successful rabies pilot projects that demonstrate the feasibility of canine rabies control/elimination producing direct benefits for human health (Dodet, 2010; Lembo et al., 2011). In the Philippines, the number of human rabies deaths also decreased from 971 cases in 2008 to 695 cases in 2009 and 584 cases in 2010 (Atienza, 2011).

Vietnam

Rabies is a public health problem in Vietnam and dogs (96.1%) and cats (3.9%) are the main reservoir of rabies (Xuyen, 2008; Hien, 2009). Most rabies cases are reported in rural areas due to large number of dogs – 80% of estimated 7 million dogs are in rural areas (WHO, 1996; Hanh, 2011). During the period 1992–1995, 414 human rabies deaths were reported and about 345 000 people received PEP each year (WHO, 1996). In 1987, a vaccine card and a rabies vaccination register were introduced in all provinces/cities of the northern part of Vietnam, to measure vaccine usage and the number of deaths from rabies. This system recorded 365 people per 100 000 PEP and 350 deaths per year (range 285–398) during 1992 and 1993 (WHO, 1996). With increased rate of PEP (reaching as high as 790.2/100 000 population in 2004), the rabies death rate in humans has decreased drastically from 0.71/100 000 population in 1994 to 0.037/100 000 in 2003, and then 0.1/100 000 population in 2008 (Xuyen, 2008; Hien, 2009). However, human rabies cases have increased later (>300 cases

reported between 2007 and 2010) with a peak in 2007 (131 cases; 12/100 000 population) then decreased again with 91 cases in 2008 and 64 cases in 2009 (Thanhniennews., 2010; Hanh, 2011). Poor public awareness of rabies, negligent health workers and the free-range breeding of dogs has been suggested to have led to a rise of rabies deaths in recent years (RIACON, 2009). Vietnam has 936 rabies vaccination centres in the whole country for rabies consultation and prophylaxis (Hanh, 2011). Besides dog bites, butchering of dogs or cats for consumption has been identified as one of the routes of rabies transmission, accounting for 1.6% of human rabies deaths in recent years (Hanh, 2011).

Vietnam produced and used mouse brain nerve tissue rabies vaccine (Fuenzalida vaccine) from 1974 to 2007, but this was replaced with modern cell-culture rabies vaccine in October 2007 (Xuyen, 2008; Dodet, 2010; Hanh, 2011). Nationwide rabies vaccination campaigns are conducted on dogs and cats with a coverage of approximately 35−50% (Hien, 2009; Hanh, 2011). Other activities for the prevention and control of rabies in humans and animals included strengthening the rabies surveillance system, intensified use of PEP in humans; awareness education campaign, training of health staff and setting up inter sectoral network of collaborators (Xuyen, 2008; Hien, 2009).

Indonesia

The first case of rabies in Indonesia was reported in West Java Province in a water buffalo in 1884, then in a dog in 1889, and in a human in 1894 (WHO, 1996). The disease then spread from Java to Sumatra in 1911, Sulawesi in 1956 and Kalimantan in 1974 (Soenardi, 1985; WHO, 1996). During 1985–1986, there was an epidemic of rabies in the province of Central Java, which had been free from rabies for about 10 years. It was believed to have spread from West Java, a rabies endemic province (Walter-Toews et al., 1990). The response included a mass vaccination of dogs and dog elimination program (210425 domestic dogs, cats and monkeys were vaccinated; 61048 dogs, cats and monkeys were destroyed by shooting and strychnine poisoning), and the epidemic was brought under control at the end of 1986 (Walter-Toews et al., 1990).

In 1989, rabies control activities (vaccination and elimination) were implemented in all infected islands of Java and Kalimantan, and then extended to infected islands of Sumatra

and Sulawesi in 1993 which resulted in decrease in human rabies cases from 117 in 1988 to 36 in 1995 (average of 80 deaths each year from 1990–1994) (WHO, 1996). An intensive dog vaccination and elimination program, with the prohibition of inter-island transportation of dogs, cats and monkeys from infected into rabies-free islands were implemented. However, canine rabies spread into East Flores district (in Flores Island) in September of 1997, which was linked to the importation of three dogs by fisherman from Butung Island (southeast Sulawesi), an area endemic for canine rabies, and resulted in human rabies deaths (1 in 1997, 10 in 1998, 13 in 1999, 1 in 2000, and 2 in 2001) (Windiyaningsih et al., 2004). As an emergency response to combat the epidemic, large numbers of dogs were killed in Flores (68,871 dogs killed in 1998, 64,728 in 1999, 147,576 in 2000 and 14,394 in 2001). At the same time, 58,980 dogs were vaccinated against rabies between 2000 and 2002 (25,054 dogs in 2000, 28,043 in 2001 and 5,881 in 2002). About 80% (n=3143) of the total dog brain samples (n=3917) examined between 1998 and 2002 were found to be positive to rabies (Windiyaningsih et al., 2004). During the period from 1998 to 2002, 113 human rabies deaths were recorded in the Flores Island (10 in 1998, 26 in 1999, 58 in 2000, 11 in 2001, and 8 in 2002). Because of vaccine shortage and logistical problems of mass dog vaccination, killing of dogs was adopted as a measure to eliminate rabies from the island. People were encouraged to kill their own dogs in addition to the mass killing done by locally hired teams. The dog elimination program was implemented in consultation with the political, religious and health care leaders and through a public educational campaign. The general public apparently accepted the strategy as many residents had lost friends or neighbours to rabies (Windiyaningsih et al., 2004). However, dog elimination program had not eliminated rabies in the Flores Island.

In 2003, rabies spread to Ambon Island, some 900 km north-east of Flores, as a result of importation of dogs from Sulawesi, and caused at least 22 human deaths by the end of 2003 (Windiyaningsih et al., 2004). In East Nusa Tenggara, rabies killed 216 people between 1997 and July 2011. In 2010, 28 people reportedly died from rabies infections in the province, whereas only one death has been reported in 2011 (January−July) (ProMED-mail, 2011a).

The latest rabies epidemic was reported in Bali province. In November 2008, rabies was first detected within the southernmost peninsula of Bali and then spread throughout the province

by June 2010 (Clifton, 2010; Putra et al., 2011). About 137 humans have died of rabies in Bali since it was first introduced in 2008, but the number could be higher than this (> 160 human cases) (ProMED-mail, 2011b). The outbreak was initially responded with mass killing of dogs (about 108,000 dogs were killed between 2008 and October 2010) (Putra et al., 2011). Later, an island-wide mass vaccination campaign was commenced in October 2010 and completed in March 2011 (phase 1) under close coordination of the Bali provincial government together with the Bali Animal Welfare Association (BAWA) and the World Society for the Protection of Animals (WSPA). During phase I, approximately 210,000 dogs have been vaccinated (70% of the Bali dog population (350,000 dogs) (ProMED-mail, 2011c). Mass dog vaccination was initially implemented with 6 teams of 50 personnel each to catch and vaccinate dogs, and later expanded to 52 teams with 420 trained personnel. There was a 48% reduction of human rabies cases in Bali in the first 3 month of 2011 (ProMED-mail, 2011c). Rabid dog cases have also declined with a report of about 11 cases per month during and after the campaign compared to about 45 cases per month before the campaign (Putra et al., 2011). Similarly the number of infected villages declined, from 19.4% (140/723 villages) to 6.6% (48/723). The second phase of the island-wide campaign started in May 2011 and was completed in October 2011 (Putra et al., 2011). Bali plans to eliminate rabies by 2015.

Myanmar

The first human rabies case was officially recorded in Yangon in 1915 when the Pasteur Institute (now the National Health Laboratory) was first established (Swe and Hla, 2001). The incidence of animal bites between 1954 and 1964 was 356/100,000 population, and the human rabies death rate was 2.2/100,000 population (Swe and Oo, 1999; Swe and Hla, 2001). About 34 human rabies deaths per year were reported during the period 1991–1995, 59 cases in 1996, 57 cases in 2000, 64 cases in 2007 and 12 cases in 2008 (WHO, 1996; Swe and Hla, 2001; Kyin, 2008). Although rabies is endemic throughout the country, most cases were reported from Yangon, but surveillance system is poor to capture all cases. Yangon Veterinary Diagnostic Laboratory and Regional Laboratories (Mandalay, Pathein, and Taunggyi) conduct the diagnostic procedures for rabies in Myanmar (Kyin, 2008).

Since the 1960s, the Veterinary Biologic Laboratory in Myanmar has been producing lyophilized avianized anti-rabies vaccine for use in dogs, but the production was stopped in 2008 because of vaccine seed problems (Swe and Hla, 2001; Kyin, 2008). Tissue culture vaccine is now imported for use in dogs. The main rabies control program includes monthly elimination of stray dogs by poisoning and shooting, and also registration and vaccination of pet dogs (Swe and Hla, 2001; Kyin, 2008). These activities are carried out by the Veterinary and Slaughter House Division of Yangon City Development Committee. Between 1998 and 2000, about 126,675 stray dogs were killed whereas 9737 dogs were registered and vaccinated against rabies in the city of Yangon (Swe and Hla, 2001). Similarly, between 2007 and 2008, 11864 dogs were killed and 3978 were vaccinated in the Yangon city area (Kyin, 2008). However, these program are only implemented in the city area and are non-existent in rural areas (Swe and Hla, 2001).

Cambodia

Canine rabies is endemic in Cambodia (WHO, 1996; Heng, 2008; Ly et al., 2009). The Institute Pasteur in Cambodia (IPC) located in the capital city of Phnom Penh is the only centre that provides free PEP in humans and also carries out laboratory diagnosis of rabies in human and animals (Heng, 2008; Ly et al., 2009). According to Ly et al., (2009), during the period 1998–2007, 124,749 patients (increase from 8,485 patients in 1998 to 14,475 patients in 2007) attended the IPC's post-exposure treatment clinic for an animal bite injury (Ly et al., 2009). The overall PEP rate was 101/100,000 with the highest rates observed in Phnom Penh and the five neighbouring provinces. During the same period (1998–2007), 63 human rabies deaths were reported and none had received PEP (Heng, 2008; Ly et al., 2009). Access to PEP is only adequate for Phnom Penh province and is not available in other areas in Cambodia. It is assumed that more cases in the countryside go unreported.

Of 22 provinces in Cambodia, rabies in dogs were confirmed in 17 provinces; but majority (95%) of them were located within 200 km from Phnom Penh (Ly et al., 2009). Cambodia has an estimated dog population of 5 million (1 dog for 3 humans) and the vaccination

coverage is estimated to be < 2% because of the lack of national rabies control program (Heng, 2008).

Lao People's Democratic Republic

Rabies is endemic in the Lao People's Democratic Republic and reported from all parts of the country (Inagaki, 1995; Douangmala, 2001; Kittiphone et al., 2008). There is only one laboratory for diagnosing rabies in Lao PDR at the National Animal Health Centre. From 1993 to April 2008, a total of 2813 dog brain samples obtained from central and southern parts of Lao were examined and 1308 (46.5%) were found to be positive for rabies (Kittiphone et al., 2008). During the period from 1993 to 2008, 33 human rabies deaths (1995=8; 1996=3, 1998=1, 1999=2, 2000=1, 2001=1, 2002=2, 2003=2, 2004=4, 2007=8, 2008=1) were reported in Laos (Kittiphone et al., 2008). The National Rabies Control Agency in Lao focuses on public awareness education and dog vaccination in the communities, but the coverage is low (Phiphakhvong, 2001; Kittiphone et al., 2008).

2.4.Rabies situation in East Asia

East Asia comprises of: China, Korean Peninsula, Japan, and Taiwan. Japan and Taiwan is free from rabies whereas there is a resurgence of rabies in South Korea bordering the demilitarized zone with North Korea mediated by wild raccoon dog. There is no information about rabies situation in DPR Korea.

People's Republic of China

Rabies was first described in China in about 556 BC (Hu et al., 2009), and is now considered a re-emerging zonoosis (Wu et al., 2009; Meng et al., 2010). Rabies is reported from all provinces in China with the exception of only two provinces (Qinghai and Tibet), and its distribution has been expanding from infected areas to previously free areas (Hu et al., 2009). Human associated activities have been responsible for the spread of disease from the infected foci (Tao et al., 2009). Currently, most (60%) human rabies cases are reported in the south

and central parts of China and about 92.5 % of human cases are reported in rural areas (Si et al., 2008; Hu et al., 2009; Tang, 2009). Over a 59-year period (1950−2008) at least 120,027 persons have died of rabies in China (Si et al., 2008).

China has witnessed three major rabies epidemics. The first epidemic occurred in the mid 1950s (1956) with a peak of about 2000 human rabies deaths. After a decline in the 1960s, the number of cases again started to increase in the early 1970s reaching a peak in 1981 (about 7037 cases), and then remained at the level of 5000–6000 cases per year. Nationwide dog vaccination and PEP in human during the period 1990–1996 largely controlled rabies with only 159 human deaths reported in 1996 (Si et al., 2008). However, the disease re-emerged again and the trend of human rabies death had increased each year with 2571 deaths reported in 2005, 3279 in 2006 and 3303 deaths in 2007 (Si et al., 2008; Wu et al., 2009; Tu, 2011). The reported number of human cases has decreased to 2466 cases in 2008, 2108 cases in 2009 and 1988 cases in 2010 (Tang, 2009; Tu, 2011). The main reasons for the re-emergence of rabies in China were the low rate of dog vaccination and failure to receive PEP (Si et al., 2008; Hu et al., 2009).

In China, rural dogs have been responsible for >95% of the human rabies cases. Wildlife such as ferret, badgers and wolves have been reported to be a reservoir of rabies and have caused human rabies, but plays a very minimal role in the epidemiology of rabies in China (Hu et al., 2009). Since the re-emergence of rabies in China, thousands of dogs have been destroyed in an effort to control rabies epidemic (Associated Press, 2006; Chinadaily, 2009; WeirdAsiaNews, 2009). However, the current approaches included dog registration and compulsory vaccination (Xinhuanet, 2009; Tu, 2011; Wang et al., 2011).

The production and use of nerve tissue vaccine was stopped in 1980 and was replaced by tissue culture vaccines. Human rabies immunoglobulin and purified equine rabies immunoglobulin are also produced in China (Hu et al., 2009).

2.5.Demonstration of Canine Rabies Elimination in Asia

The first national mass dog vaccination program against rabies began only about 35 years after Louis Pasteur developed rabies vaccine in the 1880s. The first phenol inactivated dog vaccine was developed by Umeno and Doi in Japan in 1918 (Umeno and Doi, 1921), and in 1921 Japan started a national rabies control campaign by applying mass dog vaccination in Nagasaki and Tokyo. However, Japan only became free from rabies in 1957 (Tamashiro et al., 2007; Takahashi-Omoe et al., 2008). Japan has been successful in the eradication of rabies through implementation of various Anti-Rabies legislation: (i) Communicable Diseases Prevention Law 1947 wherein rabies notification was made mandatory; and (ii) Rabies Prevention Law 1950, which required registration, confinement and compulsory vaccination of pet dogs, and elimination of stray dogs (Tamashiro et al., 2007; Inoue, 2009). The last case of indigenous human rabies was reported in 1956 and animal (cat) rabies in 1957 (Tamashiro et al., 2007; Inoue, 2009). To maintain a rabies free status, various rabies prevention programs are implemented, including application of a strict quarantine program for importation of pets, vaccination of dogs and disposal of stray dogs (Tamashiro et al., 2007; Inoue, 2009).

Hong Kong became free from rabies in 1955 (SPCA, 1980). However, the disease re-entered the colony shortly after rabies incidence had increased in the neighbouring Chinese province of Guangdong (Johnson et al., 2008). Dogs were commonly brought into Hong Kong by fishermen returning from China, a rabies endemic country. On 4th October 1980 Hong Kong experienced its first human rabies death in 25 years (SPCA, 1980). From 1980 through 1984, five human cases were recorded in Hong Kong (Wong et al., 1987). The outbreak was controlled by mass dog vaccination (71,659 dogs) and killing of dogs (34,570) (SPCA, 1980).

In Malaysia, vaccination of dogs against rabies of limited scale at the local level was implemented between 1932 and 1947. However, the dramatic increase of rabies in dogs from late 1945 and a subsequent major outbreak of rabies in the capital city, Kuala Lumpur in 1952 lead to the implementation of a national rabies eradication program (Wells, 1954). The main strategies included compulsory mass vaccination of all dogs in rabies infected states

and killing of stray and unvaccinated dogs. To prevent the spread of rabies from the infected states, a phase wise eradication program was implemented. During phase I, which started in 1952, about 30,000 dogs were vaccinated and some 10,300 dogs were killed in the most severely infected areas. Phase II started in 1953 in all rabies infected States and 50,500 dogs were vaccinated and 10,300 dogs were killed within a short (few months) period. By late 1953, 73,100 dogs were vaccinated (80% of the dog population) and 44,500 dogs were killed (Wells, 1954). At the same time, three immune belts were created: (i) along the Thai-Malaysian border at a width of 50 miles (ii) in the south opposite Singapore, and (iii) in the middle (around Kuala Lumpur). From these belts, vaccination was extended north and south thus gradually filling the gaps. Compulsory mass vaccination continued in 1954 to eliminate any cryptic pockets of the disease among dogs. No rabies cases in humans or in dogs were reported after the middle of June 1953 and the country became free of rabies in 1954 (Wells, 1954). In the immune belt area, annual vaccination campaigns are conducted, and only vaccinated dogs are licensed and unlicensed dogs are killed (Shahirudin, 2001). Malaysia is one of the best examples in Asia of eradication of rabies by mass dog vaccination, strict enforcement of legislation and killing of stray dogs, although it shares a border with rabies endemic Thailand.

Taiwan eradicated canine rabies in 1961 because of the efforts on animal quarantine, large-scale vaccination, and stray dog control program (Weng et al., 2010). The last animal case was reported in January 1961 (CDC, 2007). Taiwan has been able to maintain a rabies-free status until now, since the last rabies outbreak during 1948–1961, mainly attributable to the island's geographic isolation and also becauseof its strict border control on animal importation, which includes a 21-day isolated quarantine period and a minimum 180-day waiting period in the country of origin (Weng et al., 2010).

In Korea a rabies control program was implemented during the 1960s, resulting in decreased outbreaks to an annual average of 30 cases during the period 1950–1984. Following a mass dog vaccination and stray dog elimination program, canine rabies was eradicated in 1985 and no human rabies deaths have been reported since 1979 (Hyun et al., 2005). However, since 1993 there has been a resurgence of raccoon dog mediated wildlife rabies in Gangwon

province (northern area of South Korea bordering the demilitarized zone with North Korea) which resulted in an outbreak of rabies in cattle and dogs (Lee et al., 2001; Kim et al., 2006). The current national rabies eradication program in Korea includes mass dog vaccination throughout the country, compulsory vaccination of cattle in the rabies risk areas, distribution of bait vaccine to raccoon dogs, continuing serosurveillance of rabies in vaccinated dogs and cattle, post-exposure prophylaxis in humans and public education (Joo et al., 2011; Yang, 2011).

2.6.Economics of rabies control and cost of elimination

Mass vaccination of dogs with a minimum 70% coverage of the dog population (WHO 1987) is an expensive program in resource limited countries, both financially and on logistic grounds. And most countries in Asia rely on international agencies support to finance rabies control programs. For example, during Phase I (October 2010 to March 2011) of the rabies control program in Bali in Indonesia, approximately 210,000 dogs were vaccinated against rabies at a total cost of Rupiah 6.7 billion (~ US$ 775 000), which was funded by the World Society for the Protection of Animals (ProMED-mail, 2011c) and other agencies. Approximately 103 million Thai baht (US$ 2,575,000) were spent during an intensive dog vaccination and sterilization program in Bangkok city between 2002 and 2004 (Denduangboripant et al., 2005). In the Philippines, rabies control project have been implemented in some island groups with funding support from international organizations (e.g. Bill and Melinda Gates Foundation) (Dodet, 2010). The WHO and WSPA assisted rabies control projects have been launched in Sri Lanka (Harischandra, 2011). In Bhutan, a Bhutanese ngultrum 46 million (US$ 1 million) project is ongoing to vaccinate and sterilize 70% of the total dog population with funding support from the Royal Government of Bhutan and Human Society International (MoA, 2009; HSI, 2009). The above few examples suggest that resource limitation and relying on international agencies for funding support is one of the main constraints for sustaining the rabies control programs. To motivate initiation of control programs and secure such funding, cost-effectiveness of control and the economic benefits derived need to be demonstrated.

Although expensive, the unit cost per dog vaccination is lower than human PEP cost. A study based on a small-scale vaccination program of 36000 dogs in Thailand estimated US$ 0.52 per dog vaccinated, but this cost did not include the private cost to the dog owner (income loss, transportation cost) (Bogel and Meslin, 1990). In the Philippines, Fishbein et al. (1991) estimated that a dog rabies elimination program would cost approximately US$ 4.2 million, assuming a dog-to-human ratio of 1:10, and 60% of all dogs would be vaccinated at a cost of US$ 1.19 per dog (Fishbein et al., 1991). When the upper limit of vaccination cost (US$ 4.27 per dog) was considered, the rabies elimination program was estimated to cost US$ 15 million and the costs of an elimination campaign was estimated to be recouped within 4−11 years after the start of a one year elimination program (Fishbein et al., 1991). In addition, during a mass vaccination campaign in the Philippines, in which 26,205 dogs (76% of the total dog population) were vaccinated, the total program cost was calculated to be US$ 20,400, or US$ 0.78 per dog vaccinated (excluding the manpower cost) (Miranda and Miranda, 1997). The overall cost per dog vaccination in Asia has been estimated to be US$ 1.30, whereas the cost per dog killed is estimated to be US$ 5 (Knobel et al., 2005). Similarly, the cost of dog vaccination in other developing countries range from US$ 2.70 in Malawi (Edelsten, 1995) to US$ 0.95 in Tunisia (Bogel and Meslin, 1990) per dog vaccinated (excluding the private cost), and € 1.69 to the public and € 2.45 societal cost in N'Djamena in Chad (Kayali et al., 2006). In contrast, rabies PEP cost in humans is more expensive than dog vaccination, with an estimated cost of US$ 39.57 in Africa to US$ 49.41 in Asia; also, the bulk of the expenses are borne by the patient (Knobel et al., 2005). For example, in Vietnam approximately 400,000 people are bitten by dogs every year and the country spends at least Vietnamese dong 300 billion (US$ 15.4 million) per year on intensified PEP in humans (Dodet, 2010). In Thailand, approximately 300,000−400,000 people receive rabies PEP each year and about 17,000−25,000 doses of immunoglobulin are used annually, making the human rabies prevention program very expensive to society (Denduangboripant et al., 2005). In Sri Lanka, the Ministry of Health spent US$ 3 million in 2005 to provide rabies PEP in humans (Wimalaratne, 2007).

Although dog vaccination is labour intensive and poses logistical challenges, cost-effectiveness studies have demonstrated that dog rabies elimination is more economical than the widespread use of intensified tissue-culture vaccines in humans (Bogel and Meslin, 1990;

Zinsstag et al., 2007; Zinsstag et al., 2009). It has been stated by the World Organization for Animal Health (OIE) that just 10% of the total cost currently spent on intensified rabies vaccination in humans would be sufficient to enable the National Veterinary services throughout the world to eliminate rabies reservoir in domestic animal populations and prevent rabies cases in humans (Vallat, 2011). Interventions currently practised in Asia focussing solely on rabies prevention in humans will have no impact on reducing infection in the maintenance host, and thus rabies elimination in dogs is the key for reducing human rabies incidence and recurrent costs (Zinsstag et al., 2009; Lembo et al., 2011; Vallat, 2011).

Rabies Surveillance

Systematic and effective rabies surveillance systems are lacking in many Asian countries and rabies diagnoses in both humans and animals are mostly made based on clinical signs, history of exposure and epidemiological information (Sudarshan et al., 2007; Ly et al., 2009; Wu et al.,2009; Hossain et al., 2011; Fooks et al., 2009) with the exception of few countries (such as Thailand) that have good diagnostic facilities. Although the FAT is the 'gold-standard' for rabies diagnosis, recommended by both the World Health Organization and the World Organization for Animal Health (OIE; Dean et al., 1996; OIE, 2011), emerging technologies for the detection of rabies virus are available that are cheap, sensitive and can be used in the field condition (Fooks et al., 2009). For example, a direct Rapid Immunohistochemical Test (dRIT) can detect rabies antigen (by direct staining of fresh brain impressions) within 1 hour and has been found to be 100% sensitive and specific compared with the standard FAT (Lembo et al., 2006; Fooks et al., 2009). In addition the Rapid Immunodiagnostic test (RIDT) – which works based on the principles of immunochromatography – can also detect rabies virus antigen from brain samples (Fooks et al., 2009; Servat et al., 2012). Dog saliva samples can also be tested for rabies virus by the RIDT method using an immunochromatographic test strip and can be used outside the laboratory as an on-site testing assay with results available within 10 min (Kang et al., 2007; Nishizono et al., 2008; Fooks et al., 2009; Kasempimolporn et al., 2011). Therefore, rabies surveillance systems in Asia (or any canine rabies endemic country) can be improved using these new diagnostic tests performed as routine procedures under field conditions at greatly reduced cost (Lembo et al., 2006; Fooks et al., 2009).

2.7. Conclusions

Rabies remains a serious zoonosis and causes considerable loss of human life and economic impacts in Asia. Despite several limitations, rabies elimination is achievable in Asia, if synchronized campaigns aiming to eliminate rabies are undertaken in the same spirit as in Latin America (Belotto et al., 2005; Schneider et al., 2007) and in other countries that have brought rabies under control through mass dog vaccination and dog population control. For example, there are no insurmountable constraints to canine rabies control in Africa, as has been originally believed: elimination of canine rabies is epidemiologically and practically feasible through implementation of mass dog vaccination (Lembo et al., 2010). African nations have a similar canine rabies problem as in Asia and have similar constraints to rabies control. Rabies control in Asia should follow the 'WHO operational strategic guidelines for the control and elimination of rabies in Asia' (WHO, 2001), and by implementing the recommendations of an international consortium of rabies experts with renewed global collaborative vigour (Lembo et al., 2011). A critical issue is the funding of such control programs in Asia. International agencies are likely to fund rabies control and elimination programs, but only if such programs are shown to be cost-effective. Thus, a barrier is the lack of available information of the cost-effectiveness of controlling rabies in the reservoir – in the case of Asia, dog populations – in comparison to the funds spent treating human victims of dog bite. Defining the cost of rabies to society and communicating this information to decision-makers might be the key to achieving rabies control and potential elimination in Asia.

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CHAPTER 3

3. PATTERNS OF RABIES OCCURRENCE IN BHUTAN BETWEEN 1996 AND 2009

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CHAPTER 3

Patterns of Rabies Occurrence in Bhutan between 1996 and 2009

Abstract

This study was conducted to evaluate incidence and seasonal trends of rabies in dogs and other domestic animals in Bhutan from 1996 to 2009. Time series analysis approach was used to determine the seasonal trend and temporal association between species-specific rabies cases in animals. A total of 814 rabies cases were reported during the 14-year period, of which cattle and domestic dogs accounted for 55% (447/814) and 39% (317/814) of the cases, respectively. The remaining 6% of the cases (50/814) were reported in horses (2%), cats (2%), pigs (1%) and goats (1%). Rabies cases were reported throughout the year with more reports during spring and summer months. The annual patterns of cases were stable from 1996 to 2005, but the incidence increased during 2006 and 2008. Fifty-nine of the 205 sub-districts reported rabies in animals from 1996 to 2009 with increased incidences in the four districts in southern Bhutan, an area located close to the border towns of India. A significant ($P < 0.05$) positive cross-correlation was observed between the number of cases in dogs and other domestic animals at time lags (months) 1–3 with the highest correlation ($r = 0.94$, $P < 0.05$) observed at time lag 0 (same month) indicating that the peak in rabies incidences occur in the same month when both dogs and other domestic animal cases are reported. Regression analysis predicted rabies in other domestic animal when there are reports of rabies in dogs during the previous months. This study provides useful information about the epidemiology of rabies that can be used to plan a rabies control programme in Bhutan.

Keywords: Rabies; domestic animals; surveillance; time series analysis; Bhutan

Impacts

- This study evaluated rabies incidence and seasonal trends in domestic animals in Bhutan from 1996 to 2009.
- Rabies cases in animals were reported throughout the year with a higher incidence during spring and summer months. The number of reported cases was stable from 1996 to 2005 but increased in 2006 and 2008.
- Fifty-nine of the 205 sub-districts reported rabies between 1996 and 2009, with a higher incidence in southern Bhutan. There was a positive temporal correlation between the number of cases reported in dogs and other domestic animals.

3.1. Introduction

Rabies is an acute viral disease that affects the central nervous system, causing encephalitis and ultimately death in all warm-blooded animals including humans. Rabies is caused by a negative-stranded RNA virus within the *Lyssavirus* genus of the *Rhabdoviridae* family and is transmitted by the bite of rabid animals via saliva rich in the virus (Kaplin et al., 1986). Rabies occurs in two epidemiologic forms: urban rabies, with domestic dogs as the main reservoir and transmitter; and sylvatic rabies, with wildlife as the reservoir and transmitter of the disease (DeMattos et al., 1996). Urban rabies is endemic in most parts of the developing countries of Asia and Africa, and in Latin American and Caribbean countries, whilst North America and Europe have wildlife rabies (Finnegan et al., 2002; Belotto et al., 2005; Bourhy et al., 2005; Knobel et al., 2005). Urban dog rabies has been largely eliminated in Europe, North America and some Asian countries (Japan, Malaysia, Taiwan, Singapore) by massive dog vaccination, stray dog reduction programmes and enforcement of dog regulations (Finnegan et al., 2002; Belotto et al., 2005; Wilde et al., 2007; Takahashi-Omoe et al., 2008). Worldwide, rabies causes an estimated 55 000 human deaths annually, mostly in Asia and Africa because of endemic dog rabies and lack of healthcare and control measures (Knobel et al., 2005; Wilde et al., 2007).

Bhutan is a small Himalayan kingdom, located in South Asia between China and India, where rabies is highly endemic in both countries (Sudarshan et al., 2007; Si et al., 2008; Wu

et al., 2009). Rabies was prevalent in most parts of Bhutan until the early 1990s but has been controlled by restrictive elimination of dogs (Owoyele, 1992; Tenzin et al., 2010). Currently the disease is endemic in the southern districts of Bhutan along the border with India (Rinzin et al., 2006; Tenzin et al., 2010). Frequent outbreaks of rabies are occurring in these endemic areas affecting mostly domestic animals such as cattle and dogs (Kuensel, 2010a). Recently, outbreaks have been reported in some previously free areas in the interior as well as in southern Bhutan, indicating re-emergence of rabies in the country (Tenzin et al., 2010). Sporadic human deaths from rabies are also reported in the south rabies endemic districts of Bhutan. For instance, eight human deaths (mostly children) were reported between 2006 and June 2010, accounting for about 1.2 deaths per 100 000 population (Kuensel, 2009, 2010b; MoH, 2010). The people who are exposed to dog bites and presumed rabid animals are provided post-exposure prophylaxis free-of-charge in the hospitals, but some individuals may have failed to receive prompt treatment after exposure, resulting in death. Mass vaccination and sterilization of dogs are conducted annually in Bhutan but the coverage has been low (<20%) due to inadequate resources and rapid increase of the dog population (n = >50 000), especially free-roaming dogs (NCAH, 2007). The translocation and trans-border movement of free-roaming dogs could also explain the persistency and high incidences of rabies in southern Bhutan (Kuensel, 2010a). However, in an effort to control the free-roaming dog population and rabies in Bhutan, a nationwide dog vaccination and sterilization programme is under implementation in collaboration with an international organization – Humane Society International (MoA, 2009). Rabies is a notifiable disease in Bhutan and surveillance data for animal rabies are stored in the Veterinary Information System (VIS) database. However, no detailed analyses have been conducted on this surveillance data to describe epidemiology of rabies in Bhutan. To combat rabies successfully, a clear understanding of the disease epidemiology is required. In this study we analysed rabies surveillance data (1996 to 2009) to identify spatio-temporal patterns of reported rabies in animals and to evaluate the association between reported rabies cases in dogs and other domestic animals in Bhutan.

3.2.Materials and Methods

3.2.1. Data source

The data for this study were extracted from the national rabies surveillance database – Veterinary Information System (VIS) – maintained at the National Centre for Animal Health in Bhutan. The data contained both clinical and laboratory confirmed reported cases of rabies in animals. Brain tissue samples are collected from the field, preserved in 50% glycerine saline and sent to the laboratory for confirmatory diagnosis using only fluorescent antibody test (FAT) (Dean et al., 1996), due to constraints within the existing surveillance system. Note that for logistical reasons, field samples from all cases are usually not collected and submitted to the laboratory, particularly when at least one sample from the same herd or locality has positive laboratory confirmation. The remainders of the cases are then diagnosed by the veterinary officials based on the epidemiological investigation, history of dog bite and clinical signs consistent with rabies and subsequent death of animals (Tenzin et al., 2010). Rabies is not a difficult disease to diagnose (clinically) in endemic countries.

3.2.2. Data analysis

3.2.2.1. Descriptive and temporal analysis

Descriptive statistical analysis was performed to examine the frequencies and animal pattern of occurrences. The 14 years time series data were aggregated into monthly and yearly number of cases and time series plots were created to visualize possible trends and seasonality. The seasonal distribution was assessed by summing the frequency of cases into Bhutan's four seasons: (i) spring (March to May), (ii) summer (June to August), (iii) autumn (September to November) and (iv) winter (December to February). The expected numbers of cases were calculated under a null hypothesis that rabies incidence was independent of season in Bhutan. Observed and expected numbers were compared by chi-squared test to evaluate the association between season and rabies incidence (Kim et al., 2006).

3.2.2.2. Spatial analysis

A Geographic Information System (ArcGIS 9.3; ESRI, Redlands, CA, USA) was used to visualize the distribution of rabies in Bhutan. Case reports in any species of animals (cattle, dogs, cats, horses, pigs and goats) were aggregated at the sub-district level and thematic choropleth maps were produced demonstrating 2-yearly (1996 to 2009) distribution of rabies in Bhutan. Since rabies is mainly reported from the four southern districts (Sarpang, Chhukha, Samdrup Jongkhar and Samtse) of Bhutan, the frequencies of cases in each district were summed by year to examine the annual trend of cases in these sub-districts.

3.2.2.3. Cross-correlation analysis

The numbers of cases in dogs and other domestic animals were aggregated into monthly series of cases (n = 168 months) to evaluate the relationship between reported cases in dogs and other domestic animals by cross-correlation functions (Diggle, 1990). The cross correlation coefficients were estimated for up to 6 month lag windows (sufficient to cover the average incubation period of rabies) (Courtin et al., 2000). The approximate standard errors for the cross-correlation coefficients were calculated on the assumption that the series are not cross-correlated and that one of the series is white noise (Hartnack et al., 2009). The analysis was performed using the Applied Statistical Time Series Analysis software program (ASTSA) version 1.0 (Shumway, 1988).

3.2.2.4. Autoregression analysis

Since the times series of reported rabies cases in dogs and other domestic animals was positively cross-correlated, we performed autoregression to determine if a value in the time series of cases of rabies in dogs at time t could be predicted by cases at a previous time (*t–1, t–2...t–p*) (Wheelwright and Marridakis, 1980; Shumway, 1988; Allard, 1998).

An autoregressive model is of the form: $X_t = \delta + \varphi_1 X_{i-1} + X_{i-2} \ldots, + X_{i-p} + w_t$, where X_t is the time series, δ is the constant or intercept, φ_1 is the autoregressive model coefficient, X_{i-1} is the previous observation at time *(t–1)* and *w(t)* is the random error.

The best-fitting autoregressive model describing the number of rabies cases in dogs reported each month was chosen based on the goodness-of-fit criterion (Akaike's corrected information criteria, AICc). The selected model residuals were checked by examining the autocorrelation function (ACF) and partial autocorrelation function (PACF) plots for evidence of stationary (lack of trend or patterns) and for constant variance, independence and randomness (Shumway, 1988). A *t*-statistic was used to test the significance of estimated model coefficients.

Similarly, the best-fitting predictive model of cases in other domestic animals was fitted by a multiple regression model using reported cases in other domestic animals as the dependent variable and reported cases in dogs at some previous time lag *(t–1, t–2,..., t–p)* as the predictor variables. Akaike's corrected information criteria statistics and the residual test (as described above) were used to select the best-fitting model. A *t*-statistic was used to test the significance of estimated model coefficients (Shumway, 1988). The analysis was performed in ASTSA version 1.0.

3.3. Results

3.3.1. Descriptive analysis

From 1 January 1996 to 31 December 2009, a total of 814 rabies cases in dogs and other domestic animals were reported in Bhutan. The cases were most commonly reported in cattle (55%, 447/814) and dogs (39%, 317/ 814), with only a few cases in other species of animals (horses 17/814; cats 14/814; pigs 13/814; and goats 6/814 (Table 3.1). A total of 332 brain tissue samples were tested in the laboratory, of which 234 (70%) were confirmed positive by the FAT (Table 3.1). However, the majority (71%) of the reported cases were diagnosed based on clinical signs.

Table 3.1: Total number of reported rabies cases and the percentage of laboratory confirmed cases in different species of animals (1 January 1996 – 31 December 2009) in Bhutan (see explanation under Materials and methods section 3.2.1).

Species	Total number of reported cases	Percentage of cases in different animal species	Number of laboratory confirmed cases	Percentage of laboratory confirmed cases	Number of cases diagnosed based on clinical signs
Cattle	447	54.91	170	38.03	277
Dogs	317	38.94	45	14.20	272
Pigs	13	1.60	9	69.23	4
Cats	14	1.72	6	42.86	8
Goats	6	0.74	3	50.00	3
Horses	17	2.09	1	5.88	16
Total	814	100	234	28.75	580

3.3.2. Temporal pattern

Rabies cases in animals in Bhutan were reported throughout the year, with greater numbers reported during spring and summer months (Figure 3.1). The reported cases were above expected values during spring and summer months and below expected values in autumn months ($\chi^2 = 113.89$; $P < 0.001$) (Table 3.2).

Table 3.2: Seasonal distribution of reported rabies cases in animals in Bhutan (1 January 1996 – 31 December 2009)

Season (month)	Observed cases	Chi-square
Spring (March–May)	292	38.48
Summer (June–August)	232	3.99
Autumn (September–November)	83	71.35
Winter (December–February)	207	0.06
Total	**814**	**113.89***

* $\chi^2 = 113.89$; df =3; $p<0.001$

The annual trend of rabies cases reported in dogs and other domestic animals is illustrated in Figure 3.2. The number of reported cases was almost stable from 1996 to 2005 but increased in 2006 and 2008 ($P < 0.001$).

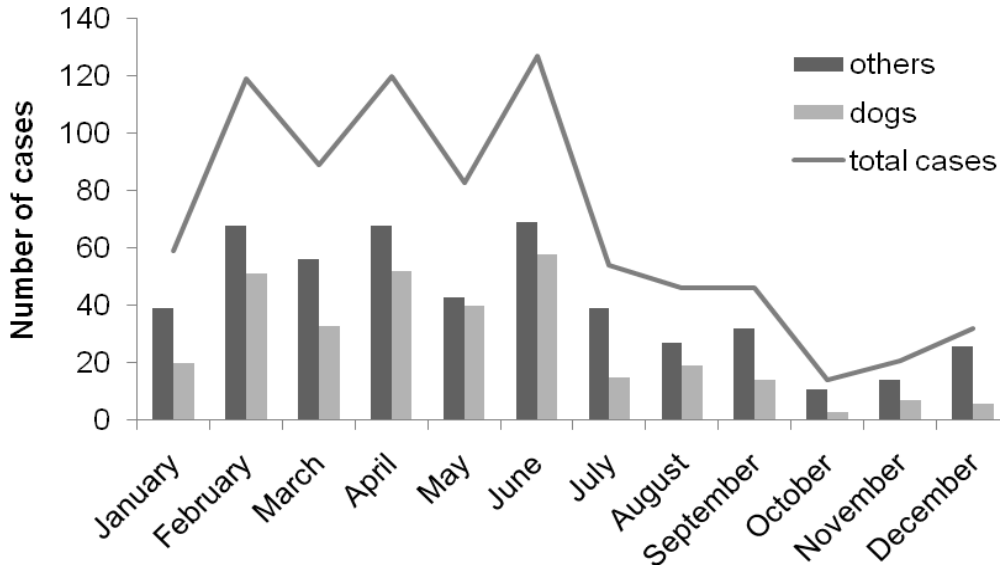

Figure 3.1: Monthly distribution of reported rabies cases in animals in Bhutan between 1 January 1996 and 31 December 2009.

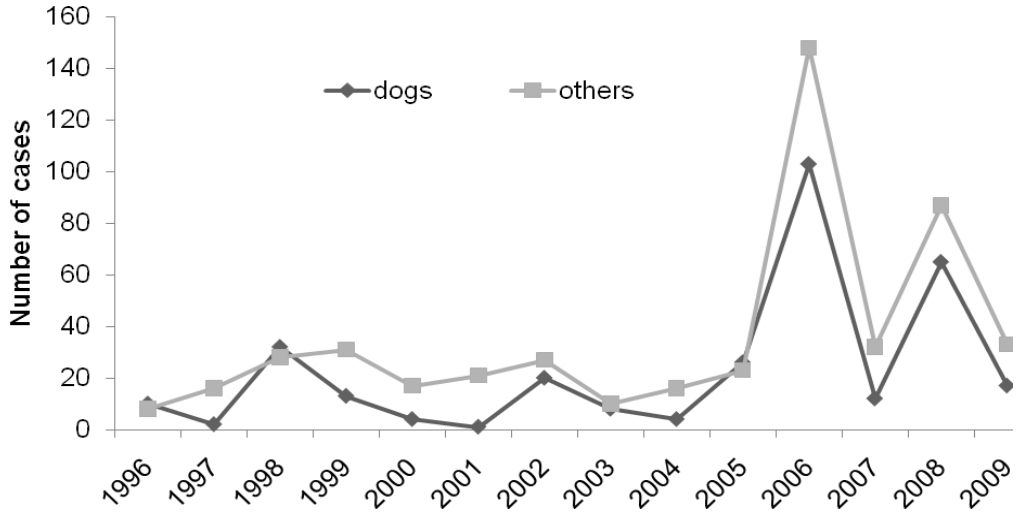

Figure 3.2: Annual trend of reported rabies cases in animals in Bhutan between 1 January 1996 and 31 December 2009.

3.3.3. Spatial pattern

Figure 3.3 shows the spatial distribution of reported rabies cases in different sub-districts in Bhutan. Of the 205 subdistricts, 59 sub-districts (29%) reported rabies in animals from 1 January 1996 to 31 December 2009. Some subdistricts reported rabies for all 14 years while others reported once and never reported again. The sub-districts that did not report subsequent outbreaks are located away from the border areas (see Figure 3.3). In general, rabies outbreaks were commonly reported in the four districts (Sarpang, Samdrup Jongkhar, Chhukha and Samtse) of southern Bhutan that share a border with India (Figures 3.3 and 3.4). Sarpang (36%) and Chhukha (36%) reported the highest number of cases followed by Samdrup Jongkhar (16%) and Samtse (13%) districts. The annual pattern of rabies cases in the four sub-districts between 1996 and 2009 is illustrated in Figure 3.4. On visual examination of the time series plots (Figure 3.4), Samdrup Jongkhar district reported a stable number of cases during 2003 to 2009, whereas the other three districts (Sarpang, Chhukha and Samtse) reported slightly increasing numbers of cases during this period. Chhukha district reported the highest number of cases in 2008.

3.3.4. Cross-correlation analysis

A significant ($P<0.05$) positive cross-correlation was observed between the number of cases in dogs and other animals at lags of 1–3 months with the highest cross-correlation ($r = 0.94$, $P<0.001$) at lag 0 (Table 3.3). This indicates that the peak in rabies incidences occur in the same month when both dogs and other domestic animals cases are reported. It also suggests that the report of rabies in dogs tends to precede the report of cases in other domestic animals.

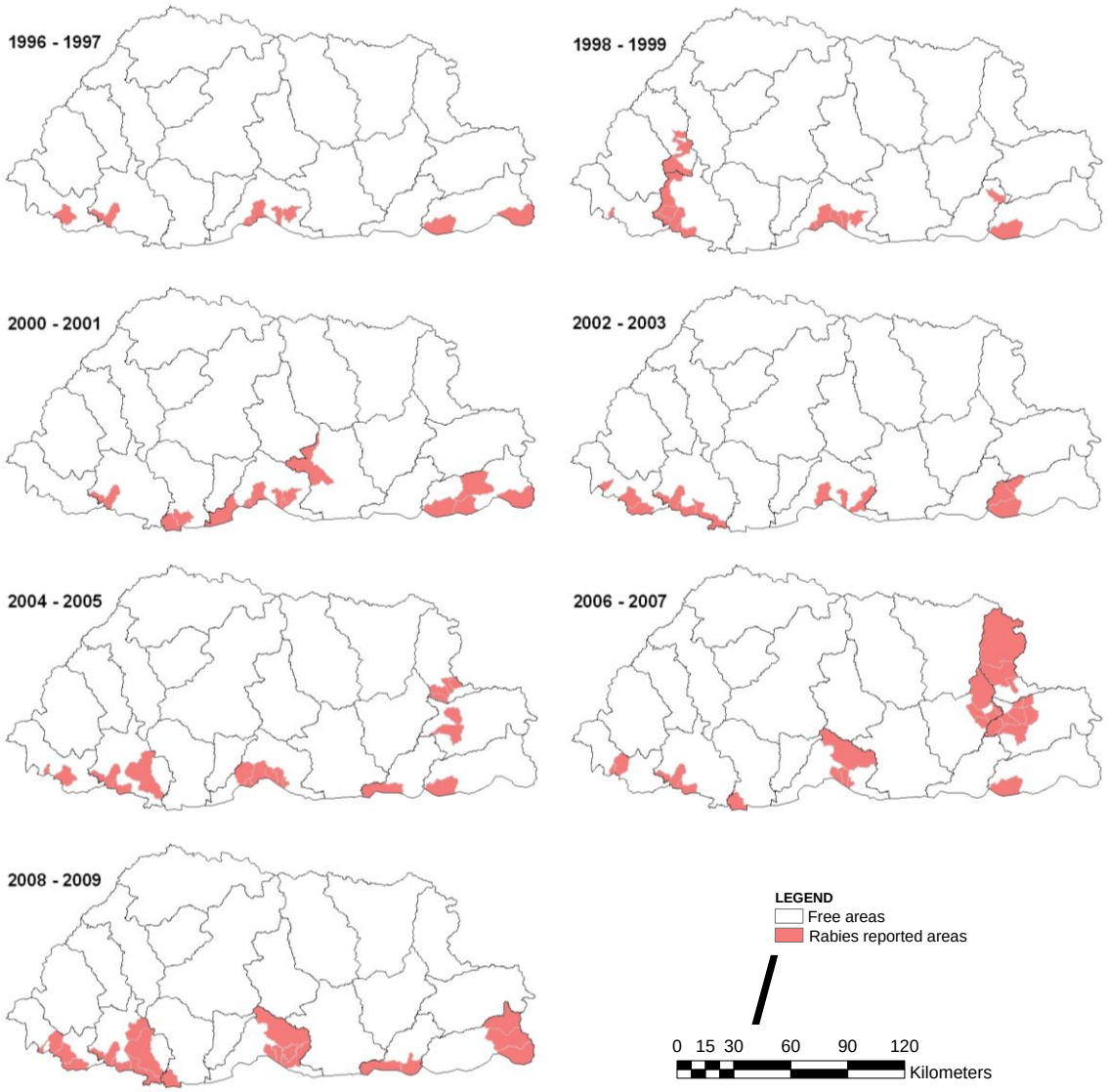

Figure 3.3: Geographical distribution of reported rabies cases in animals in different sub-districts of Bhutan between 1 January 1996 and 31 December 2009

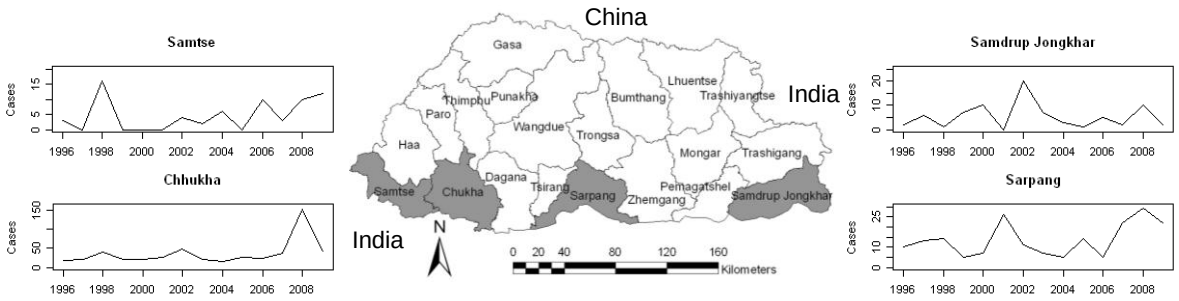

Figure 3.4: District map of Bhutan showing the four southern districts (shaded) that are highly endemic to rabies and their annual trend of reported rabies cases in domestic animals between 1 January 1996 and 31 December 2009.

Table 3.3: Cross-correlation between cases of rabies in dogs and other domestic animals reported between 1 January 1996 and 31 December 2009 in Bhutan, lagged by 0–6 months. 95% significance is r > 0.1508 and is in bold

Lag in months	CCF (r)
0	**0.935**
1	**0.542**
2	**0.417**
3	**0.215**
4	0.140
5	0.080
6	0.023

95% significance is r>0.1508 and is in bold, cross correlation function (CCF)

3.3.5. Autoregression analysis

The best-fitting (AICc = 5.045 R^2 = 0.363) autoregressive model of dog rabies cases included rabies cases reported in dogs during the previous *1–2* months indicating that an increase of one dog case at month *t–1* and *t–2* predicted an increase of 0.52 cases and 0.21 cases in dogs at month *t* (Table 3.4). The fitted autoregressive model was:

Dog rabies = 2.04 + 0.52dog[t–1] + 0.21dog[t–2] + w[t]

Table 3.4: Best fitting (AICc = 5.045 R^2 = 0.363) autoregressive model of dog cases of rabies reported between 1 January 1996 and 31 December 2009 in Bhutan

Variable	Lag (months)	*b*	S.E	*t*	*P−value*
Constant	–	2.038	0.685	2.973	0.003
dog cases	[–1]	0.523	0.077	6.734	0.000
dog cases	[–2]	0.209	0.086	2.428	0.015

The ACF and PACF of the residuals showed autocorrelation (r > 0.15) at lag 27 months. The residuals appeared to have constant variance and were independent without any fluctuation and outliers.

The final best-fitting (AICc = 3.919, R^2 = 0.31) regression model to predict the cases of rabies in other domestic animals was found to be the report of rabies in dogs during the previous 1 and 2 months indicating that an increase of one dog case at month *t–1* and *t–2* predicted an increase of 0.250 cases and 0.086 cases in other domestic animals at month t (Table 3.5). The fitted model was:

Rabies in other domestic animals = 1.325 + 0.25 dog [t–1] + 0.086 dog [t–2] + $w_{(t)}$.

Table 3.5: Best fitting (AICs = 3.919, R^2 = 0.31) regression model of other domestic animals cases of rabies reported between 1 January 1996 and 31 December 2009 in Bhutan

Variable	Lag (months)	*b*	S.E	*t*	*P-value*
Constant	–	1.325	0.382	3.466	0.000
dog cases	[–1]	0.250	0.044	5.688	0.000
dog cases	[–2]	0.086	0.044	1.957	0.050

The ACF and PACF of the residuals showed autocorrelation (r > 0.15) at lag 28 months. The residuals had constant variance and were independent without any fluctuation and outliers.

3.4.Discussion

This study describes the temporal and spatial distribution of reported animal rabies in Bhutan. The data showed that most of the reported cases are in dogs and cattle but the

number of cases in cattle exceeded that in dogs and other animals. This could be due to either under reporting of cases of rabies in free-roaming dogs or a single rabid dog might have infected many cattle during outbreaks. In Bhutan, cattle are grazed in open fields and come into contact with free-roaming dogs, increasing the risk of dog bites. Cases in free-roaming dogs are more likely to have been underreported than cases in cattle due to difficulties in tracing the cases in dogs. Moreover, rabid dogs (especially strays) are difficult to trace due to trans-border movement in the south border towns. Cases in livestock would have been captured by the reporting system, perhaps because of the greater economic value of cattle: farmers often report the illness of cattle to veterinary officials for treatment or investigation.

Rabies cases were reported throughout the year with more cases reported during spring and summer months (Figure 3.1 and Table 3.2). This finding is in agreement with a previous report in Bhutan in which more rabies outbreaks were reported during February to June compared to other months (Rinzin et al., 2006). Increased incidence of rabies during spring and summer months may be associated with the breeding season of dogs. It has been reported that the dog-breeding season is associated with increased contact rates between dogs, leading to frequent fights and increases the risk of virus transmission (Malaga et al., 1979; Ezeokoli and Umoh, 1987; Mitmoonpitak et al., 1998; Panichabhongse, 2001; Hampson et al., 2007). Malaga et al., (1979) have also suggested that the seasonality of canine rabies could be due to changes in the age structure of the susceptible dog population following the breeding season, when large numbers of puppies enter the population and are present in the streets, increasing the risk of rabies virus transmission. Study in Thailand revealed that 14% of rabid dogs were <3 months old and 42% were <6 months old suggesting that young dogs were a risk factor for rabies transmission (Mitmoonpitak et al., 1998). Nevertheless, the seasonality of rabies in wildlife (including foxes, skunks and raccoons) elsewhere has clearly been shown to follow a marked seasonal pattern due to their highly seasonal breeding pattern and strong seasonal territorial instincts (Pool and Hacker, 1982; Gremillion-Smith and Woolf, 1988; Wandeler and Bingham, 2000; Harnos et al., 2006; Zienius et al., 2007). Further studies may be necessary to substantiate the seasonality of rabies in Bhutan by establishing a proper surveillance and reporting system for rabies in free-roaming dogs. This would provide information for better planning of vaccination programmes in dogs. Currently in Bhutan,

vaccination of free-roaming dog is usually carried out in conjunction with a sterilization programme during the cooler months of the year to avoid double catching of the dogs and post-operative complications during the hot summer months. In Thailand, the mass dog vaccination campaign is generally scheduled prior to the dog breeding season (Mitmoonpitak et al., 1998).

There was no significant change in the reported number of rabies cases from 1996 to 2005, but peaks in 2006 and 2008 were observed. A series of rabies outbreaks that occurred in the east, southeast and southwest of Bhutan in recent years might be associated with these increases (Tenzin et al., 2010). Although the number of reported cases remained almost constant over the study period, the frequencies of outbreaks (data not shown) and the area of spread have increased (see Figure 3.3). For instance, places or sub-districts that were previously free of rabies have reported outbreaks recently (see Figure 3.3) (Tenzin et al., 2010). This increased incidence may be due to higher free-roaming dog population densities and the absence of a sustained vaccination programme (low vaccination coverage: <20%) resulting in the maintenance of rabies endemicity in the border areas of Bhutan, as observed elsewhere (Beran and Frith, 1988; Cleaveland and Dye, 1995; Kitala et al., 2002). It may also be due to translocation and trans-border movement of infected free-roaming dogs in southern Bhutan. Further studies are necessary to understand in detail the transmission dynamics of rabies in southern Bhutan.

We also observed a significant temporal correlation between reported cases in dogs and other domestic animals, wherein the report of cases in dogs predicts cases in other domestic animals. The relationship is biologically plausible since dogs are the reservoir and vector of rabies virus transmission to other livestock species in canine rabies endemic countries. Unlike in other countries (Courtin et al., 2000; Milius et al., 2004; Vos et al., 2009) no other intervening species (such as wildlife) have been found to be involved in the epidemiology of rabies in domestic animals, including in dogs in Bhutan (Tenzin et al., 2010). To date, no wildlife rabies cases have been reported or confirmed in Bhutan, and dogs have been the source of spillover infections of cattle, other domestic animals and sporadic infections in humans. Furthermore, the association of rabies in dogs in the previous months with the

observation of rabies incidences in other domestic animals suggests the need for enhancing rabies surveillance in the event of rabies occurrence in dogs. Similar results were obtained in a previous investigation in Namibia, in which black-backed jackals predicted rabies in dogs and domestic ruminants (Courtin et al., 2000). The association between cases in dogs and other domestic animals in Bhutan could be substantiated by conducting monoclonal antibody testing and genetic typing of the virus. Such techniques would reveal the source and transmission dynamics of rabies virus infections between reservoir and dead end hosts (such as domestic livestock).

There are some limitations in this study. The data that we used are surveillance data collected by different units and submitted to the VIS database. Although there has been no dramatic change in the surveillance system for rabies over the years, some minor differences in the reporting system from the different rabies outbreak areas may have occurred. For instance, more detailed investigation and follow-up of cases are expected to have been carried out during the period of major outbreaks than during that of the sporadic occurrence of cases. This may be also one of the reasons for observing in our analysis a strong correlation between reported rabies cases in dogs and other domestic animals at lag 0 (same month). Some underreporting of cases could have also occurred due to lack of awareness among farmers, or due to the remote location of some outbreaks. However, we assume that underreporting remained relatively constant during the study period.

It is also important to consider proper sampling and rabies laboratory diagnostic procedures being followed in Bhutan. Brain tissue samples are collected in the field, preserved in 50% glycerine saline and submitted to the laboratory for confirmatory diagnosis using the FAT (Dean et al., 1996). Because of the generally long distances from the field to the laboratory, the final confirmatory diagnosis takes some time (weeks) and it is likely that the diagnostic test is affected by the quality of the sample (McElhinney et al., 2008; Fooks et al., 2009). In some instances, the samples would be unfit for testing. All these limitations can result in poor test performance. However, rabies is not a difficult disease to diagnose (clinically) in endemic countries. Although the FAT is a standard test for rabies diagnosis, other rapid diagnostic field test kits would be useful in a country such as Bhutan so that a quick decision can be made in the field regarding implementation of control activities and also to advice people who have been potentially exposed to the disease about post-exposure treatment.

In summary, our analysis shows a stable trend in rabies reported in Bhutan from 1996 to 2005, with increased number of cases and area of spread from 2006 to 2009. There was also significant seasonal variation – increased incidences during spring and summer months. A significant correlation between rabies in dogs and other domestic animals was also demonstrated, suggesting the need to improve surveillance in the event of rabies outbreak in dogs. We recommend that the surveillance system should be improved, especially in free-roaming dog populations. Investigation of the existence of any rabies reservoirs in wildlife species should be undertaken. Because of the limited distribution of rabies in southern Bhutan, successful control and elimination of rabies is achievable by strict enforcement of dog vaccination rules and the dog population management programme.

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CHAPTER 4

4. RE-EMERGENCE OF RABIES IN DOGS AND OTHER DOMESTIC ANIMALS IN EASTERN BHUTAN, 2005–2007

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CHAPTER 4

Re-emergence of rabies in dogs and other domestic animals in eastern Bhutan, 2005–2007

Abstract

We report a major outbreak of rabies in dogs and other domestic animals that occurred in eastern Bhutan between May 2005 and November 2007. The outbreak peaked in February 2006 and subsided by the end of April 2006 with sporadic cases reported until November 2007. Rabies affected 18 of the 40 sub-districts in the three eastern districts of Bhutan. There were reportedly one human and 256 domestic animal fatalities. The outbreak affected cattle (n=141, 55%), dogs (n=106, 41%), horses (n=7, 3%) and cats (n=2, 1%). Rabies was primarily diagnosed by clinical signs but 36 cases were confirmed by fluorescent antibody test of brain samples. High densities and movements of free-roaming dogs might have been responsible for the rapid spread and persistence of the infection for a longer period than expected in dogs in eastern Bhutan.

Key words: Eastern Bhutan, epidemic, rabies outbreak.

4.1. Introduction

Rabies is an acute viral disease transmitted by the bite of a rabid animal. Each year, about 55,000 people die of rabies in Asia and Africa, mainly because of endemic canine rabies (Knobel et al., 2005). In Asia, a large number of human rabies deaths occur in India, and a national multi-centre epidemiological survey has estimated an annual burden of about 20 000 human deaths due to rabies in India (Sudarshan et al., 2007). Rabies has also been described as a re-emerging problem in China, with about 2000 human deaths reported annually (Sudarshan et al., 2007; Si et al., 2008; Wu et al., 2009). Bhutan is a small Himalayan kingdom situated in South Asia in between India and China. Until the early 1990s, rabies in dogs and other domestic animals was reported from most parts of Bhutan. A national rabies

control programme–vaccination of dogs (both free-roaming and pet dogs), implemented in 1992–controlled or eliminated canine rabies from the interior part of the country (Owoyele 1992; MoA, 2007. Since then, canine rabies has been endemic only in areas of southern Bhutan that share the porous border with India. Domestic dogs are the main reservoir of rabies in Bhutan, with spillover infection to other domestic animals, especially cattle. However, the reported incidence of rabies in humans is low and only sporadic cases occur in Bhutan (MoH, 2008)

Rabies is a notifiable disease in Bhutan and it is mandatory to report even suspected cases (RGoB, 2000). A thorough investigation is undertaken in the event of a suspected outbreak in animals. A fluorescent antibody test (FAT) – which is recommended by both the WHO and World Animal Health Organization (OIE) – is used to confirm rabies in animals (Dean et al., 1996; McElhinney et al., 2008; Fooks et al., 2009). The epidemiological data are collected and stored in the Veterinary Information System (VIS) database. As a control programme, vaccination and sterilization of dogs are conducted annually in the country focusing mostly on towns that have high, free-roaming dog densities. However, the programme coverage has been low (<20%) because of limited financial resources and the high population turnover in the free-roaming dog population (MoA, 2007).

In this report, we describe an outbreak of rabies in dogs and other domestic animals that occurred in the three eastern districts (Tashiyangtse, Trashigang, Mongar) of Bhutan (Figure 4.1a). Since the early 1990s, the villages and towns in these districts had been free from canine rabies and no wildlife-mediated rabies had ever been reported or confirmed in this region or elsewhere in Bhutan. However, between 2005 and 2007, rabies cases were reported in the region and therefore an outbreak was declared.

On 2 May 2005, the first case suggestive of rabies was reported in a cow at Gongza village, Toetsho subdistrict within Tashiyangtse district, located adjacent to the villages of Tawang district, Arunachal Pradesh state, India (see Figure 4.1b). Earlier, a suspected rabid, stray dog was sighted around a cattle herd but could not be traced subsequently. Following this incident, dog-bite cases in cattle and horses were reported in the adjacent villages of the

index case. By September 2005, the disease apparently spread into the urban centres and villages of Trashigang district, located about 30 km to the south of the index case (see Figure 4.1) and then further spread into the villages and towns of five sub-districts of Mongar district, located next to Trashigang. There was a chain of spread from Tashiyangtse to Trashigang and then to Mongar districts. Epidemiological investigation and the typical clinical signs manifested by affected animals led to suspicion of rabies which was later confirmed by laboratory testing. Of the 51 brain samples (40 cattle, 11 dogs) collected from the carcases in the affected villages, 36 (70%) samples (30 cattle, 6 dogs) were confirmed to be rabies virus positive by FAT. Samples from all suspected cases were not collected due to logistics and to avoid accidental rabies virus exposure of the people handling carcases during sample collection. When one or more samples from the infected areas were confirmed to be positive, other suspected cases showing similar clinical signs were diagnosed as rabies. Observations suggested an incursion of rabies virus and subsequent local diffusion through the susceptible dog population, with spill-over over particularly to cattle.

4.2.Materials and methods

4.2.1. Data analysis

The epidemiological data – day, month and year of occurrence; species of animals affected (cattle, horses, dogs, cats); and location (village, sub-district, district) of outbreaks were acquired. An epidemic curve was constructed for the whole study region by counting the total number of cases reported per month between May 2005 and November 2007, with the number of cases on the y–axis and the month on the x–axis. The cumulative incidence of rabies was calculated as the proportion of the population at risk that were diagnosed as rabies between 2005 and 2007. The animal census data of 2005 was used as the denominator to calculate the incidence risk for cattle, horses and cats. Since accurate dog population data for the subdistricts of the three districts were not available, we estimated the dog population based on the mean subdistrict human population using ratios proposed and validated by Knobel et al. (2005) in Asia, namely 9.5 humans to one dog (95% CI 4.5:1–14.6:1).

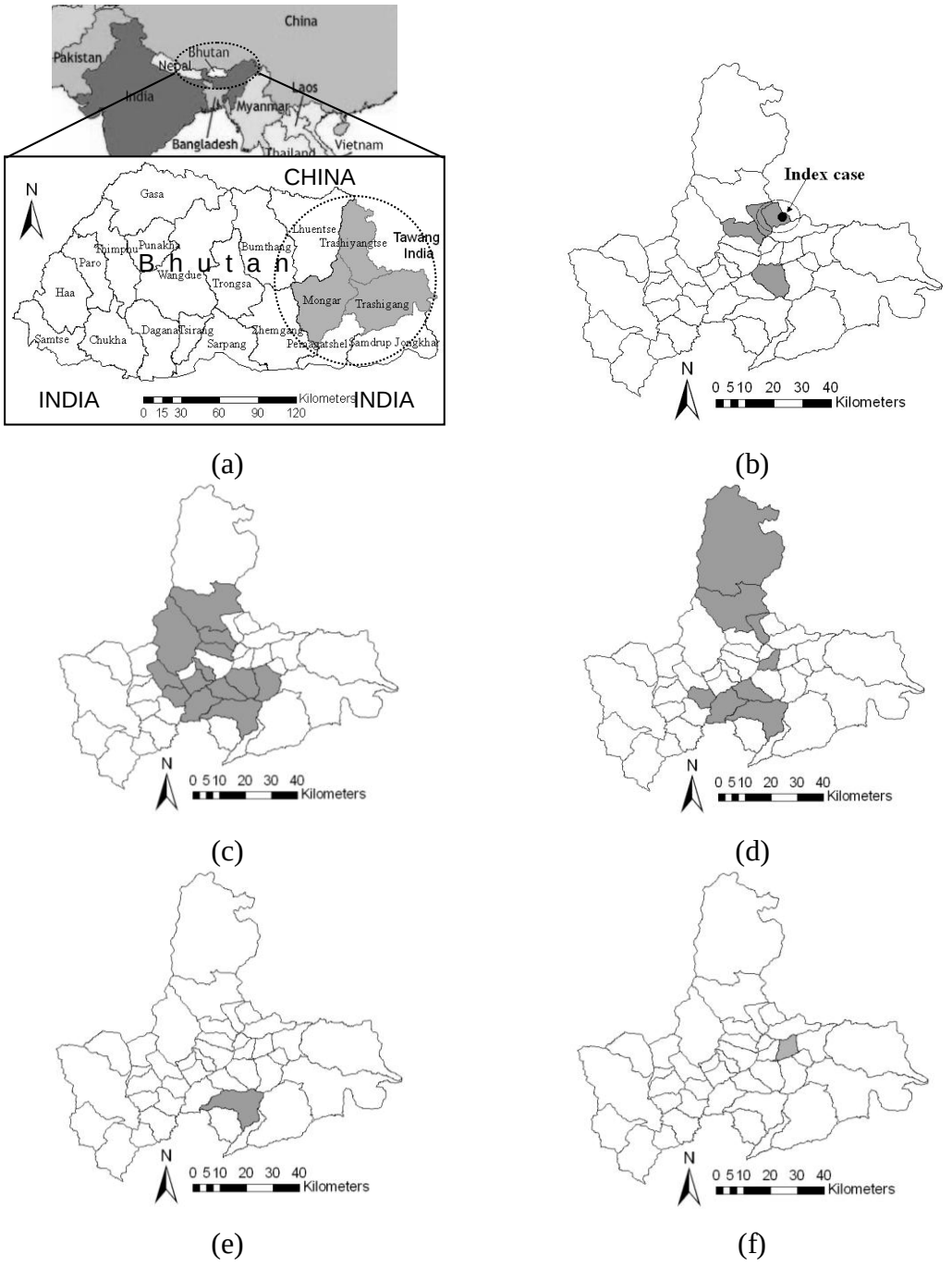

Figure 4.1: Map of South East Asia with an administrative map of Bhutan and the three eastern districts shaded; (a) inset. The monthly report and spread of rabies cases in each sub-district of eastern Bhutan between May 2005 and November 2007 is shown. (b) May 2005 to October 2005 [the index case (●) is indicated]. (c) November 2005 to April 2006. (d) May 2006 to October 2006. (e) November 2006 to April 2007. (f) May 2007 to November 2007 (the last case was reported in November 2007).

These authors calculated the ratios based on the available published reports of field surveys conducted in many Asian countries. The estimated dog population was then used to calculate the cumulative incidence of rabies in dogs. A Geographical Information System (ArcGIS 9.3. ESRI, CA, USA) was used to map and visualize the monthly reports and the spread of rabies in eastern Bhutan (Appendix 1).

4.3.Results

A total of 256 animal deaths [141 cattle, 106 dogs (38 pets, 68 free-roaming), seven horses and two cats] were reported between May 2005 and November 2007. Table 4.1 shows the cumulative incidence of rabies in cattle, dogs, horses and cats in the three districts. The animal pattern showed a much higher incidence risk in dogs compared to other species of animals.

The yearly species-specific incidence risk of rabies showed higher risk in all species of animals during 2006 than in 2005 and 2007 (Table 4.2) indicating increased transmission of infection during 2006. This is also evident from the epidemic curve (Figure 4.2).

Table 4.1: Cumulative incidence of rabies from May 2005 to November 2007, stratified by species and district, eastern Bhutan.

District	Species	Cases	Population	Incidence* (95% CI)
Mongar	Cattle	40	28158	14.2 (10.4−19.3)
	Dog	48	3902	123 (92.9−162.7)
	Horse	3	3121	9.6 (3.32−28.2)
Tashiyangtse	Cattle	28	12122	23.1 (15.9−33.3)
	Dog	27	1876	143.9 (99.1−208.5)
	Horse	2	1837	10.8 (2.9−39.6)
	Cat	2	1156	17.3 (4.7−62.8)
Trashigang	Cattle	73	37552	19.4 (15.4−24.4)
	Dog	31	5383	57.5 (40.6−81.6)
	Horse	2	3399	5.8 (1.61−21.4)

CI: Confidence interval

*Cumulative incidence, cases per 10000 population at-risk

Table 4.2: Cumulative incidence of rabies between May 2005 and November 2007, stratified by year and species in the eastern region of Bhutan.

Period	Species	Cases	Incidence* (95% CI)
May–December 2005	Cattle	20	2.5 (1.6–3.9)
	Dog	10	8.9 (4.8–16.4)
	Horse	2	2.3 (0.6–8.7)
January–December 2006	Cattle	118	15.1 (12.6–18.1)
	Dog	94	84.2 (68.8–102.9)
	Horse	5	5.9 (2.5–13.9)
	Cat	2	3.2 (0.8–11.8)
January–November 2007	Cattle	3	0.3 (0.1–1.1)
	Dog	2	1.7 (0.4–6.5)

CI: Confidence interval
* Cumulative incidence, cases per 10000 population at-risk

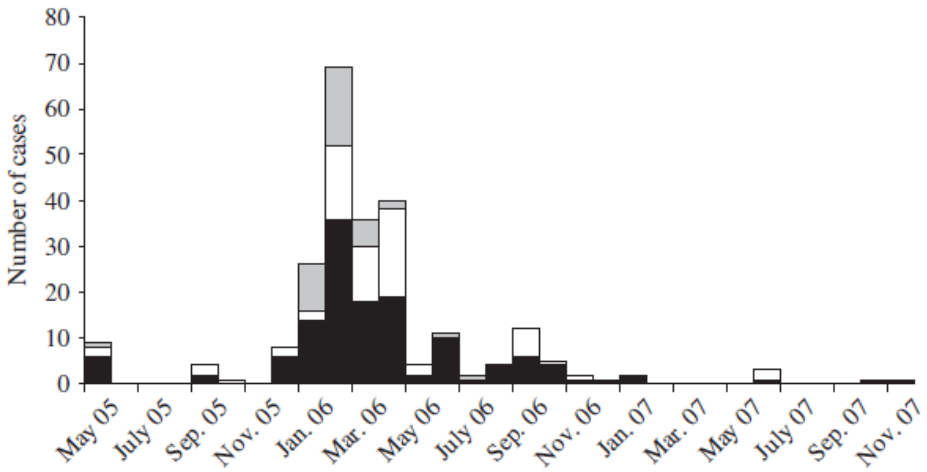

Figure 4.2: Epidemic curve of rabies cases in dogs and cattle in eastern Bhutan reported between May 2005 and November 2007. Cases in horses (n=7) and cats (n=2) are not shown because of the low numbers. ■ Cattle; □ free-roaming dogs; ▣, pet dogs.

Only one human death was reported during this epidemic, which accounted for 0.94 deaths/100 000 populations at-risk in the three districts. About 900 people were potentially exposed to rabies during the epidemic and they received 5–6 doses (Essen regimen) of rabies vaccination from the hospital. The maximum exposures were reported during 2006. The

majority of these people consumed dairy products from a suspected rabid cow or handled carcases of animals that died of rabies. However, it is not known how many of these people had actually been bitten by confirmed rabid dogs.

Figure 4.1 shows the monthly reports and spread of rabies in eastern Bhutan between May 2005 and November 2007. There was a rapid spread between January and April 2006 and a large number of subdistricts were affected during this period (Figure 4.1c). Some sub-districts reported persistence of infection for a longer period of time than other sub-districts. The rabies outbreak was eventually confirmed in 18 (45%) of the 40 sub-districts affecting 6/8, 7/15 and 5/17 sub-districts in Tashiyangtse, Trashigang and Mongar districts, respectively.

The proportion of sub-districts that reported rabies from May 2005 to December 2005, January 2006 to December 2006, and January 2007 to November 2007 were 15%, 35% and 7%, respectively. Both dog and cattle rabies was reported in all the sub-districts and almost at the same time, suggesting the role of dog bite in the transmission of disease to cattle (Figure 4.2). The source of the disease could not be established, although it is likely to have spread from across the border, since a rabies outbreak in dogs had been reported in Tawang during this period (NS Tamang, personal communication).

4.4. Discussion

The movement of infected free-roaming dogs is believed to have been responsible for the spread of infection in the region. The presence of a road network and the clustered settlement of villages and towns within the region could have facilitated the inter-mixing of dogs between the infected and non infected areas resulting in rapid spread and persistence of infection. However, landscape features such as mountains and rivers may have been physical barriers to the further movement of dogs and prevented spread into other areas in the region. For example some villages/sub-districts, although located close to infected villages, did not report rabies because they are separated by a major river.

A series of measures were taken to control the outbreak. A public awareness programme was conducted during 2005 and 2006, when the outbreak was ongoing. Consultative meetings and discussions were held with farmers and village leaders to manage the outbreak. About 5000 dogs (both pets and free-roaming dogs) in the region were vaccinated against rabies. In addition, about 900 free-roaming dogs were caught and impounded in 12 temporary shelters constructed in the three districts. Impounding was done during March and April, 2006 in an attempt to prevent the spread of disease by the movement of free-roaming dogs. They were kept for about 4 months in the shelters and were released in the vicinity of capture after vaccination and sterilization. Although impounding is not a recommended strategy for the control of rabies, it was used during this outbreak as an alternative measure to mass culling of dogs, in response to the religious (Buddhist) sentiment of people in the community. No rabies cases were reported or confirmed in the dogs in the shelters. Further studies are necessary to determine the beneficial or deleterious effects of impounding dogs during an outbreak. Although mass culling of free-roaming dogs was not implemented during this outbreak, rabid and some in-contact dogs were eliminated after people realized the seriousness of the disease (many people had lost their valuable animals because of rabies). Such restricted culling may have removed the reservoir of infection resulting in fade-out of the epidemic. A similar major rabies epidemic that occurred in Chukha district in the south western part of Bhutan between January and July 2008 was controlled by immediate culling of in-contact free-roaming dogs in the outbreak areas. This outbreak affected three sub-districts and resulted in the death of 42 cattle, 52 dogs and three horses. Another rabies outbreak that occurred during 2008 in Samtse district – which is located adjacent to Chukha district (Figure 4.1a) – was also controlled by immediate culling of in-contact free-roaming dogs in the outbreak areas.

The experiences and the review of this epidemic suggest either the lack of a clear rabies outbreak control strategy for a major outbreak or the failure to implement the strategy in eastern Bhutan. Effective control of any disease depends on clear policy guidelines and their implementation, with legal support as well as effective communication. Therefore, control of any rabies outbreak in the future should be aimed at eliminating the reservoir of infection by immediate culling of in-contact and unvaccinated free-roaming dogs in the outbreak areas.

An awareness programme on the public health importance of rabies is also necessary to promote good community participation in the control programme. As a preventive measure, sustained sterilization and vaccination of dogs with >70% coverage would induce herd immunity and eliminate rabies in canines (WHO, 2005). In addition, dog population management should be targeted towards habitat control by proper waste management and promoting a sense of responsible dog ownership in the community. Blanket mass culling or removal of dogs may not be a practical solution since it will be neither acceptable to the community (as evidenced from this outbreak) nor scientifically justifiable. We also recommend that the collection of good quality epidemiological data such as estimates of the dog population, combined with laboratory surveillance, would provide accurate information for the planning and management of rabies in Bhutan. In this report, the risk estimates in dogs could be biased due to errors in estimating the population at risk in the region or inclusion of non-rabies cases in the data. Therefore, attempts should be made to collect all suspected samples in any future outbreak for laboratory confirmation of the cases. In addition, since no genetic characterization was done during this outbreak, it is also important to study phylogenetic relationships in order to understand rabies virus variants circulating in Bhutan.

The analyses presented in this report are descriptive and provide a basis for further analytical investigations to explain the evolution of the epidemic and generate hypotheses for the spread of rabies in eastern Bhutan.

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Declaration of interest: None.

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CHAPTER 5

5. RE-EMERGENCE OF RABIES IN CHHUKHA DISTRICT, BHUTAN, 2008

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CHAPTER 5

Re-emergence of Rabies in Chhukha district, Bhutan, 2008

Abstract

From January through July 2008, rabies reemerged in the Chhukha district of southwestern Bhutan. To clarify the distribution and direction of spread of this outbreak, we mapped reported cases and conducted directional tests (mean center and standard deviational ellipse). The outbreak resulted in the death of 97 animals (42 cattle, 52 dogs, and 3 horses). Antirabies vaccine was given free of charge to ≈674 persons suspected to have been exposed to rabies. The outbreak spread south to north and appeared to follow road networks, towns, and areas of high human density associated with a large, free-roaming, dog population. The outbreak was controlled by culling free-roaming dogs. To prevent spread into the interior of Bhutan, a well-coordinated national rabies control program should be implemented in disease-endemic areas.

5.1. Introduction

Rabies is a fatal zoonosis caused by rabies virus or rabies-related viruses (genus *Lyssavirus*) and transmitted by the bite of a rabid animal (Cleaveland et al., 2006). Domestic dogs are the main (>95%) source of human rabies infection. An estimated 55,000 persons die of rabies in Asia and Africa each year (Knobel et al., 2005), >20,000 in India alone (Sudarshan et al., 2007). In Bhutan, rabies is endemic to the southern districts that border India (Rinzin et al., 2006; Tenzin et al., 2010). Domestic dogs are the main reservoir and are responsible for spillover infection of other domestic animals, especially cattle. Sporadic human deaths have also been reported in south-central and southwestern rabies-endemic areas of Bhutan (MoH, 2008; Kuensel, 2009).

On January 23, 2008, a clinical case of rabies in a cow in Dala, a subdistrict of the Chhukha district, was reported and later confirmed by fluorescent antibody test (Dean et al., 1996;

McElhinney et al., 2008). The cow had reportedly been bitten ≈3 weeks earlier by a stray dog with suspected rabies. On the same day, another case was reported (and later confirmed by fluorescent antibody test) in a stray dog in the town of Tshimalakha, Bjachho subdistrict. A retrospective epidemiologic field investigation found that an unreported rabies outbreak in dogs had occurred in the southern villages of Dala subdistrict during November and December 2007.

We report a rabies outbreak in the 3 subdistricts of Chhukha district, Bhutan: Dala, Bongo, and Bjachho (Figure 5.1). To help develop future control programs, our objectives were to 1) describe the spatio–temporal patterns of the outbreak, 2) generate hypotheses about rabies introduction and spread, 3) assess the relationship between animal rabies and public health, and 4) estimate the cost of the outbreak

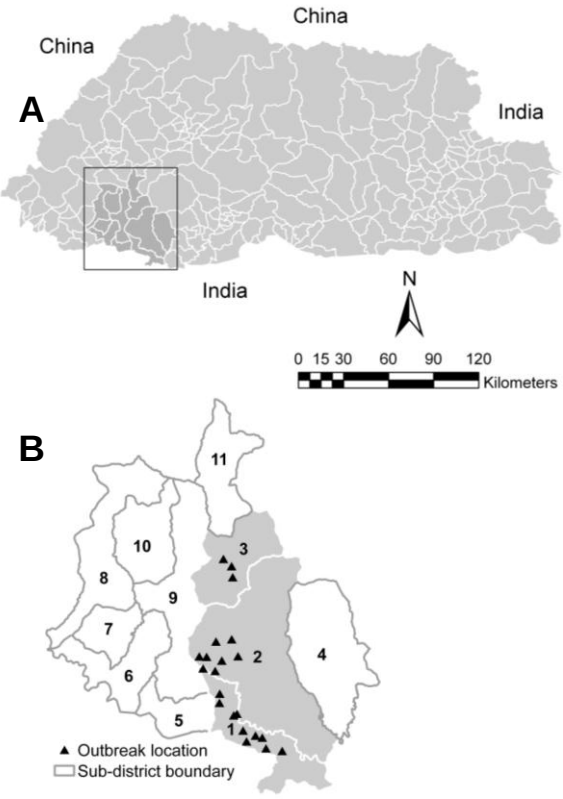

Figure 5.1: (A) Bhutan, with the Chhukha district enclosed. B) The 11 sub-districts of Chukha district: 1. Dala; 2. Bongo; 3. Bjachho; 4. Genata; 5. Sampheling; 6. Phuentsholing; 7. Logchina; 8. Dungna; 9. Geling; 10. Metap; 11. Chapcha. Dray shading indicates the study areas (1-3); the triangles (▲) indicate locations of rabies outbreaks.

5.2.Materials and Methods

5.2.1. Data Sources

Outbreak data were obtained from the Veterinary Information System database and included case date, number and species of animals affected, location (village X and Y coordinates), subdistrict, and date and type of intervention activities implemented during the outbreak. Data on number of human exposures, reasons, and type and number of postexposure prophylaxis doses administered were acquired from local hospitals. The study was conducted January 23–July 31, 2008.

5.2.2. Data Analysis

5.2.2.1. Animal Patterns

The attack rate (no. rabies cases/1,000 animals at risk) was calculated for the outbreak period (Smith 1995). Animal census data for 2008 or dog population data recorded during a vaccination campaign in 2006 in the main towns of Chhukha district were used to calculate attack rates (Table 5.1) (MoA, 2008). The Chi-square test was used to compare differences in dog and cattle attack rates among subdistricts. We tabulated the number of persons exposed in each subdistrict, number of persons who received postexposure prophylaxis, and reasons for doing so.

5.2.2.2. Temporal Patterns

The distribution of cases over time was investigated by counting the biweekly number of cases. The relationship between intervention measures (e.g., culling and impounding of free-roaming dogs) and the time series of cases was assessed by counting the number of cases before and after implementation of these control measures.

5.2.2.3. Spatio-Temporal Patterns

The reported cases were mapped (ArcGIS 9.3; ESRI, Redlands, CA, USA) by using a Bhutan shape file (datum: GRS [Geodectic Reference System] 1980, Spheroid; projection: GCS [Geographic Coordinate System] Bhutan Drukref03, Transverse Mercator). The mean center of cases (average X and Y coordinates, useful for tracking changes in distribution) and a standard deviational ellipse (a measure of directional spread), weighted by date of report of cases (Ward and Carpenter 2000; Ward 2007; Ward et al., 2008; Guerra et al., 2003; Svensson et al., 2009), was calculated (Spatial Statistics Tools; ArcGIS 9.3).

5.2.2.4. Economics

The cost of the outbreak was analysed by using 3 simple, direct-cost calculation methods (Meslin 1994). The first calculation was direct cost associated with cattle deaths = number of cattle deaths (n = 42) × average cost of cattle (existing market price of local cattle in Bhutan was Bhutanese ngultrum [Nu.] 10,000). The second calculation was cost of postexposure prophylaxis for humans = total number of human exposures (n = 674) × 5 vaccine doses/ person × cost of vaccine (Nu. 450/dose in Bhutan), which was provided free of charge and paid for by the Ministry of Health, Bhutan. The third method was cost of the surveillance and control program, which was calculated on the basis of actual expenditure incurred (removal and impounding of dogs, awareness program), vaccination of ≈200 dogs (at Nu. 25/vaccine dose), and travel and logistics costs for the outbreak response team (paid by the Department of Livestock, Bhutan).

5.3. Results

5.3.1. Animal patterns

During the study period, 97 cases of rabies (42 in cattle, 52 in dogs, 3 in horses) were reported in the subdistricts of Dala (18 cattle, 12 dogs), Bongo (21 cattle, 32 dogs and 3 horses), and Bjachho (3 cattle, 8 dogs) (Table 5.1). Incidence was 5 (95% confidence interval 4–7) and 20 (95% confidence interval 15–26) cases per 1,000 population at risk for cattle and dogs, respectively. Incidence did not differ significantly between the 3 subdistricts for dogs $\chi^2 = 3.65$, $p = 0.16$) or cattle $\chi^2 = 3.12$, $p = 0.21$) (Table 5.1).

Table 5.1: Attack rates (per 1000) for reported rabies, Chhukha district, Bhutan January 23−July 31, 2008

Sub-district and species	No. cases/total population	Attack rate (95% confidence interval)
Dala		
Cattle	18/4194	4 (3−7)
Dogs	12/601	20 (11−34)
Bongo		
Cattle	21/2898	7 (5−11)
Dogs	32/1343	24 (17−33)
Bjachho		
Cattle	3/772	4 (1−11)
Dogs	8/707	11 (6−22)

5.3.2. Temporal patterns

The epidemic peak occurred during weeks 11 and 12 (April 3–16), and 65% of cases were reported between weeks 9 and 18 (April and May). The epidemic lasted for 27 weeks and ended in July (Table 5.2) (see Appendix 2).

5.3.3. Spatio-temporal patterns

The outbreak (mean center X = 2,700,680 meters; Y = 1,014,350 meters) had an ellipsoid (south-to-north) distribution (Figure 2, panel A). The mean center during consecutive 2-month intervals moved northward (Figure 5.2, panel B). These distributions overlapped and had a south-to-north direction; however, during the final phase (June– July), the outbreak was distributed west to east and spread in the main town areas of Gedu in Bongo subdistrict and its surrounding villages (Figure 5.2, panel B). The distribution of cases followed the road network and towns with high human density and high numbers of free-roaming dogs (Figure 5.2, panel A; some road network data not shown).

Table 5.2: Number of rabies cases in animals reported biweekly by sub-district, Chhukha district, Bhutan, January 23–July 31, 2008*

	Dala*		Bongo†			Bjaccho‡		
Weeks	Cattle	Dogs	Cattle	Dogs	Horses	Cattle	Dogs	Total
1−2	2	0	0	0	0	3	3	8
3−4	1	0	0	0	0	0	0	1
5−6	0	0	0	0	0	0	2	2
7−8	5	0	0	0	0	0	0	5
9−10	1	1	4	3	0	0	1	10
11−12	4	3	6	4	0	0	1	18
13−14	1	2	2	5	0	0	1	11
15−16	0	2	0	9	0	0	0	11
17−18	3	4	0	6	0	0	0	13
19−20	1	0	0	3	0	0	0	4
21−22	0	0	2	0	0	0	0	2
23−24	0	0	3	1	0	0	0	4
25−26	0	0	4	1	2	0	0	7
27	0	0	0	0	1	0	0	1
Total cases	18	12	21	32	3	3	8	97

* Free-roaming dogs were culled during weeks 6-9 (March 27-Feb 27) and weeks 24 (July 3-9); † Free-roaming dogs in Gedu town were culled during weeks 15-20; dogs were impounded during week 17; ‡ Dogs in Tshimalakha and Tshimasham were impounded during weeks 16 and 17 (See Appendix 2).

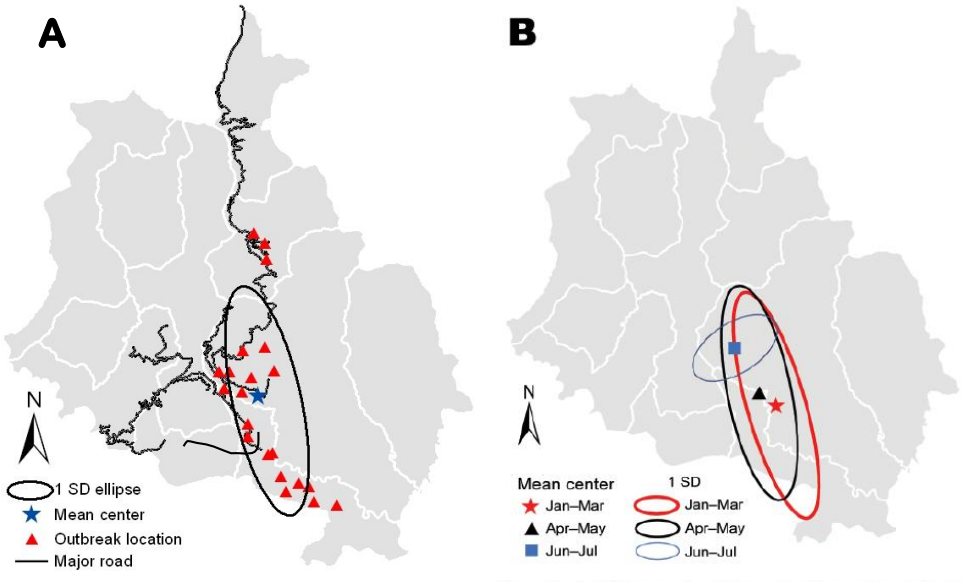

Figure 5.2: Spatio-temporal pattern of rabies outbreaks in the Chhukha district, Bhutan, January 23–July 31, 2008. A) Pattern for the complete outbreak period. B) Patterns during consecutive 2-month intervals during the outbreaks period. Jan–Mar period includes January 23–31 (total 69 days; total for other periods 61 days).

5.3.4. Human exposure patterns

A total of 674 persons were reported to have been exposed to animals with suspected rabies. Most (77%) exposures were related to contact with rabid animals while either conducting zoosanitary measures or feeding sick animals and by consuming meat and dairy products derived from suspected rabid animals (Table 5.3). All persons were given anti-rabies vaccine (5 doses/person) in the hospital. No human deaths were reported during this outbreak.

Table 5.3: Numbers of human exposures to suspected rabies, Chhukha district, January 23−July 31, 2008

Sub-district and exposure	Number of person exposed
Dala	
Contact with rabid animals	30
Bongo	
Dog bite	130
Contact with rabid animals	132
Consumption of meat/dairy products from rabid animals	120
Other animal bites	22
Bjachho	
Consumption of meat from rabid animals	116
Consumption of dairy products from or contact with rabid animals	124
Total	674

5.3.5. Outbreak control

In the outbreak areas, free-roaming dogs were culled during weeks 6–9, 15–20, and 24; a total of 500 dogs were impounded during weeks 16 and 17 and remained in shelters until the outbreak subsided. In the adjacent unaffected areas, ≈200 dogs were vaccinated against rabies. The general public and school students in the outbreak areas were made aware, through public meetings and media announcements, of the dangers of rabies. Culling and impounding of free-roaming dogs is believed to have controlled the outbreak; no cases were detected after July (Table 5.2) (See Appendix 2).

5.3.6. Outbreak cost

The direct outbreak cost was estimated to be Nu. 2.75 million (≈US $59,923; 1 US $ = Nu. 46). This cost included cattle deaths (≈Nu. 42,000; 15%); postexposure prophylaxis for humans (≈Nu. 1,516,500; 55%); and implementation of the rabies control program (Nu. 820,000; 30%). The control program cost included ≈Nu. 500,000 for culling, impounding, and awareness programs; ≈Nu. 5,000 for vaccination of domestic dogs; and ≈Nu. 315,000 for the rapid response team (field surveillance and control activities). Because other indirect costs were not taken into account, these costs are likely underestimates.

5.4. Discussion

The rabies outbreak in the Chhukha district initially occurred in dogs in the villages in the southern parts of Dala. The index case dog probably bit several other dogs, resulting in sustained animal-to-animal spread among the free-roaming dog population. After this initial focus of infection, infected free-roaming dogs might have spread the disease by biting cattle and other dogs.

The outbreak spread from south to north and seemed to follow the road network and town areas (Figure 5.2)(Appendix 3) that had many free-roaming dogs. High numbers of free-roaming dogs would have provided opportunities for infected dogs to transmit the virus to susceptible dogs and then to cattle in the region. Later, the movement of infected free-roaming dogs from some of these towns might have been responsible for the spread of the disease and spillover infection to cattle in surrounding villages (Tenzin et al., 2010). However, the culling of free-roaming dogs possibly removed this rabies reservoir from the outbreak areas, resulting in a drastic reduction in the number of cases by June 2008 (WHO, 2005). This corroborates anecdotal field evidence that immediate removal of reservoirs can facilitate the control of a rabies outbreak. In contrast, in similar large rabies outbreak in eastern Bhutan from May 5, 2005, through the end of 2007, mass culling was not implemented because of the religious sentiments of the local people (Tenzin et al., 2010); in this outbreak, widespread dissemination of rabies persisted for much longer.

Postexposure prophylaxis is crucial for preventing rabies in humans after exposure to any rabid animals. Globally, >10 million persons (mostly in Asia) receive postexposure vaccination against rabies (WHO, 2005; WHO, 2007). In the Chhukha district outbreak, ≈674 persons were given full courses (at 0, 3, 7, 14, and 28 days; Essen regimen) of antirabies vaccine, provided free by hospitals. However, most exposures likely carried low risk, e.g., feeding sick (rabid) cattle, touching carcasses while conducting zoosanitary procedures, and consuming cooked meat and dairy products derived from cattle that had died of rabies (because of lack of knowledge about rabies). Except for the few who handled meat or carcasses or were bitten by dogs, others fell under the World Health Organization exposure category I (touching or feeding animals, licks on intact skin); rabies vaccination is usually not recommended for such exposures (WHO, 1997; WHO, 2007; McGuill et al., 1999; Rotz et al., 1998). Probably the fear of rabies sensitized the public and ultimately led to mass vaccination of people. Similar mass vaccination after consumption of dairy products from cattle with suspected rabies or handling of rabid animals and contact with confirmed rabies patients has been reported in Bhutan and elsewhere in the world (Tenzin et al., 2010; McGuill et al., 1999; Rotz et al., 1998; Rimhanen-Fenne et al., 2010). There are no specific guidelines to assess such non-bite exposure groups. Should a large-scale exposure occur in the future, use of specific criteria and risk assessment for anti-rabies vaccination may prevent unnecessary use of scarce vaccine resources, whereas public awareness education might prevent future episodes and potential food borne transmission (WHO, 2005; McGuill et al., 1999; Rotz et al., 1998). Furthermore, in addition to the existing 5-dose intramuscular Essen regimen followed in Bhutan, other postexposure prophylaxis regimens, such as the intradermal method approved by the World Health Organization Expert Committee, should be reviewed because this method is immunogenic, effective, requires fewer doses of vaccine, and costs 70% less than the conventional intramuscular regimen (WHO, 1997; WHO, 2007; Wilde et al., 2005; Shantavasinkul et al., 2010).

The estimated cost of this outbreak was large by Bhutan standards and reflects the extent of rabies in a resource-limited country (Knobel et al., 2005; Rinzin et al., 2006; Meslin 1994). Globally, it has been estimated that > US$ 1 billion per year is spent on rabies prevention programs, mostly on postexposure prophylaxis (WHO, 2005; WHO, 2007). Similarly, in the

Chhukha district outbreak, 55% of the estimated total costs were associated with post-exposure prophylaxis for humans. Although vaccinations were free for the recipients, the cost to the Ministry of Health was high. Because the program to eliminate rabies in dogs contributes to the elimination of rabies in humans (or reduces the cost of post-exposure prophylaxis), public health and animal health efforts should emphasize the need for control and elimination of rabies in animal reservoirs. In Bhutan, the resources allocated to rabies control in the animal health sector are inadequate and often lead to low vaccination coverage of dogs. Therefore, financial resources should be shared by the public health sector for effective implementation of rabies control and dog management programs.

In conclusion, the Chhukha district rabies outbreak spread consistently from south to north, following the distribution of roads and towns that had large free-roaming dog populations. Rapid culling of in-contact and unvaccinated free-roaming dogs controlled this outbreak. A similar strategy should be considered for any future rabies outbreaks in Bhutan. Because of the risk for spread of rabies from the southern rabies-endemic zone to the rabies-free interior of Bhutan, a well-coordinated national rabies control program should be implemented to prevent and control rabies in Bhutan.

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CHAPTER 6

6. EPIDEMIOLOGY OF RABIES IN BHUTAN:GEOGRAPHIC INFORMATION SYSTEM BASED ANALYSIS

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CHAPTER 6

Epidemiology of Rabies in Bhutan: Geographic Information System based analysis

Abstract

In this study, we describe the spatio-temporal patterns of rabies in domestic animals in Bhutan. Surveillance data were analysed using Geographical Information System and traditional and spatial statistics. Rabies cases in animals (mostly dogs and cattle) were mainly reported in southern parts of Bhutan, bordering India. The trend of reported cases was relatively stable until 2005, but increased during both 2006 and 2008, due to major outbreaks in eastern and south-west Bhutan. Significant clusters ($P<0.05$) of high numbers of cases were observed in south-central and south-west Bhutan. There was also a significant ($P<0.05$) spatial correlation between reported cases in dogs and in cattle. The rabies prevention program should be focussed on the highly endemic areas of southern parts of Bhutan. Mass vaccination of dogs in this region would create an immune buffer (cordon sanitaire) and prevent rabies incursions into the interior of Bhutan.

Key words: Bhutan, case study, domestic animals, epidemiology, spatial distribution.

6.1. Introduction

Rabies is one of the oldest infectious diseases known to mankind and still remains a major public health problem, causing an estimated 70,000 human deaths every year, particularly in Asia and Africa (Hampson et al., 2011). Although effective post-exposure prophylaxis is available, these measures may not be affordable in many resource-limited countries (Knobel et al., 2005; Zinsstag et al., 2009). Control of rabies in the domestic dog − the primary source of human rabies − can interrupt the transmission cycle and prevent further transmission to

humans (Zinsstag et al., 2009). For example, mass vaccination of dogs in the Serengeti District of North-western Tanzania with vaccination coverage of 64.5%; 61.1%;, 70.6% and 73.7% following each of the four campaigns between October 1996 and February 2001 resulted in significant decline of rabies incidence in dogs, by 70% after the first campaign and by 97% after the second campaign (Cleaveland at al., 2003). Similarly, the incidence of human bite injuries from suspected rabid dogs declined significantly in Serengeti District after dog vaccination and reduced demand for human post-exposure prophylaxis but not in adjacent unvaccinated districts (Cleaveland at al., 2003). In the canine rabies endemic area of the Phetchabun Province of Thailand, mass vaccination of dogs with an annual vaccination coverage ranging from 64% to 78%, along with post-exposure prophylaxis in humans, resulted in a decline of dog rabies incidence, and no human rabies deaths have been reported during the last three years (1999 to 2001) of the campaign period between 1996 and 2001 (Kamoltham et al., 2003). Similarly, in Latin America, mass dog vaccination campaigns and human post-exposure prophylaxis carried out between 1982 and 2003 have resulted in a significant decline in the number of rabies cases in dogs from 15,686 cases to 1,131 (93% drop), and human rabies cases, declining from 355 to 35 (91% drop) (Belloto et al., 2005; Schneider et al., 2005). These findings in the field clearly demonstrate that the human rabies incidence can be reduced or eliminated by controlling rabies in animal reservoirs.

Bhutan is a small (38,394 km^2) Himalayan landlocked country, located between India to the south and the Tibetan province of the Peoples' Republic of China to the north. While Bhutan shares a porous border with India in the south, the Himalayan mountain ranges in the north act as a natural barrier with China. There are increasing trade activities with free movement of people between Bhutan and India. Canine rabies is endemic in the areas of southern Bhutan that border India (Tenzin et al., 2011a), but sporadic occurrences have been reported in other, previously free areas (Tenzin et al., 2010a, Tenzin et al., 2010b). This indicates that there is a risk of the disease re-emerging (an increased distribution or incidence) in Bhutan, if proper surveillance and control programmes are not implemented. In addition, human rabies deaths are also reported every year from the southern endemic areas of Bhutan (Kuensel, 2009). For instance, there were 15 reported human rabies deaths from 2006 to July 2011, equivalent to a cumulative incidence of 2.1 deaths per 100 000 at-risk population (with an

average annual incidence of 0.28 deaths per 100 000 people) (MoH, 2010). The presence of rabies in southern Bhutan results in substantial financial implications for its society in the form of farm animal deaths, the expense of post-exposure prophylaxis in humans, and the cost of prevention and control programmes in animals (Tenzin et al., 2010a, Tenzin et al., 2010b). There are large numbers of stray dogs in Bhutan − particularly in urban centres (freely cohabiting with humans). This poses the risk of dog bites, and results in increasing usage of post- exposure prophylaxis because all dog bites are considered a rabies risk, due to the presence of rabies in southern Bhutan (Tenzin et al., 2011b). Although shooting and oral poisoning of dogs has been implemented for both rabies and dog population control in the past, it is notpractised any more in Bhutan. A nationwide dog population control programme (capture−neuter−vaccinate−release) is ongoing in Bhutan, with the objective of sterilizing and vaccinating more than 80% of the estimated 50,000 dogs, thereby reducing the stray dog population to a manageable level (HSI, 2010). Domestic dogs play a principal role in the transmission of rabies, and no wildlife rabies cases have been reported so far in Bhutan.

In this study, we describe the distribution of domestic animal rabies cases in Bhutan, using a Geographic Information System (GIS). These systems are increasingly used in public health and epidemiologic research as tools to visualize, manage, explore and analyse spatial data, providing useful information about the distribution and risk areas of disease that can inform control programmes (Ward 2007; Frössling et al., 2008; Ward et al., 2008; Zhang et al., 2008). Similarly, the information generated from this study can help to identify the risk areas which need to be prioritised in the allocation of resources for the surveillance and control of rabies in Bhutan.

6.2.Material and methods

Rabies surveillance data for the period between January 1996 and December 2009 were retrieved from the Veterinary Information System database maintained at the National Centre for Animal Health and the Regional Veterinary Laboratories of the Department of Livestock, Bhutan, as described elsewhere (Tenzin et al., 2011a)**.** These data included: the number of rabies cases reported in each animal species (cattle, horses, pigs, goats, cats and dogs), date

of occurrence, and location (village, sub-district and district). A case is defined as an individual of any species of domestic animal (dogs, cattle, horses, pigs, goats, cats) in which rabies has been clinically confirmed, based on epidemiologic investigations or laboratory examination of brain specimens (Tenzin et al., 2011a). The reported rabies cases in cattle and dogs over a period of 14 years (1996 to2009) were then aggregated at the sub-district level and used in subsequent analysis. A Geographic Information System (ArcGIS 9.3 ESRI, Redlands, CA, USA) was used to visualize the spatial distribution of rabies cases in dogs and cattle. A global spatial autocorrelation (Moran's I test) statistic (ArcGIS™ 9.0 Spatial Statistics) was used to determine whether rabies case distribution, in dogs and cattle, exhibited spatial patterns. Local clusters of cases were investigated by estimating Anselin's local indicator of spatial autocorrelation statistic (Anselin 1995; Anselin 2005). A spatial interpolation analysis was also performed, using the centroid of each sub-district as a point layer, to estimate a continuous distribution of rabies cases. An inverse distance weighing (IDW) method (Spatial Analyst Tools; ArcGIS™) was used to interpolate reported rabies cases in dogs and cattle separately. The IDW is a moving average or distance weighted average method and assumes that each interpolating surface should be influenced most by the nearby points and least by the more distant points. The IDW assumes that each measured point has a local influence that diminishes with distance. For example, to predict a value for any unmeasured location, IDW uses the measured values surrounding the prediction location (Spatial Analyst Tools; ArcGIS™ 9.3). The resulting interpolation was then displayed as a continuous, graduated colour surface of the centroid of each reported sub-district for cattle and dogs separately.

6.3.Results

A total of 814 animal rabies cases were reported between 1996 and 2009, with a majority of the cases in cattle (55%, 447/814) and dogs (39%, 317/814). Only a few cases were reported in other species (horses, 17; cats, 14; pigs, 13; and goats, 6) (see Table 6.1) (Tenzin et al., 2011a). The number of reported cases was relatively stable until 2005, but increased in 2006 and 2008, due to major outbreaks in eastern and southwest Bhutan. Figure 6.1 illustrates the monthly distribution of reported rabies cases in animals.

Table 6.1: Total number of reported rabies cases in domestic dogs and other domestic animals in Bhutan, 1996–2009.

Year	Cattle	Dogs	Horses	Cats	Pigs	Goats	Total
1996	8	10	0	0	0	0	18
1997	16	2	0	0	0	0	18
1998	28	32	0	0	0	0	60
1999	26	13	2	0	2	1	44
2000	15	4	1	0	1	0	21
2001	19	1	0	0	1	1	22
2002	26	20	1	0	0	0	47
2003	9	8	0	0	1	0	18
2004	12	4	2	0	2	0	20
2005	21	26	2	0	0	0	49
2006	138	103	5	3	2	0	251
2007	28	12	0	1	1	2	44
2008	72	65	4	7	2	2	152
2009	29	17	0	3	1	0	50
Total	447	317	17	14	13	6	814

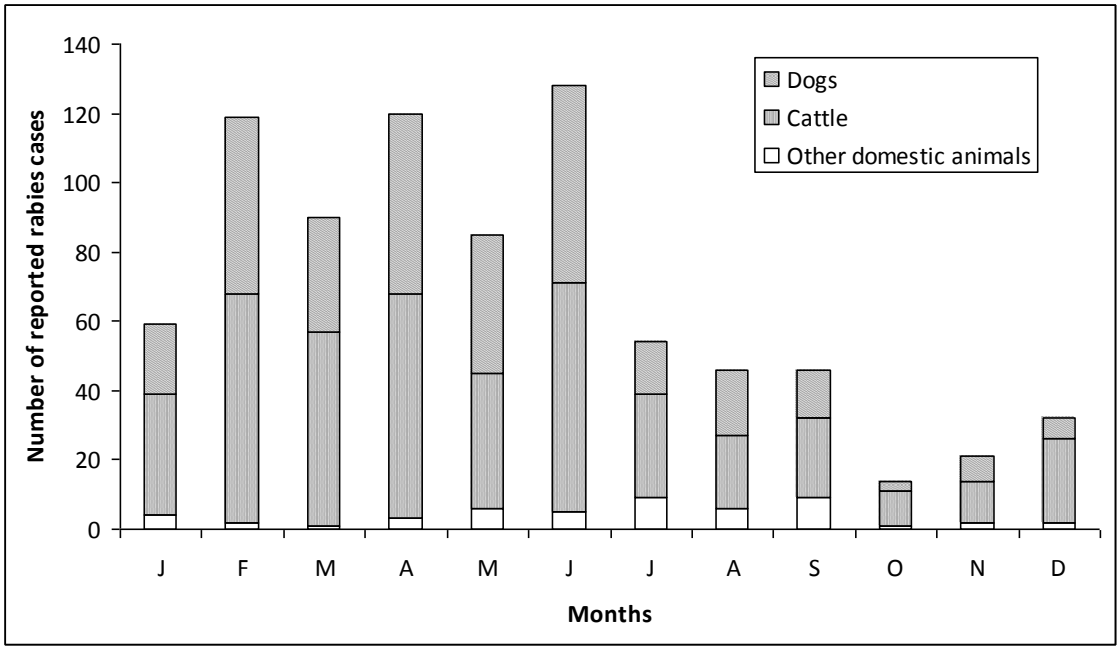

Figure 6.1: Cumulative monthly distribution of reported rabies cases in domestic animals in Bhutan, 1996–2009

Of the total 20 districts and 205 sub-districts in Bhutan, 11 districts and 59 sub-districts reported rabies between 1996 and 2009. Cases were commonly reported in southern Bhutan which shares a border with India. The distribution of reported rabies cases was strongly clustered in both dogs (I = 0.153 P=0.004) and cattle (I = 0.144 P=0.004). Local indicator of spatial autocorrelation (LISA) statistics identified significant clusters (P<0.05) of high numbers of cases in both dogs and cattle in south-central and south-west Bhutan. The interpolated map shows high numbers of reported cases in southern and eastern Bhutan (Figures 6.2 and 6.3). There was a significant (P<0.05) spatial correlation of reported cases in both dogs and in cattle, as illustrated in Figures 6.2 and 6.3.

6.4. Discussion

In this study, we conducted descriptive and GIS-based exploratory spatial analyses to describe the spatial distribution of rabies in domestic animals and to identify rabies risk areas in Bhutan. There were a higher number of reported cases in cattle than in the reservoir species (domestic dogs). This can be explained by the surveillance system used, in which there is no active surveillance of rabies in domestic dogs, especially stray dogs. Rabid stray dogs are difficult to trace, due to transborder movement in the southern border towns of Bhutan. In addition, rabid stray dogs can be killed by vehicles or people, and remain unreported to the veterinary authorities. In contrast, cases in cattle and other domestic animals are effectively captured by the reporting system, because of the greater economic value of these animals (Tenzin et al., 2011a). It is also possible that a single rabid dog might be involved in infecting many cattle that are usually let out on open field grazing areas. This could happen, especially during major rabies outbreaks, as had been observed during outbreaks in eastern Bhutan and in south-west Bhutan, where large number of cattle have died of rabies following exposure to rabid dog bites (Tenzin et al., 2010a, Tenzin et al., 2010b).

Our analysis confirmed a significant spatial distribution of rabies in Bhutan, with increased incidences in southern Bhutan. The results suggest that rabies endemicity has been maintained among stray or free-roaming dogs in the south border areas, or that this

endemicity could be the result of transborder movement or translocation of infected dogs between South Bhutan and Indian border towns.

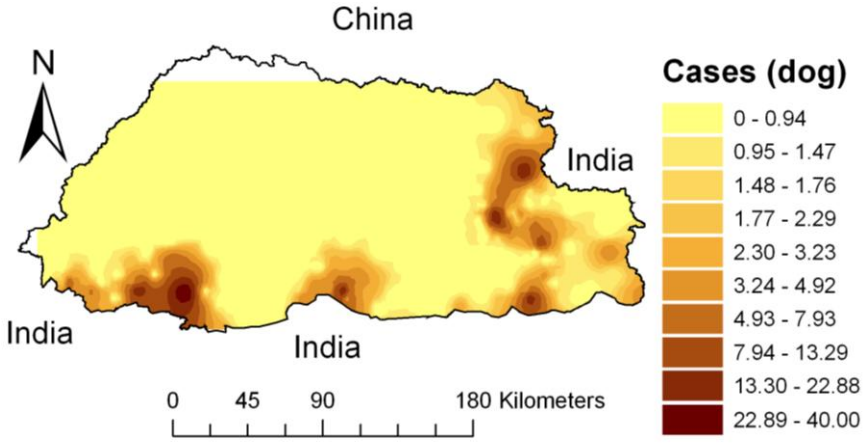

Figure 6.2: Spatial distribution of rabies cases in domestic dogs in Bhutan during the period 1996–2009, interpolated by inverse distance weighing (IDW)

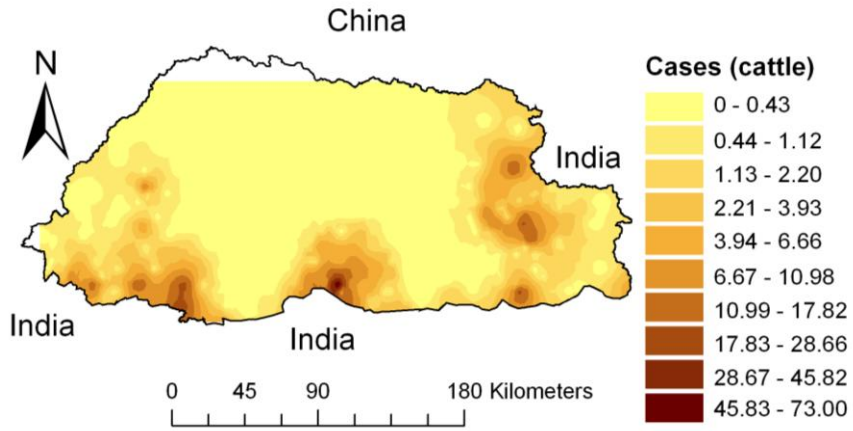

Figure 6.3: Spatial distribution of rabies cases in cattle in Bhutaduring the period 1996–2009, interpolated by inverse distance weighing (IDW)

This is confirmed by phylogenetic studies which show that the rabies virus strain circulating in southern Bhutan is closely related to Indian rabies virus strains (Tenzin et al., 2011c). According to LISA statistics, clusters of rabies cases (in both cattle and dogs) were identified in the south-central and south-western regions of Bhutan. Identification of these clustered areas will help to focus the investigation of risk factors and direct the organisation of an effective control programme. We recommend that mass vaccination of dogs with >70% coverage should be focussed and prioritised in these highly endemic areas in southern Bhutan to optimise the use of limited resources. Prevention of rabies in these 'hot spots' and endemic areas would eventually eliminate and reduces the incidence of rabies in dogs and humans' since no wildlife reservoir of rabies has been reported in Bhutan. Mass vaccination of dogs in these areas would create an immune belt (*cordon sanitaire*) and prevent rabies incursions into interior of Bhutan. For example, Malaysia successfully eradicated canine rabies by mass vaccination and a stray dog reduction programme in the 1950s and a rabies- free status has been maintained by vaccinating dogs within an immune belt along the Thailand-Malaysia border, despite rabies remaining endemic in Thailand (Wells 1954). This should serve as a model for Bhutan to plan for a rabies elimination programme at the India−Bhutan border. Furthermore, disease surveillance and monitoring programme should be prioritised in rabies endemic-southern Bhutan.

In conclusion, the GIS-based spatial analysis clearly indicates that the risk of rabies incidences in dogs and other domestic animals exhibits a regional trend, particularly in south-east, south-central and south-west Bhutan. The identification of rabies risk areas and their spatial visualization as a risk map in this paper would be useful for prioritisation of rabies surveillance and control activities in Bhutan. Therefore, disease risk map is a valuable tool for understanding the spatial distribution of rabies cases and to identify highly endemic areas for future public health planning and prioritising resource allocation for control programmes.

Recommendations

- Mass vaccination of dogs with >70% coverage should be focussed and prioritised in the highly endemic areas in southern Bhutan to create an immune belt (*cordon sanitaire*) and prevent rabies incursions into the interior of Bhutan

- A regionally coordinated rabies control programme is necessary in the border areas between Bhutan and India to eliminate rabies in Bhutan.
- Inter-sectoral coordination and cooperation is necessary for the prevention and control of rabies in Bhutan
- Epidemiological surveillance of rabies should be improved by the laboratory confirmation of the cases, and the data thus generated should be shared between the public health and veterinary sectors and also internationally through the World Organization of Animal Health (OIE).
- Rabies surveillance should be extended to wildlife to confirm the presence or absence of rabies or rabies-related viruses in wildlife in Bhutan

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CHAPTER 7

7. ANTHROPOGENIC AND ENVIRONMENTAL RISK FACTORS FOR RABIES OCCURRENCE IN BHUTAN

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CHAPTER 7

Anthropogenic and environmental risk factors for rabies occurrence in Bhutan

Abstract

Anthropogenic and environmental factors were assessed as predictors of sub-districts in Bhutan that reported rabies in domestic animals during the period 1996−2009. Rabies surveillance data were retrieved from the Veterinary Information System database. Anthropogenic and environmental information were obtained from public data sources. Using the total number of rabies cases reported in domestic animals, the 205 sub-districts of Bhutan were categorized as those sub-districts that reported rabies and those that did not report rabies (n=146). Logistic regression models were fit to the data and odds ratios and 95% confidence intervals were estimated. Sub-districts that share a border with India (OR 10.43; 95% CI: 4.42–24.64; P<0.001); sub-districts connected by major roads (OR 3.09; 95% CI: 1.24–7.68; P=0.015); and greater human population density (OR 3.26; 95% CI:1.48–7.21, P=0.003) were significantly associated with a sub-district reporting animal rabies in Bhutan during 1996−2009. Results suggest that human population characteristics play an important role in rabies occurrence.

Keywords: Rabies, domestic animals, anthropogenic and environmental risk factors, Bhutan

7.1. Introduction

Rabies remains a serious public health hazard in Bhutan like in many other developing countries where canine rabies is endemic and dog bite is the main mode of transmission of virus to humans (Knobel et al., 2005; Wilde et al., 2007; Dodet et al., 2008). Rabies outbreaks are mainly reported in southern parts of Bhutan, affecting domestic dogs with spill-over infection in farm animals (Kuensel, 2010a; Tenzin. et al., 2011a). Rabies outbreaks in dogs and other domestic animals have also been reported in previously free areas in the east and southwest Bhutan (Tenzin et al., 2010a,b). Rabies cases in animals are reported throughout the year in Bhutan, with a higher incidence during spring and summer months. A positive temporal correlation between the number of cases reported in dogs and other domestic animals has been observed (Tenzin et al., 2011a). Phylogenetic studies have demonstrated that the rabies virus variant circulating in Bhutan is similar to Indian virus strains, and belongs to Arctic-like virus 1 (Tenzin et al., 2010c). Although infrequent, sporadic human deaths due to rabies are reported in Bhutan following bites by rabid dogs (Kuensel, 2009; 2010a,b; 2011a,b; BBS, 2011a,b). Post exposure prophylaxis (PEP) is provided free of charge to people by the government medical hospitals (Tenzin. et al., 2011b) and vaccination of dogs and animal birth control are the main rabies control strategy implemented in Bhutan (MoA, 2009; HSI, 2010). In a previous hospital-based questionnaire survey in Bhutan, involving interviews of 324 dog bite victims, males and children aged 5–9 years were found to be more likely to be bitten. Using a decision model, an annual incidence of 4.67 rabies deaths/100,000 population at-risk was predicted in two rabies endemic areas of south Bhutan. In the absence of post exposure prophylaxis, the mortality was predicted to be 19.24 rabies deaths/100,000 population at risk (Tenzin et al., 2011c). A community-based study of rabies knowledge, attitudes and perception found that rabies knowledge in Bhutan can be predicted by gender, educational level and dog ownership status, while the health seeking behaviours of people with animal bite injuries can be predicted by dog ownership status, presence of children in the household and occupation of the respondents (Tenzin et al., 2012a).

It is understood that rabies endemicity is maintained in areas that have a high dog density with inadequate vaccination coverage (or lack of a control program) (Cleaveland and Dye, 1995; Lembo et al., 2008). Human rabies is associated with social and environmental conditions that bring people into contact with dogs. In rabies endemic countries, rabies disproportionately affects the poorer sections of the rural community and children below 15 years of age (Pancharoen et al., 2001; Knobel et al., 2005; Cleaveland et al., 2006). In Bhutan, there is a clear regional trend of rabies distribution in which it is very common in some areas and not reported in other areas (Tenzin. et al., 2011d). It is therefore important to understand the risk factors for disease occurrence. Socio-demographic, anthropogenic and environmental factors have been assessed to understand the epidemiology of various infectious diseases in epidemiologic research and have provided useful information as predictors of disease occurrence (Glass et al., 1995; Weiss and McMichael, 2004; Hu et al., 2007; Mongoh et al., 2007; Ward et al., 2009). For example, the estimated equine West Nile Virus attack rate in Texas (USA) was best described by environmental features such as lakes, forests and cultivated areas (Ward et al., 2009). Highly pathogenic avian influenza H5N1 occurrence has been associated with road connectivity in Romania (Ward et al., 2008a). Similarly, land use and demographic data were used to predict large or small raccoon rabies epizootics in the US (Jones et al., 2003). Therefore, a better understanding of disease spread using human social ecology and landscape features may be important for designing better control programs (Weiss and McMichael, 2004, Carey et al., 1978).

In this study, we examined the association between a range of anthropogenic and environmental factors as predictors of the risk of a sub-district reporting animal rabies occurrence in Bhutan.

7.2.Materials and methods

7.2.1. Data source

The data were retrieved for the period 1 January 1996 to 31 December 2009 from the Veterinary Information System (VIS) database maintained at the National Centre for Animal

Health. This database contains all reports of animal rabies events in Bhutan. Data are submitted by the Regional Veterinary Laboratories and the Satellite Veterinary Laboratories as 'flash reports' when outbreaks or other disease cases are detected. The data in the VIS database include the number of rabies cases reported by animal species (cattle, horses, pigs, goats, cats and dogs); and location (village, sub-district and district) as described elsewhere (Tenzin. et al., 2011a). Rabies cases are diagnosed based on clinical signs, epidemiological investigation and laboratory testing, as described previously (Tenzin et al., 2011a). Administratively, Bhutan is divided into 20 districts and further sub-divided into 205 sub-districts. The smallest administrative unit is the village (five or more per sub-district). The number of rabies cases in any species of domestic animals reported during the period 1996−2009 was summarized for each of the 205 sub-districts in Bhutan. Based on this, the sub-districts were classified into two categories: a sub-district that reported rabies in any species of animals during the period 1996–2009 (coded as '1'), and that did not report rabies (coded as '0'). This was used as the outcome variable in the logistic analyses.

7.2.2. Data analysis

A polygon shape file of all sub-districts (that reported rabies and that did not report rabies) was created using ArcGIS™ 9.3 (ESRI Inc., Redland CA). This shape file was overlaid on raster coverage in a Geographical Information System (DIVA-GIS version 7.3.0.1, http://www.diva-gis.org) and relevant information was extracted. The coverage described a range of land use and environmental variables (independent variables) that might explain the sub-district risk of animal rabies reporting. Data extracted were elevation and land cover (gData, beta version; http://biogeo.berkeley.edu, Accessed 30 March, 2011). The resolution of the raster data was 30 seconds (~833 m). All classes represented in the land cover dataset were individually selected and separate raster files were created: tree cover (broadleaved evergreen; broadleaved deciduous; needle-leaved evergreen), mosaic cover (tree cover or other natural vegetation; cropland, shrub and/or grassland), shrub and herbaceous cover (closed-open evergreen; closed-open deciduous; sparse herbaceous and herbaceous cover), and cultivated and managed areas. For each of these, the total area per sub-district (km^2) was calculated (Spatial Analyst, ArcGIS 9.3. ESRI Inc., Redland CA) and binary variables were then created based on the median values ('0' represented ≤ median value and '1' represented

> median value) because of highly skewed distributions and low sample size in each stratum. The average elevation (in meters) of each sub-districts was extracted and coded as '0' for ≤ median value of all sub-districts and '1' for > median value. Additional anthropogenic data were included as predictor variables: sub-district human population and cattle population density (coded as '0' ≤ median and '1' > median value), presence or absence of a border with India (yes/no), connected by major road network and presence of major towns in sub-districts where rabies was reported (yes/no) (MoA, 2000; NSB, 2005).

7.2.3. Statistical analysis

The associations between the outcome variable and potential risk factors were estimated by fitting logistic regression models (SPSS version 11.5. SPSS Inc., Chicago, IL). Initially, univariable analyses were conducted and variables that were unconditionally statistically significant at *P* value <0.25 were selected for further evaluation. Multicollinearity was assessed and for pairs of predictor variables that were highly collinear, the variable that had the higher P-value and which had less biological relevance was excluded from further analysis. A multivariable logistic regression model was then fit to the data. A variable was considered to be significantly associated with the outcome variable if the *P* value was ≤ 0.05. First order interaction terms were added to the model and tested for significance (P ≤ 0.05). Odds ratios (OR) and 95% confidence intervals were calculated from the final model. The fit of the model was assessed using the Hosmer-Lemeshow goodness-of-fit test (Hosmer and Lemeshow, 2000). Moran's autocorrelation statistic (*I)* (Ward and Carpenter, 2000) for model residuals was calculated to assess whether the model residuals were spatially correlated (GeoDA^{TM} 0.9.5.i5. Anselin, 2005). The rook and queen contiguity of first and second order of the sub-districts were investigated as spatial weights (GeoDa™ 0.9.5-i5. Anselin, 2005). Absence of spatial autocorrelation (P>0.05) was considered evidence that the model had adequately incorporated the spatial dependence in the observed data (Ward and Carpenter, 2000).

7.3. Results

Eleven of the 20 districts (59%) and 59 of the 205 sub-districts (29%) reported rabies in domestic animals – mainly in cattle (55%) and in dogs (35%) – during the period 1996–2009 (Tenzin. et al., 2011a). Rabies incidents were reported more in areas of southern Bhutan that share a border with India, with sporadic outbreaks in other areas (Figure 7.1).

The following variables were associated (P<0.25) with the risk of reporting rabies occurrence in sub-districts on univariable analysis: sub-districts sharing border with India, sub-districts that have a major road network connection and have major towns, high human and cattle population density. Of the land use and environment variables, sub-districts that have high arable agriculture land cover were at greater risk of reporting rabies while sub-districts with higher elevation and tree cover were at lower risk of reporting disease (Table 7.1).

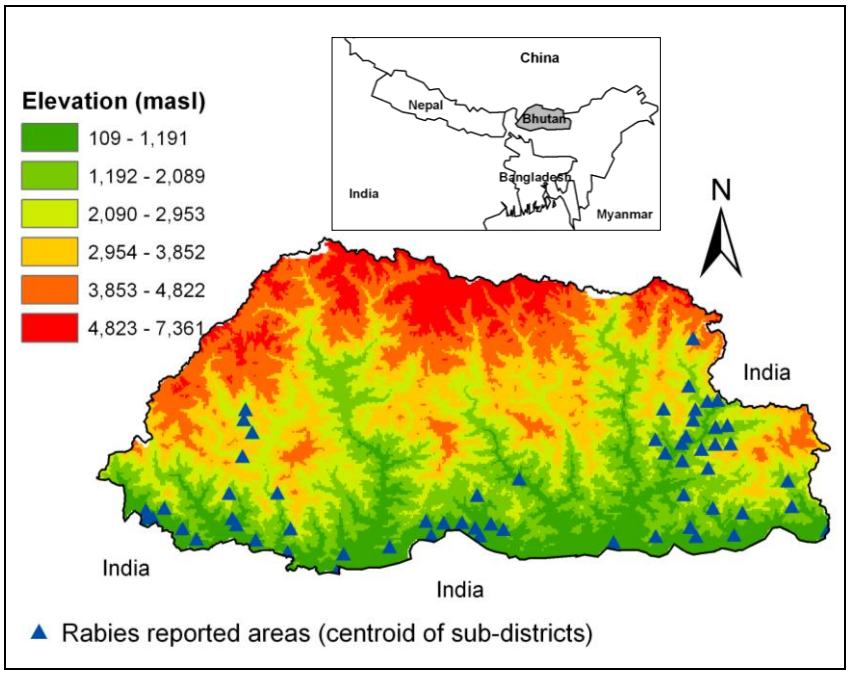

Figure 7.1: Map of Bhutan showing the elevation and the distribution of rabies occurrence in domestic animals, 1996–2009. The (▲) indicates the centroid location of sub-districts that reported rabies in domestic animals during the period 1996–2009.

The environmental variables (shrub and herbaceous cover and mosaic cover) were not significant in the univariable analysis. Agriculture land cover density and cattle population density were highly correlated (P<0.001) with border with India and human density and therefore were excluded from the final model.

The final best fitting multivariable logistic regression model included three variables: sub-districts sharing a border with India (OR 10.43; 95% CI: 4.42–24.64; P<0.001); sub-district connected by a major road network (OR 3.09; 95% CI: 1.24–7.68; P=0.015); and high (>20.12 per km^2) human population density (OR 3.26; 95% CI: 1.48–7.21, P=0.003) (Table 7.2). No interaction terms were significant (P>0.05). The model fitted the data well according to the Hosmer–Lemeshow's goodness-of-fit test (Chi-square test = 1.476; P=0.831). The model standardized residuals showed significant (P=0.001) spatial autocorrelation (I=0.11).

Spatial data (centroid XY coordinate of the sub-district) was included in the model and the model residual autocorrelation was re-estimated. The model residuals showed autocorrelation (I=0.061, P=0.03) when the spatial relationship between sub-districts were defined using the first order rook or queen contiguity weight matrix, but it was less than the autocorrelation estimated from the earlier model (I=0.11). Then the local indicator of spatial autocorrelation (LISA) statistic was estimated (Spatial Analyst, ArcGIS^{TM} 9.3. ESRI Inc., Redland CA) to detect any clusters of model residuals (Anselin, 2005; Ward et al., 2008b), and LISA analysis identified significant (P<0.05) clusters of residuals in the east and southwest of Bhutan. However, no significant (P=0.068) spatial autocorrelation (I=0.042) was detected in the model standardized residuals when the spatial relationship between sub-districts were defined using the second order rook contiguity weight matrix.

Table 7.1: Univariable logistic regression analyses of factors associated with occurrence of rabies in animals at the sub-districts level in Bhutan during 1996–2009 (*P*<0.25).

Variables/categories	*b*	SE	*P*-value	OR	95 % CI
Sub-district sharing borders with India					
No	0	-	-	1	
Yes	2.065	0.377	<0.001	7.89	3.77–16.52
Presence of major town					
No	0	-	-	1	
Yes	1.427	0.382	<.001	4.17	1.97–8.81
Sub-district connected by major road that reported rabies					
No	0	-	-	1	
Yes	1.286	0.385	0.001	3.62	1.70–7.70
Sub-district human population density (per km^2)					
≤ 20.12	0	-	-	1	
> 20.12	1.377	0.338	<0.001	3.96	2.04–7.69
Sub-district cattle population (number)					
≤ 1363	0	-	-	1	
> 1363	0.944	0.322	0.003	2.57	1.37–4.83
Sub-district arable land cover (acreage)					
≤ 1180	0	-	-	1	
> 1180	1.465	0.343	<0.001	4.33	2.21–8.48
Sub-district average altitude (masl)					
≤ 1914	0	-	-	1	
> 1914	-1.020	0.325	0.002	0.36	0.19–0.68
Sub-districts tree cover (per km^2)					
≤ 54	0	-	-	1	
> 54	-0.888	0.322	0.006	0.41	0.22–0.77

Table 7.2: Final multivariable logistic regression model of risk factors associated with occurrence of rabies in animals at the sub-district level in Bhutan during 1996–2009.

Variables/categories	*b*	SE	*p*-value	OR	95% CI
Constant	-2.984	0.456			
Sub-district share border with India					
No	0	-	-	1	-
Yes	2.345	0.438	<0.001	10.43	4.42–24.64
Road network connection to sub-district that reported rabies					
No	0	-	-	1	-
Yes	1.128	0.464	0.015	3.09	1.24–7.68
Sub-district human density (per km^2)					
≤ 20.12	0	-	-	1	-
> 20.12	1.183	0.404	0.003	3.26	1.48–7.21

Log likelihood ratio chi squares test = 19.03, $P<0.001$, Hosmer–Lemeshow goodness of fit test (Chi-square 1.476; $P=0.831$).

7.4. Discussion

The study identified three sub-district level socio-demographic and anthropogenic risk factors significantly associated with reporting of rabies in domestic animals in Bhutan. Sharing a common border with India was found to be the most important individual predictor of the overall distribution of sub-districts rabies occurrence in Bhutan. Of the 59 sub-districts that reported rabies in Bhutan, 43 (73%) shared a border with India. The southern parts of Bhutan are mostly lowlands and have an open border with India. The transborder movement of stray dogs and a lack of an adequate control program may be responsible for the maintenance of rabies endemicity among the large dog population in these border areas (Coleman and Dye, 1996; Tenzin. et al., 2010b). Human rabies incidents are also reported from south Bhutan–India border towns because of rabid dog bites and failure of immediate post exposure treatment (Bhutantimes, 2011; Kuensel, 2009; 2011a, b), suggesting that a rabies control and surveillance program may need to be focussed in these areas. Similarly, other livestock diseases such as foot-and-mouth disease have also been found to be reported

mostly in those sub-districts that share a border with India, compared to those sub-districts that do not (Dukpa et al., 2011). Therefore, cross-border coordinated efforts are necessary for elimination of human rabies transmitted by dogs and other diseases of public health and economic importance.

Our results also suggest that human population characteristics – such as high human population density and road network accessibility are associated with animal rabies occurrence at the sub-district level. In a country such as Bhutan, the domestic dog population density (e.g. stray dogs) may be directly influenced by the human population density and availability of food, which provide continuity of habitat suitable to dogs. Therefore, public awareness education on waste management and dog ownership is important. The combination of a high dog density and contiguous dog populations and low vaccination coverage can result in the persistence of rabies virus in such populations (Kitala et al., 2002; Lembo et al., 2008), resulting in frequent disease outbreaks. Similar situations have been reported in many other canine rabies endemic countries (Cleaveland and Dye, 1995; Wilde et al., 2007; Lembo et al., 2008; Wu et al., 2009). The combined effect of sharing a common border with India and road network connectivity– after controlling for spatial autocorrelation – has not been described previously in Bhutan. This finding supports the hypothesis that the movement of dogs from endemic regions could be maintaining rabies in southern Bhutan. Directly observing and measuring dog movements in a developing country such as Bhutan is problematic. However, with knowledge of the combined effects of sharing an international border and the local road network, surveillance and control programs can be better targeted.

The passive rabies surveillance system operating in Bhutan between 1996 and 2009 captured more reported rabies cases in cattle than in dogs. Rabies cases in dogs – especially in the stray dog population – are more likely to have been underreported than cases in cattle because of the difficulties in tracing cases in dogs and because of the trans-border movement of stray dogs within the south border areas of Bhutan. Cases in cattle or other livestock are more likely to be captured by the reporting system in place because of the greater economic value of these species: for example, farmers routinely report the illness of cattle to veterinary centres for treatment or investigation. Since no wildlife rabies cases have been reported in

Bhutan, it can be concluded that rabies in cattle or in other domestic animals is due to rabies in dogs. In developing countries, including Bhutan, the domestic dog is the main source of exposure and a primary vector for human rabies and other domestic livestock (Knobel et al., 2005; Zinsstag et al., 2009; Tenzin and Ward, 2012b). Therefore, rabies cases in cattle were used as proxy for the dog rabies problem in Bhutan since the reporting of rabies cases in cattle is more sensitive, reliable and timely than that of dog reported cases.

These study findings have to be interpreted with caution since the model did not adequately captured the spatial dependence between observations (despite removing the multicollinear variable in the model and inclusion of spatial data) until a spatial weight matrix of the model standardized residuals based on the second order rook contiguity of sub-districts was included (I=0.042; P=0.068). This indicates that a complex spatial structure existed in the reporting of rabies by sub-districts in Bhutan. The aim of this study was not to explain the spatial structure of rabies reports *per se*, but rather to identify risk factors for sub-district rabies reports. Thus, spatial structure in this context was a nuisance variable. However, one explanation of the finding that a second order rook contiguity of sub-districts accounted for the spatial structure observed is the nature of the passive surveillance system operating in Bhutan. Results of model residual analysis suggest that such correlation might be operating within districts, but not at the first order nearest neighbour level. That is, sub-districts separated by one other sub-district were more likely to report rabies in dogs and cattle. Regardless of the reasons for this complex spatial structure and even though modelling of it was not perfect, the final model coefficients and odd ratios are not expected to be greatly biased.

An understanding of the effect of anthropogenic risk factors on rabies occurrence can be used to design and target disease control and surveilance programs. Sub-districts bordering India in the south were at higher risk of reporting rabies than the interior of Bhutan. More resources for rabies control programs and surveillance should be targeted in the towns and villages of southern Bhutan that have higher risk of rabies occurrence. Prevention of rabies in high risk areas would create an immune belt (cordon sanitaire) and prevent rabies incursions into the interior Bhutan. Surveillance targeted on the trans-border movement of dogs and the road networks in this region are likely to be more efficient for detecting the spread of rabies.

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CHAPTER 8

8. RABIES VIRUS STRAINS CIRCULATING IN BHUTAN: IMPLICATIONS FOR CONTROL

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CHAPTER 8

Rabies virus strains circulating in Bhutan: implications for control

Abstract

We report a molecular epidemiological study of rabies virus strains circulating in animal populations in Bhutan, and investigate potential origins of these viruses. Twenty-three rabies virus isolates originating from dogs and other domestic animals were characterized by sequencing the partial nucleoprotein (N) gene (395 *bp)*. Phylogenetic analysis was conducted and the Bhutanese isolates were compared with rabies viruses originating from other parts of the world. Phylogenetic analysis showed that Bhutanese isolates were highly similar and were closely related to Indian strains and South Asian Arctic–like–1 viruses. Our study suggests that the rabies viruses spreading in southern parts of Bhutan have originated from a common ancestor, perhaps from the Indian virus strain.

Keywords: molecular epidemiology, rabies, Arctic–like virus, Bhutan

8.1. Introduction

Rabies is caused by a virus within the genus *Lyssavirus* of the family *Rhabdoviridae.* According to the official *ICTV* Master Species List 2009-version 6, the genus *Lyssavirus* is composed of twelve species: Aravan virus (ARAV), Australian bat lyssavirus (ABLV), Duvenhage virus (DUUV), European bat lyssavirus 1 (EBLV-1), European bat lyssavirus 2 (EBLV-2), Irkut virus (IRKV), Khujand virus (KHUV), Lagos bat virus (LBV), Mokola virus (MOKV), rabies virus (RABV), West Caucasian bat virus (WCBV), and Shimoni bat virus (SHIBV) (ICTV 2009; ICTV 2011). RABV is the only lyssavirus present in terrestrial mammals throughout the world and associated with bats only in the Americas.

The RABV genome consists of a single-stranded, non–segmented, negative-sense RNA of approximately 12 kb in size and encodes for five structural proteins: the nucleoprotein (N

protein), phosphoprotein (P protein), matrix protein (M protein), glycoprotein (G protein), and the RNA-dependent RNA-polymerase (L protein). The N, P, and L proteins form the internal helically packaged ribonucleocapsid complex (RNP) whilst the M and G proteins form the inner and outer lipid-bilayer envelopes surrounding the RNP core, respectively (Wunner et al., 1988). Many of these genes have been targeted for molecular studies. The N genes are highly conserved and have been extensively employed for rabies diagnosis using RT-PCR and other genetic analyses (Smith et al., 1992; Kuzmin et al., 2004; Denduangboripant et al., 2005; Bourhy et al., 2008). These studies have provided clearer understanding about the epidemiology of rabies virus distribution in the world.

Rabies is a fatal zoonotic disease. It is endemic in Asia, where it causes about 31,500 human deaths each year despite the availability of effective vaccines (Knobel et al., 2005). Of these cases, an estimated 20,000 human deaths occur every year in India alone. Lack of comprehensive rabies control programs and an inability to pay for post exposure treatments after dog bites are responsible for high incidences of rabies in Asian countries (Knobel et al., 2005; Wilde et al., 2007). Over the past decade many molecular epidemiological studies have been conducted in Asian countries, and have provided a better understanding of the RABV variants circulating in Asia and the transmission dynamics of the disease (Denduangboripant et al., 2005; Nanayakkara et al., 2003; Nagarajan et al., 2006; Susetya et al., 2008; Gong et al., 2010; Hyun et al., 2005). This information is needed for implementation of effective rabies control programs in the region.

Bhutan is located between China and India and canine rabies is prevalent in southern Bhutan along the border with India (Tenzin et al., 2010). As in other Asian countries, domestic dogs in Bhutan play an important role in the maintenance and transmission of the disease to other domestic animals and occasionally to humans. In recent years, rabies outbreaks have increasingly occurred in endemic southern Bhutan as well as in some other areas where rabies had not been reported previously (Tenzin et al., 2010). Despite frequent outbreaks, molecular epidemiological studies of rabies in Bhutan have not been undertaken and the rabies virus variants circulating in the country are unknown.

In the present study, we performed genetic characterization of RABV based on the partial nucleoprotein (N) gene. A phylogenetic analysis of these N gene sequences was performed to investigate their genetic relationship with other RABV variants circulating in the world, especially in Asia. The information generated from this research could help in planning a more effective rabies control program in Bhutan.

8.2.Materials and methods

8.2.1. RABV sample

Twenty-three fresh brain tissue samples were obtained from cattle (16), dogs (4), cat (1), and pigs (2) that died of clinically confirmed rabies from four southern districts (Samtse, Chhukha, Sarpang, and Samdrup Jongkhar) in Bhutan during 2008 and 2009 (Table 8.1, Figure 8.1). These samples were collected from areas near the border between Bhutan and the Indian states of Assam and West Bengal. Rabies was confirmed in each case by the fluorescent-antibody test (FAT). For RT-PCR and nucleotide sequencing, about 10 mg of each FAT-positive brain tissue sample was smeared onto FTA® Gene Guard System (a commercial product consisting of filter paper impregnated with patented chemicals supplied by Whatman, USA), air-dried, and then transferred to the laboratory (WHO Collaborating Centre for Research and Training on Viral Zoonoses, Faculty of Medicine, Chulalongkorn University) in Thailand.

8.2.2. Elution of RABV from filter paper

The brain tissue sample from the filter paper was eluted by rocking each dried brain spot in 9 ml of lysis buffer (NucliSens; BioMerieux) at room temperature with a rotator-mixer (rotor size, 60; BioSan) at 60 rpm for a period of 2 hours. The filter paper was then removed from the buffer solution.

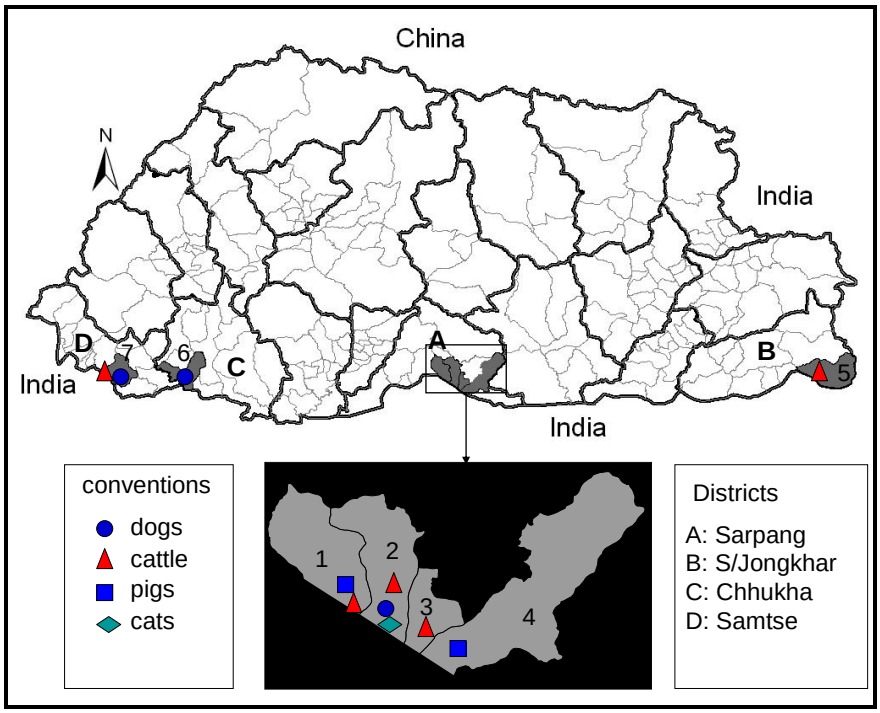

Figure 8.1: Map of Bhutan showing the geographical locations where the RABV isolates were obtained from different species of animals. Thick and thin lines on the map indicate the district and sub-district boundaries, respectively. Numerical values on the map indicate the names of sub-districts where RABV isolates were obtained (1) Bhur; (2) Gelephu; (3) Chhuzargang; (4) Umling; (5) Langchenphu; (6) Phuentsholing; (7) Samtse; see Table 1 for details). Symbols represent the species from which the virus was isolated.

8.2.3. RNA extraction and Reverse Transcriptase Polymerase Chain Reaction

The total RNA extraction from eluted specimens was performed by using a silica-guanidine thiocyanate protocol, NucliSense isolation reagent (Biomerieux, Boxtel, The Netherlands) according to the manufacturer instructions.

Single-step reverse-transcription polymerase chain reaction (RT-PCR) was performed using the One Step RT-PCR Kit (Qiagen GmbH). Specific sense primer CN8 (3' GT(TC) GGA TGT TAT ATG GG 5', nt 1013 - nt 1029) and an antisense primer CN4 (3' GGA TTG AC(AG) AAG ATC TTG CTC AT 5', nt 1514-nt 1536) were used for the N gene amplification. Binding sites of the primers were referred to the positions of total genomic sequence of the Pasteur Virus (PV) strain of RABV (GenBank accession no. M13215). One cycle of reverse transcription was done at 50°C for 35 min, followed by denaturation at 95°C

for 15 min. PCR was followed by 40 amplification cycles of denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min, and extension at 72°C for 1 min. Finally a 10 min extension step at 72°C was done to complete the amplification of the target gene. The final PCR products were run in a 2% agarose gel electrophoresis in TBE 1x buffer stained with ethidium bromide (at 1µg/ µl), and viewed under UV light to observe the specific bands (Hemachudha et al., 2003).

8.2.4. Gene sequencing

Gel slices containing RT-PCR products were excised from the gel and the RT-PCR products were purified using QIAquick PCR Purification Kit (Qiagen Ltd., Crawley, UK) according to the manufacturer's instruction. Direct sequencing of the N gene was performed using primers CN4 and CN8 with ABI PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) and ABI PRISM 310 DNA sequencer (Applied Biosystems, Foster City, CA, USA) (Hemachudha et al., 2003). For each RABV isolate, 395 nucleotides of N gene corresponding to position 1101 – 1495 of the Pasteur Virus genome (GenBank accession no. M13215) were analysed. All nucleotide sequences generated in this study were submitted to GenBank and their accession numbers were assigned (Table 8.1).

8.2.5. Phylogenetic analysis

Multiple sequence alignment was performed by using MUSCLE version 3.8 program (Edgar 2004). A neighbour-joining (NJ) analysis employing Kimura-2 parameter model with bootstrap statistic test of the phylogenetic tree (1000-replicates) was performed in MEGA version 4.0.2 program (Tamura et al., 2007). Thirty four additional N gene sequences of RABV previously published were retrieved from GenBank and used for comparison. The GenBank accession numbers and other details of the sequences are shown in Figure 8.2. The bootstrap values of >50% are shown on the tree branches. Tree Explorer module in MEGA 4.0 was used to obtain the graphic output. Phylogenetic analysis using Bayesian Markov Chain Monte Carlo (MCMC) method was also implemented in Mr Bayes version 3.1 program (Huelsenbeck et al., 2001) with a *GTR+I+gamma* evolutionary model. The analysis was run for 2,000,000 generations to get 200,000 samples from the posterior probability

distribution. The posterior probability values >0.95 was considered significant. The estimated tree topology was illustrated using Tree view program.

The geographical distribution of the Bhutanese rabies isolates were mapped using a Bhutan boundary shape files in ArcGIS 9.3 (ESRI, Redlands, USA, CA).

Table 8.1: Rabies virus isolates obtained from Bhutan and used in the present study

Isolate ID	District	Sub-district	Location	Host species	Year of isolation	GenBank accession
BHT 2445	Sarpang	Gelephu	Puranobasti	Cattle	2008	HQ166002
BHT 2685	Sarpang	Gelephu	Tankabasti	Cattle	2008	HQ166003
BHT 88	Sarpang	Gelephu	Lodrai	Dog	2008	HQ166004
BHT 183	Sarpang	Gelephu	Lekithang	Cattle	2008	HQ166005
BHT 187	Sarpang	Bhur	Majuwa	Cattle	2008	HQ166006
BHT 202	Sarpang	Bhur	Majuwa	Pig	2008	HQ166007
BHT 220	Sarpang	Gelephu	Majuwa	Cattle	2008	HQ166008
BHT 317	Sarpang	Gelephu	Lekithang	Cattle	2008	HQ166009
BHT 573	Sarpang	Gelephu	Pelrithang	Cattle	2008	HQ166010
BHT 333	Sarpang	Bhur	Jarwa	Cattle	2008	HQ166011
BHT 2	Sarpang	Gelephu	unknown	unknown	2008	HQ166012
BHT 101	Sarpang	Gelephu	unknown	unknown	2008	HQ166013
BHT 2000	Sarpang	Gelephu	Tankabasti	Dog	2009	HQ166014
BHT 2053	Sarpang	Gelephu	Puranobasti	Cattle	2009	HQ166015
BHT 2169	Sarpang	Chhuzargang	Pemaling	Cattle	2009	HQ166016
BHT 2196	Sarpang	Chhuzargang	Chhuzargang	Cattle	2009	HQ166017
BHT 2719	Sarpang	Umling	Dungmin	Pig	2009	HQ166018
BHT 630	Sarpang	Gelephu	Puranobasti	Cat	2008	HQ166019
BHT 631	Samtse	Samtse	Gerigoan	Cattle	2008	HQ166020
BHT 68	Samtse	Samtse	Lamatar	Dog	2009	HQ166021
BHT 481	Samdrup Jongkhar	Langchenphu	Talabasti	Cattle	2009	HQ166022
BHT 469	Samdrup Jongkhar	Langchenphu	Talabasti	Cattle	2009	HQ166023
BHT 450	Chhukha	Phuentsholing	P/ling town	Dog	2009	HQ166024

8.3.Results

Figure 8.2 shows the Bayesian phylogenetic tree inferred by comparing the partial N gene sequences of 23 Bhutanese isolates with 34 RABV N gene sequences available in GenBank. The topology of the NJ tree (result not shown) was similar to that of the Bayesian phylogeny and only its bootstrap values were mapped on the Bayesian tree. The tree shows four significant genogroups which was supported with posterior probability values >0.95. Group I consisted of RABV isolates from Iraq, Iran, Pakistan, India, and Nepal with 88% bootstrap supporting value. These isolates were previously described as Arctic–like–1 viruses circulating in the Middle East and South Asia (Kuzmin et al., 2004; Nadin-Davis et al., 2007; Kuzmin et al., 2008; Mansfield et al., 2006). The Bhutanese isolates formed a monophyletic cluster (labelled as Group I–a) within this Arctic–like–1 virus group. Group II is composed of six Arctic virus isolates originating from Russia, Canada, Alaska, and Greenland (bootstrap value of 99%). Group III consisted of seven viruses originating from Korea, Japan (formerly), southern Siberia, Far East Russia, and northern China (Inner Mongolia) with 97% bootstrap value. These isolates were previously described as an Arctic–like–2 viruses circulating in Northeastern Asia (Hyun et al., 2005; Kuzmin et al., 2004; Nadin-Davis et al., 2007; Kuzmin et al., 2008). Three isolates from Europe formed Group IV with 99% bootstrap support value, and the other four isolates from Asia (Thailand and Sri Lanka) formed separate clusters according to the country of origin. The phylogenetic analysis clearly revealed that all Bhutanese isolates were closely related to Indian strains and formed a large cluster with the Arctic–like–1 viruses, and were distinct from other Asian rabies virus isolates from Thailand and Sri Lanka.

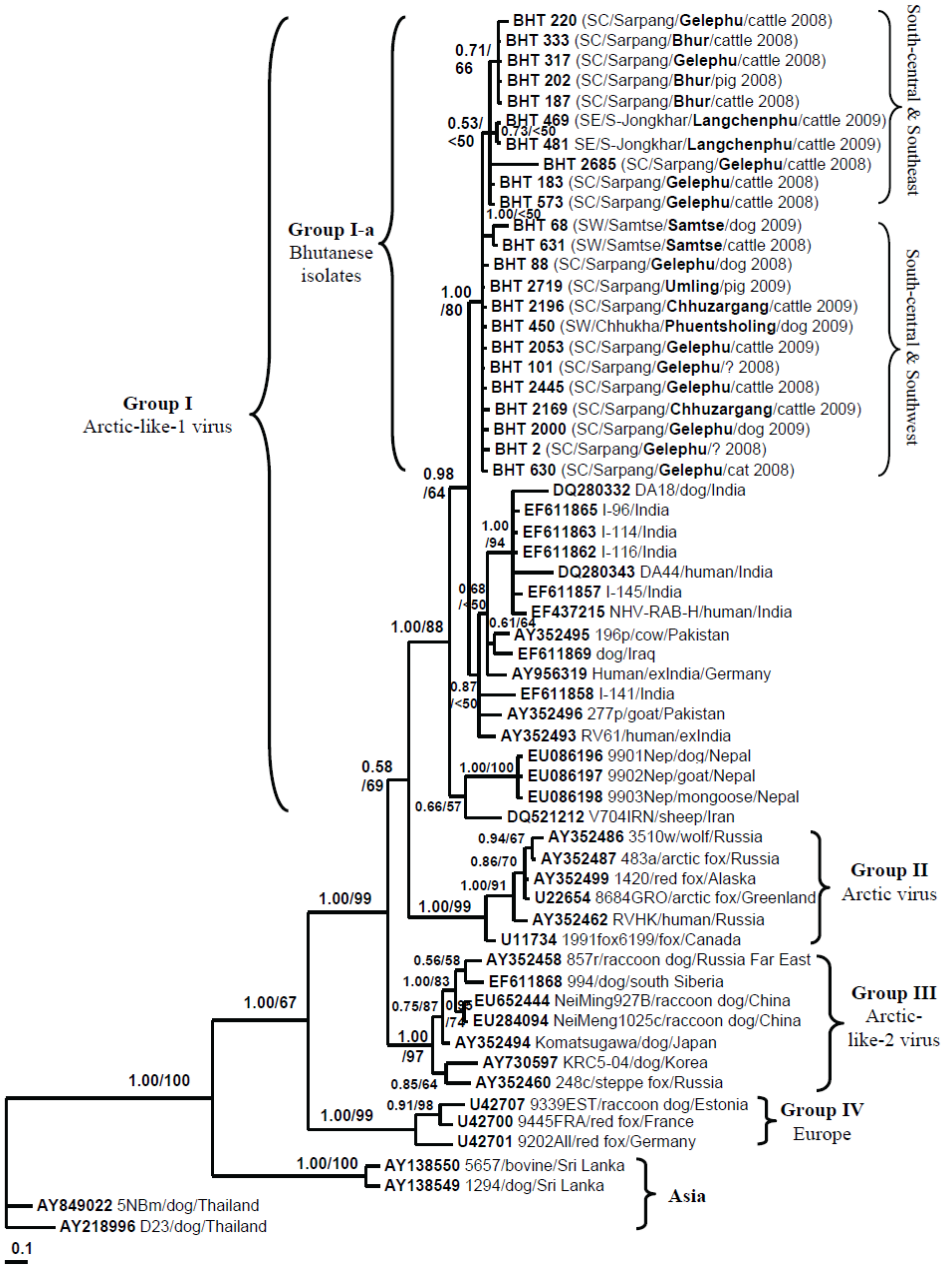

Figure 8.2: A phylogenetic tree of 60 RABV isolates analysed in this study. The tree was inferred by the Bayesian MCMC phylogenetic analysis method using a partial nucleoprotein (N) gene (395 *bp*) of 23 Bhutanese isolates compared to other N rabies sequences obtained from the GenBank database. The posterior probability value of each node is shown along the tree branches. Bootstrap branch-support values >50% (with 1000 replicates) generated from neighbour-joining (NJ) analysis is also indicated. A scale of base substitution number per site is shown at the bottom of the tree. Additional information of each Bhutanese sequence is mapped in parentheses as region/district/sub-district/host species/year of isolation (SW, Southwest; SC, South-central; SE, Southeast). The isolate ID, host species, country of isolation, and GenBank accession numbers of other rabies sequences are also given for examination. Various groupings found in this study are described in the text.

8.4.Discussion

This is the first report characterizing the molecular epidemiology of rabies virus isolates in Bhutan. The result confirmed that all isolates from Bhutan belong to the rabies virus and were not related to other rabies related viruses (e.g. from bats) of *Lyssavirus* genus. Our analysis showed that the Bhutanese isolates were highly similar and did not form any distinct subgroups although the isolates originated from different geographical areas. This suggests that the rabies virus variants circulating in southern Bhutan originated from a single common ancestor. Even though the Bhutanese isolates did not form clear subgroups due to low bootstrap values along most branches, they tended to separate into two subgroups based on the geographical locations of the isolates: the Southeast-and-Central and Southwest-and-Central subgroups (Figure 8.2, see Table 8.1 for details). However, more sequences from both Southeast and Southwest areas of Bhutan would be needed to provide greater phylogenetic support and confirm our hypothesis.

Phylogenetic analyses revealed that Bhutanese RABV isolates were more closely related to the RABV strains from India and Arctic–like–1 viruses circulating in South Asia (Nadin-Davis et al., 2007; Kuzmin et al., 2008), and could be grouped together as a large cluster of the South Asian Arctic–like–1 viruses (Group I in Figure 8.2). Geographically, Bhutan lies in the same Himalayan region of the Indian sub-continent where the emergence and extensive circulation of the Arctic–like–1 rabies viruses has been confirmed in India (Nadin-Davis et al., 2007). However, it should be noted that the Indian rabies sequences used for comparison in this study originated mostly from the southern part of India and ex-India (a foreigner who died of confirmed rabies after being bitten by a dog in southern India). Even though South India is quite far away from Bhutan, it is interesting to observe that Bhutanese and Indian RABV isolates share close genetic relationships, and suggests that they have originated from a common ancestor, probably the Indian strain. Translocation of infected dogs via human activities may be responsible for the spread of rabies virus between the two countries. In studies (Denduanboripant et al., 2005; Susetya et al., 2008; Gong et al., 2010), the translocation of dogs together with their owners migrating for work and trades purposes have been hypothesized as a probable mechanisms for the spread of rabies virus from country-to-country or region-to-region in some Southeast Asian countries (e.g. in Thailand, Vietnam and Indonesia).

In this study, a direct comparison between rabies isolates of Bhutan and those from Northeast India (which share a porous border) was not possible because there are no published sequence data available from Northeast India. Perhaps a similar rabies virus variant is circulating in dogs in Northeast India. This hypothesis needs to be confirmed by surveillance and conducting molecular studies and genetic analyses.

The limited distribution of rabies virus within the southern areas of Bhutan provides important information for planning and implementing a rabies control program in the country. It implies that a successful control and even elimination of rabies is promisingly practical in Bhutan if a sustained vaccination campaign of domestic dogs (>70% coverage) and dog population management is carried out efficiently. Control of rabies within the domestic dog cycle would break the chain of transmission (spill over) into other animals, and would eventually eliminate the disease from the country.

In conclusion, we recommend that more sampling of virus from potential reservoirs from different rabies outbreak areas and time periods is needed to provide detailed information about rabies virus transmission dynamics in Bhutan.

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Declaration of interest: None

8.5.References

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CHAPTER 9

9. HUMAN RABIES POST EXPOSURE PROPHYLAXIS IN BHUTAN, 2005–2008: TRENDS AND RISK FACTORS

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CHAPTER 9

Human rabies post exposure prophylaxis in Bhutan, 2005–2008: Trends and risk factors

Abstract

The aim of this study was to understand the use and distribution of human rabies post exposure prophylaxis (PEP) vaccine in Bhutan and to identify risk factors for receiving an incomplete course of the vaccine. We analysed post exposure treatment records from 28 medical hospitals from 2005 to 2008. Males (59%) accounted for significantly more PEP events than females (41%) across all age groups ($P < 0.001$). Children–particularly 5–9 years of age – received more rabies PEP than other age groups. Animal bite and non-bite accounted for 27% (n = 2239) and 16% (n = 1303) of rabies PEP, respectively, whilst 57% (n = 4773) of the PEP events had no recorded information about the reasons for post exposure treatment. Post exposure treatment was provided throughout the year with a higher number during the winter and spring months. The number of PEP events significantly ($P < 0.001$) increased between 2005 and 2008, from <1000 to >2800 events, respectively. Significantly ($P < 0.001$) more PEP events were reported from the southern parts of Bhutan that are endemic for rabies or those areas in eastern Bhutan that have reported rabies outbreaks than other parts of Bhutan. Forty percent (n = 3360) of the patients received an incomplete course of vaccine (<5-doses of vaccine intramuscular). Results suggest that patients with animal bite injury were less likely to receive an incomplete vaccine course than non-bite recipients, and patients presented to hospitals in rabies endemic or outbreak areas were less likely to receive an incomplete course than in non-rabies areas or rabies free areas. Similarly, patients presenting to hospitals for PEP during spring and summers months were less likely to receive an incomplete vaccine course than those during other seasons. Public education campaigns need to be conducted in Bhutan to reduce dog bite incidents and also to prevent mass exposures to rabies. A thorough assessment of each individual case based on the WHO guidelines would reduce unnecessary PEP (and therefore costs) in Bhutan.

9.1.Introduction

Rabies remains a significant public health problem in the world, with an estimated 55 000 human deaths occurring each year, mainly in developing countries of Asia and Africa (Knobel et al., 2005). Although rabies is inevitably fatal once clinical symptoms develop, the disease is preventable with timely treatment following an exposure to a rabid animal. The recommended post-exposure treatment consists of a thorough washing or flushing of the bite wound with soap and water or with viricidal agents, administration of rabies vaccine and infiltration of rabies immunoglobulin into and around the bite wound (WHO, 1996; WHO, 2010a). The main aim of post exposure prophylaxis (PEP) is to neutralize or inactivate inoculated virus in the wound before it can enter the nervous system of the patient (Warrel 2004). Therefore, a quick decision – based on a thorough assessment of the risk – must be made by the physician about whether to initiate rabies PEP. Post exposure prophylaxis is unnecessary and is a waste of resources if the biting animal is not rabid. As per the recent estimates of the World Health Organization (WHO), ≥15 million people receive PEP for rabies worldwide each year, mostly in India and China (WHO, 2010a). Children are at the greatest risk of rabies exposures and approximately 40% of PEP is given to children aged 5–14 years old (WHO, 2010a).

In Bhutan, rabies in animals is mainly prevalent in the southern districts that border India. Between 1996 and 2009, 814 cases of rabies were reported in dogs and other domestic animals in Bhutan (Tenzin et al., 2011). Similarly, from January 2006 to January 2011, nine human rabies deaths have been reported (eight cases in southern Bhutan and one in eastern Bhutan, 1.2/100000 population at-risk) (MoH, 2010; Kuensel 2009a; Kuensel 2010a; BBS, 2011). Because of the frequent outbreaks of rabies in dogs and farm animals in southern Bhutan, medical hospitals in all regions provide rabies PEP free of charge to those who have been bitten by animals (category II and III exposure) and also for category I exposure (touching/feeding animals, licks on intact skin) or for ingestion of meat and dairy products derived from suspected rabid animals. Currently, the human diploid cell vaccine (HDCV) is used and treatment follows the standard 5-dose (1 ml each) intramuscular administration regimen (Essen regimen) on days 0, 3, 7, 14 and 28 (WHO, 2010a). Although the number of human rabies cases in Bhutan is low and sporadic, the number of persons seeking rabies PEP is increasing because of the large number of stray and free-roaming dogs and increased incidents of dog bite, and because

of mass exposures (likely category I exposure) during periods of rabies outbreaks in animals (BBS, 2010, Bhutan Observer 2010; Kuensel 2009b). However, little is known about the epidemiologic characteristics of rabies PEP use in Bhutan.

In this study, we explored the epidemiology of human rabies PEP use based on treatment records between January 2005 and December 2008 from 28 selected hospitals and Basic Health Units, in Bhutan. The aim of this study was to describe the use and distribution of human rabies post exposure prophylaxis in Bhutan and to identify risk factors for an incomplete course of PEP. The results from this report are intended to assist medical practitioners and public health policy makers to reduce the incidences of human exposures and prioritizing the use of PEP based on the WHO guidelines. This is also likely to improve the national surveillance for rabies post exposure prophylaxis in Bhutan.

9.2.Materials and methods

9.2.1. Data sources

Bhutan is administratively divided into 20 districts and 205 subdistricts and has a population of about 0.68 million (NSB, 2005). There are 30 medical hospitals, 181 Basic Health Units (BHUs) and 38 indigenous medicine units, distributed in the districts and sub-districts in the country (MoH, 2010). Human animal bites are not a reportable condition in Bhutan, but bite victims visit hospitals for treatment and medical advice. Post exposure rabies prophylaxis vaccine is mainly provided via hospitals, but is also given at some of the BHUs located in rabies endemic areas or during rabies outbreaks. These hospitals and BHUs record basic information about patients provided with post exposure prophylaxis. For our analysis, we acquired PEP case data from 18 hospitals and 10 BHUs in Bhutan for the period January 2005 to December 2008. There was no PEP record (no data) in five of the civilian hospitals contacted. The PEP data from the remaining seven hospitals were not included due to logistic reasons of data collection (five military hospitals and two civilian hospitals). Similarly, majority of the BHUs were not considered for data collection due to logistic reason (remote location and also absence of rabies cases in those areas, assuming no PEP course would have been given). Only sampled BHUs from rabies endemic/outbreak areas were included for our analysis. There was no

reporting system of PEP data (especially individual case) to the main data management unit in the Health Ministry in Bhutan from the respective health centers and was difficult to access this information. Available information that we collected included demographic data (age and gender of victims), mode of contact and circumstances of exposure, date of patient presentation to the hospital or BHU and date of administration of PEP. The study was approved by the Ethics Committee of the Human Research and Epidemiology Unit, Ministry of Health, Bhutan.

9.2.2. Data analysis

The data were entered into a Microsoft Excel spreadsheet (Microsoft Excel, Redmond, WA). Descriptive analyses of the data were performed using Microsoft Excel. Chi-square tests were used to compare the difference in proportions of PEP recipients between gender, age groups, season and years. To make meaningful comparisons between age groups, observed frequencies of PEP for various age groups were compared with expected frequencies calculated from the Bhutan census data of 2005 (NSB, 2005). For comparing gender, seasonal and annual differences, equal expected frequencies were assumed between groups. A P-value of <0.05 was considered statistically significant.

Although the complete course schedule of post exposure prophylaxis (Essen regimen) is five doses (WHO, 2010a), many patients received an incomplete course of the vaccine (i.e. <5 doses). We conducted logistic regression analyses (GenStat Version 11.1 (VSN International Ltd., UK) to identify possible risk factors for incomplete PEP as the outcome variable (incomplete versus complete). The risk factors investigated included age group, gender, type of exposure (animal bite versus non-bite), rabies risk area (rabies outbreak or endemic area versus non-outbreak or free areas), season and year. Initially, we constructed contingency tables between explanatory variables and the outcome, calculated unadjusted odds ratios (OR) and the corresponding 95% confidence intervals and P-values. The variables that had a significant crude association with the outcome ($P < 0.25$) were selected for multivariable logistic regression model, using a forward stepwise selection approach. The selected variables were examined for collinearity in pairs by calculating Spearman's rank correlation coefficient (ρ) and no highly correlated pairs of variables ($\rho > |0.70|$) were observed and all variables were retained for further analysis. Variables with P-values <0.05 (based on the likelihood-ratio chi-squared test) in the

multivariable model were considered to be significantly associated with incomplete PEP. Model diagnostics were checked by examining the standardised Pearson residuals, leverage values, and delta betas (Dohoo et al., 2009).

To control for effects of clustering of observations from the same hospitals (and BHUs), we refitted the final model using a generalized linear mixed model by adding hospitals as a random effect term. Intra-class correlation (ICC) was calculated for the random effect term using the latent variable approach (Dohoo et al., 2009).

A Geographic Information System (ArcGIS 9.3, ESRI, Redland, CA, USA) was used to illustrate the distributions of human rabies post exposure prophylaxis given in different hospitals and BHUs in Bhutan. Here, the total number of patients that were given PEP in each selected hospital/BHU was mapped and represented by proportional symbols for visualization and for understanding the geographic distribution of PEP.

9.3.Results

Data were collected from a total of 9084 patients from 18 hospitals and 10 Basic Health Units in Bhutan. Data for pre exposure prophylaxis (117 cases) were excluded from the analysis because the aim of the study was to understand the use and distribution of post exposure prophylaxis only. Of the 8967 PEP events, 8315 (92.7%) patients were given various courses of rabies post exposure prophylaxis, 504 patients were given tetanus toxoid injection, and there was no information about the type of treatment provided in the remaining 148 patients. Thus for the analyses reported in this paper, we used a dataset of 8315 patients that were given PEP.

9.3.1. Gender

A total of 8302 patients (99.8%) had complete information for gender. Of these, 58.6% (n = 4864) were male and 41.4% (n = 3438) female. The proportion of rabies PEP recipients was significantly higher in males than females during the study period (χ^2 = 277; P < 0.001).

9.3.2. Age

Of the 8315 patients that received PEP, age was not recorded for 208 patients. The median age of patients receiving PEP was 21 years (range <1 to 96 years) and the modal age was 8 years. The observed and the expected frequencies of PEP recipients differed significantly across age groups (χ^2= 320; P < 0.001). The majority (n = 5438, 67.8%) of the patients that received PEP were below 30 years of age, and the maximum number of cases was observed in the 5–9 years (n = 1235; 15.2%) and then the 10–14 years (n = 1049; 12.9%) age groups. Figure 9.1 illustrates the distribution of rabies PEP by age and gender between 2005 and 2008 in Bhutan, in which the proportion of PEP recipients in males was significantly higher than females across all age groups.

9.3.3. Seasonal and annual trend of post exposure prophylaxis

Data on date of PEP administration were available for 8187 patients. The first date (day 0) of the 5-dose vaccine course was used to examine the seasonal pattern. Post exposure prophylaxis were given throughout the year, but more were provided during the winter (n = 2367; 28.9%) and spring (n = 2256, 27.6%) months (Figure 9.2). The overall proportion of vaccine recipients across seasons was significantly different (χ^2 = 156; P < 0.001). The proportion of PEP across each season in each year was also significantly different compared to that expected (P < 0.001). The number of patients that received PEP increased from <1000 patients in 2005 to >2800 patients in 2008 (χ^2 = 1059; P < 0.001).

9.3.4. Mode of contact and type of exposures

Both animal bite and non-bite incidents were presented to the hospitals and BHUs for post exposure prophylaxis. The majority (n = 4773; 57.4%) of the PEP case records did not have information about the reasons for PEP (data not recorded in the treatment register); 26.9% (n = 2239) were given PEP because of animal bites and 15.7% (n = 1303) for non-bite incidents. The non-bite incidents mainly included category I exposure (touching and feeding of animals) and also ingestion of meat and dairy products derived from rabid animals. The details of the various reasons for PEP are illustrated in Table 9.1.

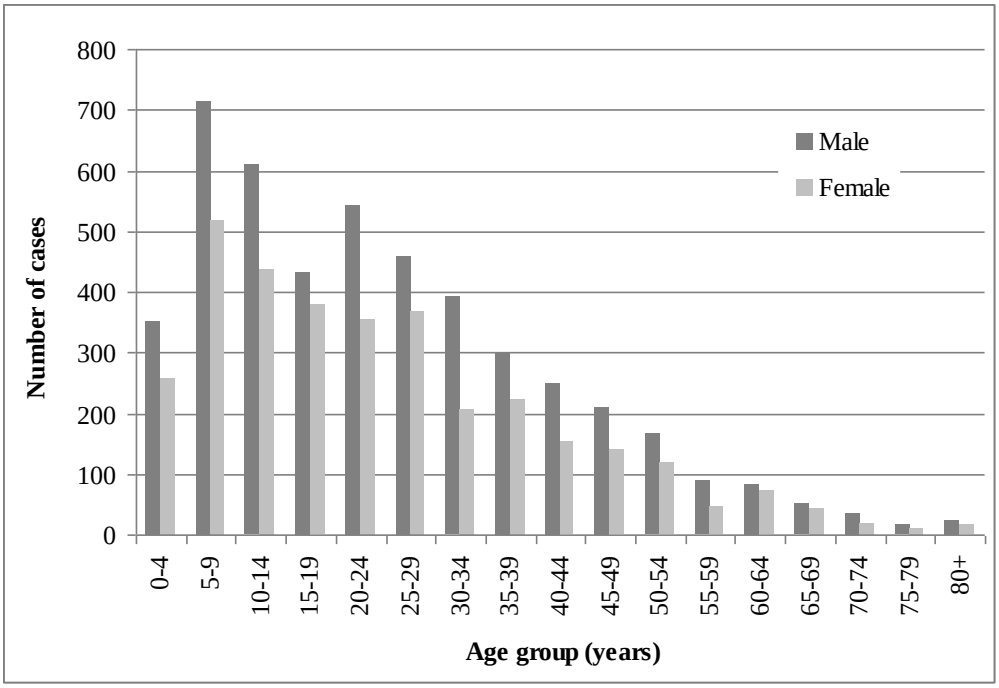

Figure 9.1: Distribution of rabies post exposure prophylaxis in people by age and gender in Bhutan from 2005 to 2008.

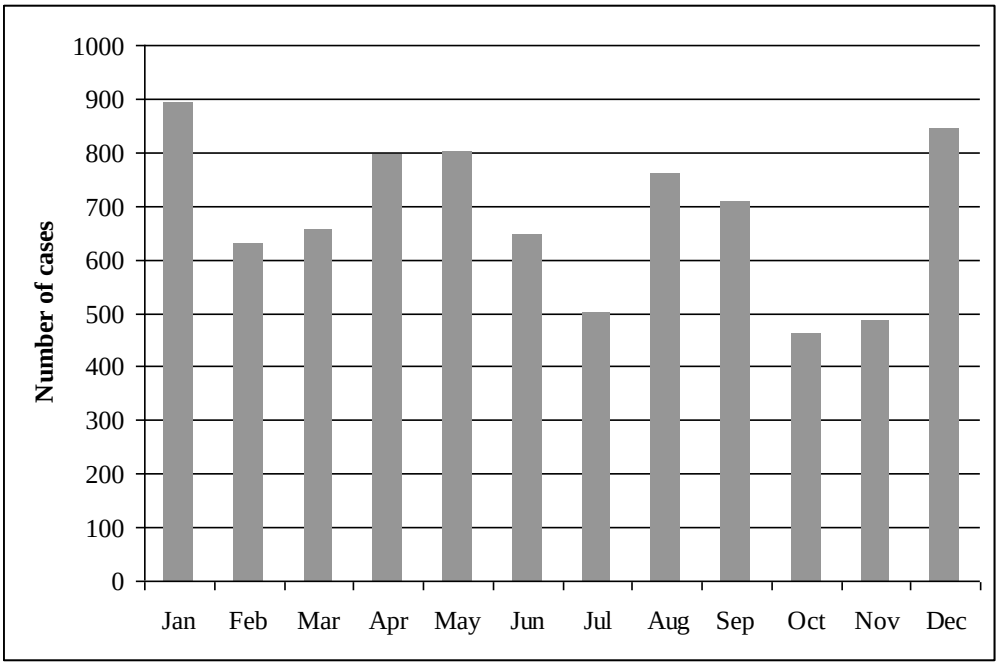

Figure 9.2: Monthly pattern of human rabies post exposure prophylaxis in Bhutan, 2005–2008.

Table 9.1: Descriptive statistics for mode of (likely) exposure and reasons for receiving rabies post exposure prophylaxis by people in Bhutan for a study period 2005 to 2008.

Reasons for PEP rabies vaccination	Number of PEP rabies vaccine given	% of PEP given
Dog bite	2099	25.24
Cat bite	71	0.85
Cat scratches	16	0.19
Rat bite	43	0.52
Other animal bite (monkey, bear, horse, goat, pig)	10	0.12
Contact with rabies patient	135	1.62
Contact/handled rabid cattle (feeding, touching and handling of carcasses during zoo sanitary measures)	234	2.81
Contact/handled rabid dog (during zoosanitary measures)	142	1.71
Dairy product consumption (milk, butter, cheese) derived from rabid or rabies suspected cow	607	7.30
Meat consumption (meat derived from rabid or rabies suspected cattle)	156	1.88
Other mode of contacts	29	0.35
Missing information	4773	57.40
Total	8315	100

9.3.5. Patterns of post exposure prophylaxis due to dog bites

Of the animal bite incidents, dog bites formed a major (93.7%) component of PEP in humans. Of the 2099 dog bite incidents in humans, 59.4% (n = 1243) of patients were male and 40.6% (n = 849) female, and the difference between the gender was significant ($\chi^2 = 74.20$; $P < 0.001$). The majority of the PEP due to dog bite was reported in children, especially within the age group of 5–14 years. The proportions of observed and expected PEP in humans due to dog bite were significantly different across age groups ($\chi^2 = 98.44$; $P < 0.001$). Figure 9.3 illustrates the age and sex distribution of PEP due to dog bites. It indicates that males received more PEP than females across all age groups due to dog bites in Bhutan. Dog bites were reported throughout the year, but more were reported in the spring months. The difference between the observed and expected cases was significant across all seasons ($\chi^2 = 22.17$; $P < 0.001$).

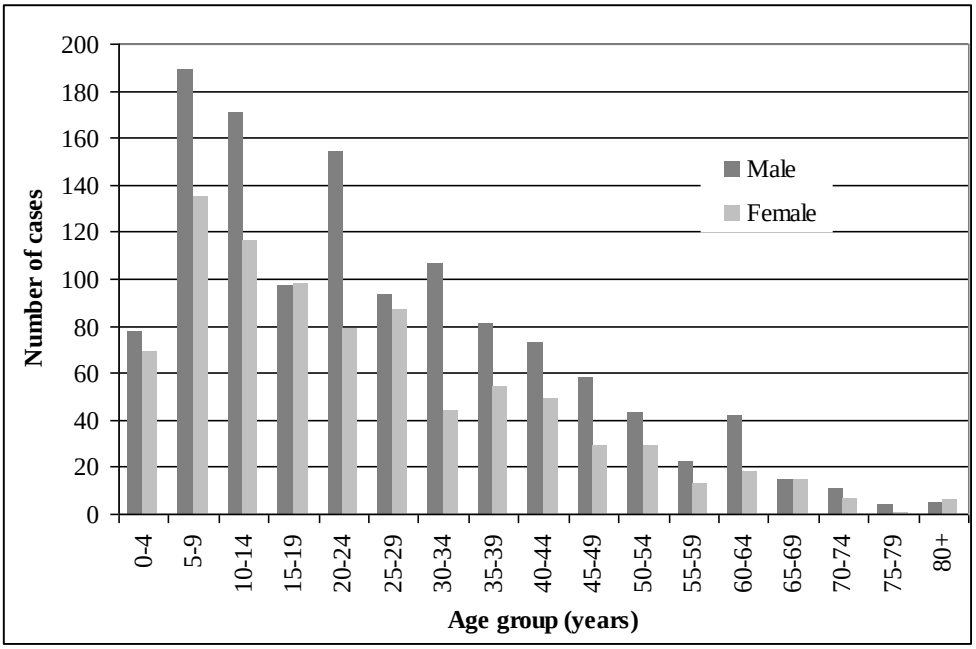

Figure 9.3: Distribution of dog bite victims classified by age and gender who received rabies post exposure prophylaxis in Bhutan, 2005–2008.

9.3.6. Rabies post exposure prophylaxis course

Of the patients receiving rabies PEP, 3859 (46.4%) received the standard 5-dose intramuscular injection (Essen regimen) on day 0, 3, 7, 14 and 28; 1096 (13.2%) patients received a 6-dose course (including day 90); 705 (8.5%) patients received a 4-dose course; 1337 (16.1%) patients received a 3-dose course; 551 (6.6%) patients received a 2-dose course; and 767 (9.2%) patients received only 1-dose of the vaccine. Overall, 3360 (40.4%) patients received an incomplete course of the vaccine (<5-dose course) based on the Essen regimen. Of the 3360 incomplete vaccine course recipients, 18.8% (n = 631) of the patients had animal bite injuries, 15.5% (n = 520) of the patients had non-bite incidents, and there was no information about the type of exposures for the remaining 2209 (65.7%) patients.

9.3.7. Risk factors for incomplete PEP course

Five variables (Table 9.2) were unconditionally associated ($P < 0.001$) with having an incomplete PEP vaccine course – age, type of exposure (animal bite versus non-bite), rabies risk area (rabies outbreak/endemic versus non-outbreak/free area), season and year. Gender was not associated with an incomplete PEP course ($P = 0.499$). Except for the

type of exposure variable (due to much missing data) and the gender, all other variables were included in the multivariable model. Patients that presented to hospitals in rabies endemic or rabies outbreak areas were more likely to complete the vaccine course than patients in non-rabies risk areas. Compared to 0–14 years, other age groups were more likely to have an incomplete vaccine course; patients that visited a hospital for PEP during winter and autumn months were more likely to have an incomplete vaccine course than in spring and summer months. The proportion of incomplete course of vaccine recipients significantly increased over the study years (2006 through 2008). Table 9.3 illustrates the final generalized linear mixed model in which the hospital was included as a random effect in the model. Coefficients for all variables were almost similar to that of the unadjusted multivariable model except for area (rabies outbreak or endemic area versus non-outbreak or free areas) which became non-significant in the model adjusted for clustering. The estimated intra-class correlation (ICC) in the final model was 0.63, indicating that a greater proportion of total variation was clustered at the hospital level. Examination of the residuals showed no evidence of unusual influence of any observation on the model predictions.

9.3.8. Spatial distribution of post exposure prophylaxis

The distribution of the total number of patients given rabies PEP in different hospitals (and BHUs) in Bhutan is shown in Figure 9.4. The map illustrates an unequal distribution of PEP patients among the hospitals. As expected, hospitals located in areas of southern Bhutan that are endemic for rabies and those hospitals with catchment areas covering reported rabies outbreaks during the study period (e.g. eastern Bhutan) had given significantly more (χ^2 = 1847, P < 0.001) PEP (n = 6117, 73.57%) compared to hospitals located in the interior of Bhutan that did not report rabies (n = 2198, 26.43%). Within the interior parts of Bhutan, some of the hospitals located in western Bhutan (e.g. Thimphu and Paro) that have no history of presence of rabies for at least 18 years had provided a large number of rabies PEP to patients (see Figure 9.4).

Table 9.2: Contingency table for explanatory variables with incomplete rabies post-exposure prophylaxis in Bhutan, 2005 to 2008, and odd ratios based on univariable logistic regression analysis.

Variables/categories	Incomplete	Complete	OR	95% CI	P-value[a]
Age group					<0.001
0–14	1012	1893	1.00	-	
15–29	1095	1438	1.42	1.27–1.59	
30–44	609	918	1.30	1.14–1.47	
> 45	456	686	1.23	1.07–1.41	
Gender					0.499
Female	1403	2035	1.00	-	
Male	1949	2915	0.97	0.88–1.06	
Type of (likely) exposure (animal bite vs. non bite)					<0.001
No	520	631	1.00	-	
Yes	783	1608	0.59	0.51–0.68	
Rabies outbreak/risk areas					<0.001
No	1452	1908	1.00	-	
Yes	746	4209	0.23	0.21–0.26	
Season of PEP					<0.001
Winter (Dec–Feb)	1041	1326	1.00	-	
Spring (Mar–May)	865	1391	0.79	0.67–0.91	
Summer (Jun–Aug)	637	1273	0.63	0.51–0.76	
Autumn (Sep–Nov)	762	892	1.08	0.96–1.21	
Year					<0.001
2005	238	641	1.00	-	
2006	846	1206	1.88	1.59–2.24	
2007	1139	1623	1.89	1.59–2.23	
2008	1137	1485	2.06	1.74–2.44	

[a] P-value based on likelihood ratio chi-square test of significance.

Table 9.3: Final generalized linear mixed logistic model with hospitals added as random effect for incomplete rabies post exposure prophylaxis course in Bhutan for the study period 2005 to 2008

Variables/categories	*b*	*s.e (b)*	OR	95% CI	P-value [a]
Hospital-level random effect	5.78	2.45	-	-	-
Constant	0.17	1.02	-	-	-
Age group					
0–14	-	-	1.00	-	
15–29	0.24	0.06	1.27	1.12–1.44	<0.001
30–44	0.26	0.07	1.29	1.11–1.49	
> 45	0.20	0.08	1.22	1.04–1.43	
Rabies outbreak/risk areas					0.287
No	-	-	1.00	-	
Yes	-1.26	1.18	0.28	0.03–2.89	
Season of PEP					<0.001
Winter (Dec-Feb)	-	-	1.00	-	
Spring (Mar-May)	-0.24	0.07	0.78	0.67–0.90	
Summer (Jun-Aug)	-0.39	0.07	0.67	0.57–0.78	
Autumn (Sep-Nov)	0.06	0.07	1.06	0.91–1.23	
Year					<0.001
2005	-	-	1.00	-	
2006	0.30	0.11	1.35	1.08–1.69	
2007	0.32	0.10	1.37	1.12–1.69	
2008	0.23	1.02	1.96	1.59–2.42	

[a] P-value based on likelihood ratio χ^2 test of significance.

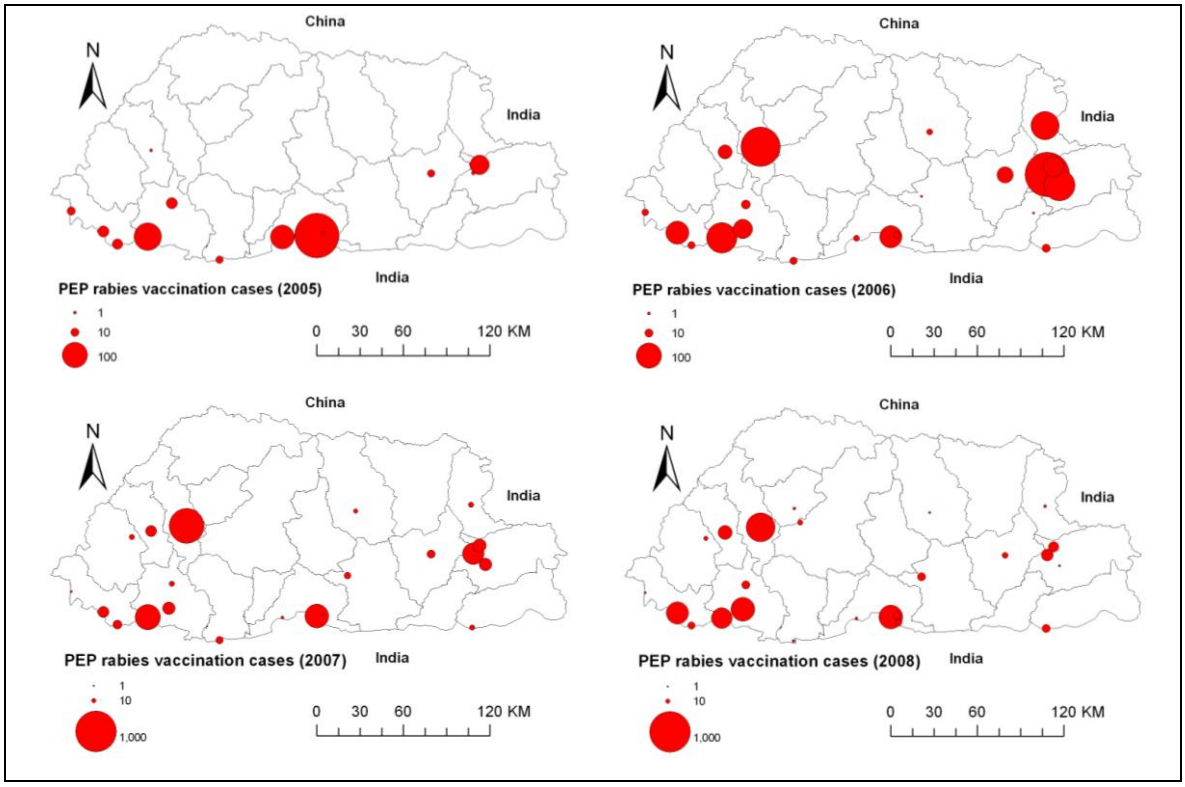

Figure 9.4: A district map of Bhutan showing the total number of persons (shown as proportional symbol) that were given rabies post exposure prophylaxis in different hospitals between 2005 and 2008.

9.4.Discussion

This report describes human rabies exposure and post exposure prophylaxis in Bhutan from 2005 to 2008. Our study showed that the overall prevalence of PEP was higher for children, especially for those up to 14 years of age, and then the trend decreased as age increased (Figure 9.1). This is in agreement with several other studies (WHO, 2010a; Martin et al., 1969; Helmick 1983; Pancharoen et al., 2001; Sriaroon et al., 2006, Blanton et al., 2005; Edison et al., 2010; Wyatt et al., 1999). It has also been suggested that animal bites in children are more likely to be reported to hospitals for wound treatment and possible vaccination because of parental concern (Martin et al., 1969). However, rabies experts from the Asian countries believe that children are at high risk of exposure to rabies, but less likely to report animal exposure, such as scratches or licks from dogs and cats, to their parents (Dodet, 2010). In this study, dog bite incidents were also more common in children than adults (Figure 9.3) suggesting that children received higher PEP than adults. We also identified that dog bites and PEP are significantly greater in males than females across all age groups (Figures 9.2 and 9.3), a finding observed in other

studies (Martin et al., 1969; Helmick 1983; Wyatt et al., 1999; Khokhar et al., 2003; O'Bell et al., 2006)

Post exposure treatment was provided throughout the year in Bhutan, with higher number in the winter and spring months (Figure 9.2). This may be associated with increased dog bite incidents and also mass exposure (likely category I exposure) to rabies outbreaks occurring during these seasons. A previous study revealed a significantly higher number of rabies cases in animals during late winter through to the summer months in Bhutan (based on data from 1996 to 2009) (Tenzin et al., 2011), and it is likely that more PEP would have been given during these months. However, PEP data from 1996 to 2004 were not analyzed (data not available) to determine if a correlation exists between the number of PEP events and the seasons. A trend in PEP administration is evident, with the number of cases increasing from <1000 in the year 2005 to >2800 cases in 2008. This suggests that the number of PEP events is associated with the increased incidence of rabies outbreaks and also dog bite incidents (BBS, 2010; Kuensel, 2007). For instance, mass anti-rabies vaccination of people (n > 900) was implemented at the time of a major rabies outbreak in eastern Bhutan from 2005 to 2007 (Tenzin et al., 2010a) and in south-west Bhutan (n > 600) during 2008 (Tenzin et al., 2010b), following contact with rabid animals (likely category I exposure) or ingestion of meat and dairy products derived from rabid animals resulting in increased post exposure treatment over this period. Similarly, other studies in the US have observed more post exposure treatment during summer or warmer months, which is usually associated with increased animal exposure (e.g. dog bite) (Helmick 1983; Blanton et al., 2005; Wyatt et al., 1999; O'Bell et al., 2006)..

Although the recommended PEP regimen may differ based on the category of exposures and the prior history of immunization (WHO, 2010a), we found no reports of rabies immunoglobulin being regularly administered to dog bite victims in Bhutan. This may be due to the high cost of the biological and limited supply in the market (Warrell 2004; Wilde et al., 2005; WHO, 2010a). Although many lives may have been saved by using rabies vaccine alone, there are published reports of PEP failures in the absence of rabies immunoglobulin and when PEP administration methods had deviated from recommended PEP protocols (Shill et al., 1987; Wilde et al., 1989; WHO, 1995; Wilde et al., 1996; Gacouin et al., 1999; Sriaroon et al., 2003; Matha and Salunke 2005; Wilde 2007; Rupprecht et al., 2009) or occurred as true failure even when correct post exposure

treatment protocol have been followed (Hemachudha et al., 1999; Wilde et al., 2005; Shantavasinkul et al., 2010). Considering the prevalence of rabies in domestic dogs in the border towns of southern Bhutan, it is imperative that the human rabies immunoglobulin (HRIG) (or less expensive equine rabies immunoglobulin (ERIG) or $F(ab)^2$ products of equine rabies immunoglobulin) (Quiambao et al., 2008) be administered to proven rabid dog bite cases (WHO category III exposure) in southern Bhutan, along with rabies vaccine and proper wound treatment (WHO, 2010a).

The rabies post exposure treatment is a complex decision making process for clinicians, especially in a country where there are large numbers of stray and free-roaming dogs, when the biting animal is not available for observation, where no quarantine of biting animals is practiced and laboratory testing is not normally done (McCombie 1989 ; Sriaroon et al., 2005)

Our study indicates that a large amount of rabies PEP is administered to patients whose risk of exposure to rabies virus is low (or non-existent) based on the WHO guidelines for post exposures prophylaxis (WHO, 2010a). It is also likely that the majority of people who were administered PEP for dog bites may in fact have been bitten by normal healthy dogs/pet dogs, and the anti-rabies vaccine may have been provided as a precautionary measure either due to pressure from the victims or the physician on duty cannot take the risk (Kuensel 2007; McCombie, 1989). We also found that approximately 16% of PEP was given to people with non-bite incidents, including touching and feeding of suspected/confirmed rabid animals, and ingestion of meat and dairy products (milk, butter, cheese) derived from suspected or confirmed rabid cattle. As per the WHO guidelines (WHO, 2010a), this entire group falls under category I exposure and no treatment is required. WHO guidelines clearly state that ingestion of raw meat or other tissues from animals infected with rabies is not a source of human infection (WHO, 2010a; 2010b). Although there have been no well-documented reports of human rabies transmission through such non-bite incidents (WHO, 2010a; Warrell 2004; ProMED-mail 2010), we believe that the PEP may have been administered by the clinician on duty because of the heightened concerns and anxiety of the people rather than in response to a true exposure (Blanton et al., 2005; McCombie 1989; Kuensel 2010b). For instance, recently, the death of a suspected rabid cow in one of the villages in southern Bhutan caused panic among the people after milk from the cow was consumed, but it was not

laboratory examined and confirmed due to logistic reason. This resulted in increased demand for post exposure anti-rabies vaccination of more than 200 people (Kuensel 2010b). Similar incidents have occurred in Bhutan during previous outbreaks, requiring mass post exposure vaccination of people (normally category I exposure) (Kuensel 2009b; Tenzin et al., 2010a; Tenzin et al., 2010b). Therefore, following WHO recommendations, based on the category of exposure and the epidemiological likelihood of the implicated animal being rabid to make a decision about PEP would avoid unnecessary PEP and reduce the expenditure (WHO, 2010a).

Furthermore, our study also shows that 43 people were given rabies PEP following rat bites (Table 9.1). Rat rabies is a rare phenomenon and may represent only incidental infection of rats by dog or cat attacks or by eating infected dog or cat carrion (Sriaroon et al., 2005; Kamoltham et al., 2002). House rats and mice (and rodent species in general) are not a natural reservoir of rabies and post exposure prophylaxis is seldom indicated for rat bites (Sriaroon et al., 2005; Corey and Hattwick 1975). In addition, several field studies in other countries have not recovered rabies virus from sampled rats (Sriaroon et al., 2005; Kantakamalakul et al., 2003; Patabendige et al., 2003; Wincewicz 2002).

Unlike other vaccines, PEP for rabies requires repeated visits by the patient to the hospital to complete a full course within 28 days (e.g. Essen regimen). Therefore, patient compliance is important for adequate immunization (Madhusudhana et al., 2002). Our analysis shows that about 40% of patients received an incomplete vaccine course (<5-dose course) between 2005 and 2008. Analysis of the dataset shows that patients presenting to hospitals in rabies risk areas (rabies outbreak or endemic area) were less likely to have an incomplete course of the vaccine. Among the incomplete recipients of vaccine, almost half of the patients were from the interior of Bhutan where the risk of infection – even if they were left untreated – was low or nonexistent since there have been no reported rabies cases in dogs in the interior of Bhutan for at least 18 years (Tenzin et al., 2010a). Similarly, animal bite victims are less likely to have an incomplete course than non-bite exposures (which pose less risk of infection). One study in Thailand (Sriaroon et al., 2005) have shown that almost one-third of the dog bite patients neglected to come for the last dose (day 90) of the vaccine series (Thai Red Cross ID regimen) and did not complete a full vaccine series. Asian Rabies Expert Bureau (AREB) members emphasized the need for a simplified PEP protocols-requiring reduced number of clinic

visits (Dodet, 2010). The shortening of time to complete the PEP vaccination schedule would help in increasing the patient compliance for completion of the PEP course and also would reduce the burden on patients, in terms of loss of time for work and transportation cost (Dodet, 2010; Shantavasinkul et al., 2010). However, research is ongoing to develop shorter schedules of PEP regimen. For example, most recently, a reduced dose rate – 1ml dose each on day 0, 3, 7, 14 (4 intramuscular injection) that complete the course within two weeks was introduced and recommended by US-CDC (Rupprecht et al., 2009; Rupprecht et al., 2010), and even shorter schedule "one week, 4-site" (4-site intradermal injections on day 0, 3 and 7), developed by the Thai Red Cross and the Queen Saovabha Memorial Hospital in Bangkok, Thailand (based on their preliminary findings) have shown a significantly ($P < 0.001$) higher geometric mean titer of rabies neutralizing antibodies on days 14 and 28 than the WHO approved Thai Red Cross (TRC) ID regimen (2-site ID injections on each days 0, 3, 7, and 28) (Shantavasinkul et al., 2010; Warrell et al., 2008) .

Post exposure treatments are provided free of charge, resulting in substantial cost to the primary health care system in Bhutan. For instance, the government spent about Bhutanese ngultrum (Nu.) one million for rabies vaccine from 2002 to 2005 and the expenditure increased to Nu.5.878 million in 2006 (Nu.45 = 1 US$) (Kuensel 2007). Similarly, it has been estimated that post exposure treatment (rabies vaccine) in humans accounted for most of the cost during the time of rabies outbreak in Bhutan (Tenzin et al., 2010b). Although WHO guide lines do not recommend PEP for category I exposure (WHO, 2010a), PEP is still being provided in Bhutan for these categories of exposures. If rabies vaccine injection is at all necessary and demanded by the people (BBS, 2010; McCombie 1989) (in situations such as ingestion of cooked meat and dairy products derived from a rabies suspected or confirmed animal), we recommend that intra-dermal regimens be considered for these category of exposure and can reduce the cost of treatment by about 70%, compared to conventional intramuscular regimens (Khawplod et al., 2006; Wilde et al., 1999). The intra-dermal regimes have been successfully used in many rabies endemic countries in Asia – including Thailand, Sri Lanka, The Philippines, and India – and have been found to be equally immunogenic and as effective as that of the standard intramuscular regimen (WHO, 1996; Madhusudhana et al., 2002; Khawplod et al., 2006; Wilde et al., 1999; Brown et al., 2008; Brown et al., 2011).

Finally, the overall goal of public health policy should be to reduce post exposure treatment in humans by conducting proper risk assessment, public awareness education programs and by increasing vaccination coverage in dogs (WHO, 2010a). Mass vaccination of dogs have been documented to be a more beneficial, less expensive, logical and ethical way to control rabies in animals than mass post exposure treatment of people alone in resource-limited countries (Bodel and Meslin 1990; Zinsstag et al., 2007; Kayali et al., 2006). A One-Health approach should be encouraged and strengthened by collaboration and pooling of resources between public health and veterinary services for rabies control program in Bhutan. Sharing of information about the local epidemiology of rabies in animals between animal and human professionals can help the clinician to make appropriate decisions for post exposure treatment in Bhutan. A thorough assessment of each individual case by following the WHO guidelines and decision making pathway algorithm (a flow-chart with a decision-making tree) (Corey and Hattwick 1975; Moran et al., 2000; Rupprecht and Gibbons 2004; Dubnov et al., 2006; WHO, 2010a; Dodet, 2010) would reduce the overuse or misuse of the post exposure treatment, and therefore reduce expenditure in Bhutan. We also recommend improving the country-wide PEP rabies surveillance system in hospitals and Basic Health Units by updating the database management and reporting system. This would provide a means to assess the expenditure and status of the rabies control program.

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9.5.References

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CHAPTER 10

10. DOG BITES IN HUMANS AND ESTIMATING HUMAN RABIES MORTALITY IN RABIES ENDEMIC AREAS OF BHUTAN

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CHAPTER 10

Dog bites in humans and estimating human rabies mortality in rabies endemic areas of Bhutan

Abstract

Background: Dog bites in humans are a public health problem worldwide. The issues of increasing stray dog populations, rabies outbreaks, and the risk of dogs biting humans have been frequently reported by the media in Bhutan. This study aimed to estimate the bite incidence and identify the risk factors for dog bites in humans, and to estimate human deaths from rabies in rabies endemic south Bhutan.

Methods: A hospital-based questionnaire survey was conducted during 2009−2010 among dog bites victims who visited three hospitals in Bhutan for anti-rabies vaccine injection. Decision tree modeling was used to estimate human deaths from rabies following dog bite injuries in two rabies endemic areas of south Bhutan.

Results: Three hundred and twenty four dog bite victims were interviewed. The annual incidence of dog bites differed between the hospital catchment areas: 869.8 (95% CI: 722.8–1022.5), 293.8 (240–358.2) and 284.8 (251.2–323) per 100,000 people in Gelephu, Phuentsholing and Thimphu, respectively. Males (62%) were more at risk than females ($P<0.001$). Children aged 5–9 years were bitten more than other age groups. The majority of victims (71%) were bitten by stray dogs. No direct fatal injury was reported. In two hospital areas (Gelephu and Phuentsholing) in south Bhutan the annual incidence of death from rabies was 3.14 (95% CI: 1.57–6.29) per 100,000 population. The decision tree model predicted an equivalent annual incidence of 4.67 (95% CI: 2.53–7.53) deaths/100,000 population at risk. In the absence of post exposure prophylaxis, the model predicted 19.24 (95% CI: 13.69–25.14) deaths/year in these two areas.

Conclusions: Increased educational awareness of people about the risk of dog bites and rabies is necessary, particularly for children in rabies endemic areas of Bhutan.

Author Summary

Dog bites in humans are a public health problem worldwide. We conducted a hospital based questionnaire survey and described the incidence and risk factors for human dog bites in Bhutan. We also estimated the human death rate attributable to rabies in two rabies endemic areas of south Bhutan. Our study shows that dog bites incidents in humans are common in the survey areas. There were significant gender and age differences in bite incidents; males and the children are affected the most. The majority of the victims were bitten by stray dogs, increasing the risk of rabies infection if not treated in time. Our decision tree model predicted 2.23 (95% CI: 1.20−3.59) human deaths from rabies/year, equivalent to an annual incidence of 4.67 (95% CI: 2.53–7.53) deaths/100,000 in the two rabies endemic areas of south Bhutan. In the absence of post exposure prophylaxis, the model predicted 19.24 (95% CI: 13.69–25.14) deaths/year in these two areas. The public should be encouraged to visit hospitals for post exposure prophylaxis following dog bite injury in south Bhutan.

10.1. Introduction

Dog bites in human are a serious public health problem and have been well documented worldwide (Overall and Love, 2001; Ozanne-Smith et al., 2001). In the United States, 4.7 million people were estimated to have been bitten by dogs in 1994 (an incidence rate of 16.1/1000 in adults and 24.5/1000 in children), of whom 800,000 required medical treatment (Sacks et al., 1996a). Later, a survey conducted during 2001–2003 in the USA estimated 4.5 million dog bites each year (an incidence rate of 16.6/1000 in adults and 13.1/1000 in children), an increase of 3% in adults and a decrease of 47% in children (Gilchrist et al., 2008). There have been similar reports of human dog bites in the United Kingdom (Morgan and Palmer, 2007), Belgium (Keuster et al., 2006), Spain (Rosado et al., 2009), Switzerland (Horisberger et al., 2004), Australia (Kreisfeld and Harrison, 2005), India (Sudarshan et al., 2006), and in the United Republic of Tanzania (Cleaveland et al., 2002). There are also several reports of dog bites incidents from other countries (Chomel and Trotignon, 1992; Bhanganada et al., 1993; Fe`vre et al., 2005; Georges and Adesiyun, 2008; Cornelissen and Hopster, 2009; Hossain et al., 2011) but most cases are believed to be unreported, especially in developing countries.

The consequences of dog bites to humans are many. Although the most common issue is the direct physical injury, sometimes the injuries may cause permanent disfigurement of the victims requiring reconstructive surgery (Thomas and Banks, 1990; Wolff, 1998; Gilchrist et al., 2008), psychological trauma and post traumatic stress (Peters et al., 2004; Keuster et al., 2006; Schalamon et al., 2006), and rarely attacks can be fatal (Sacks et al., 1996b; Sacks et al., 2000; Ozanne-Smith et al., 2001; Raghavan, 2008). Dog bites also result in a large monetary expense for treatment, emergency hospitalization and post-exposure treatment for rabies (Weiss et al., 1998; Quinlan and Sacks, 1999; Overall and Love, 2001; Sriaroon et al., 2006; Daniels et al., 2008). For instance, the annual medical cost and other expenses associated with dog bites in the USA were estimated to be between $235.6 and $ 253.7 million in 1994 (Quinlan and Sacks, 1999) while the French Postal Services reported 58,000 days of sick leave resulting from 3,357 bites to postal workers costing about US $ 2.5 million in 1985 (Chomel and Trotignon, 1992). Globally ≥ 15 million people receive rabies prophylaxis annually, mainly for dog bite injuries (WHO, 2010). In addition, dog bite incidents also have direct impacts on the dogs involved in the bites, resulting in their relinquishment to shelters and euthanasia (Miller et al., 1996; Patronek et al., 1996; Scarlett et al., 1999; Schalamon et al., 2006). Legislative action (e.g. Dangerous Dog Acts) have also been implemented in some developed countries to ban specific breeds of dogs because of the issue of increased bite incidents (Dangeriousdogacts, 1991; Jackson, 2005); such legislation does not appear to have been effective in reducing the incidence and severity of the bites. However, the severity of dog bite incidents is striking in developing countries: a vast majority of victims die from rabies infection (Knobel et al., 2005). There are an estimated 55,000 human deaths annually, particularly in Asia and Africa, due to endemic canine rabies (Knobel et al., 2005).

Like other countries, dog bites are common in Bhutan because of the presence of a large number of stray dogs in the streets (Bhutan Observer, 2008; Tenzin. et al., 2011a). Although no cases of directly fatal dog attacks on humans have been documented, deaths due to rabies from dog bites have been reported in south Bhutan (Kuensel, 2009, 2011a). Dog bites and the presence of rabies in the south border areas of Bhutan also results in substantial cost to the government (Tenzin et al., 2010a; Tenzin. et al., 2010b). For example, approximately Bhutanese ngultrum (Nu.) one million was spent on rabies vaccine from 2002 to 2005 and the expenditure increased to about Nu.5.878 million in

2006 alone (Nu. 45 = 1US$) (Kuensel, 2007b; Tenzin. et al., 2011a). In addition, rabies outbreaks also cause a substantial cost to farmers from the deaths of farm animals as a result of spill over infection from dogs (Tenzin et al., 2010a; Tenzin. et al., 2010b). Recently, there has been considerable media coverage on the stray dog population, the risk of dog bites to humans, and public nuisance in Bhutan (Kuensel, 2007ab; Bhutan Observer, 2008; Kuensel, 2010abc), yet there is no clear information about the epidemiology of human dog bites in Bhutan. Therefore, understanding the epidemiology of dog bites and the number of human deaths caused by rabies is important for public health planning program.

One of the approaches to understanding the scale of human deaths due to rabies is the use of decision tree models. This methodology has been developed by Cleaveland et al. (Cleaveland et al., 2002) using active rabies surveillance data in Tanzania. The model is designed based on a series of probability steps using the distribution of bite injury on different body parts and the probability of developing rabies. More recently this decision tree model has been used by the World Health Organization to estimate human deaths from rabies in Asia and Africa overall. Globally, a total of 55,000 (90% CI: 24,000–93,000) human rabies deaths annually was predicted (Knobel et al., 2005). Fevre et al. (Fe`vre et al., 2005) have also used this model to estimate human rabies deaths in Uganda using passive surveillance dog bite data. All these studies have provided clear information about the burden of rabies for proper planning program. In this study, we report the results of a dog bite survey conducted in three hospital areas of Bhutan. The objectives of this study were to:

(1) estimate the incidence of human dog bites, describe characteristics of bites and to identify risk factors for dog bites in some areas of Bhutan;

(2) understand the level of general knowledge and practice about rabies among bite victims; and

(3) estimate the number of human deaths due to rabies in two hospitals areas of south Bhutan (rabies endemic areas) using a decision tree model and compare the estimates to the observed data.

The findings from this study are expected to guide dog bite and rabies prevention and control programs in Bhutan.

10.2. Materials and methods

10.2.1. Study area and conduct of the survey

This study was conducted during 2009−2010 at three government medical hospitals in Bhutan – Jigme Dorji Wangchuk National Referral Hospital (JDWNRH), Gelephu Regional Referral Hospital (GRRH) and Phuentsholing General Hospital (PGH). These hospitals were selected because they provided the highest numbers of courses of rabies post exposure prophylaxis (PEP) to people during the preceding four years (2005–2008) (Tenzin. et al., 2011a). JDWNRH is located in the capital city of Bhutan – Thimphu (interior western Bhutan), whereas GRRH and PGH are located in Bhutan–India border towns in the south-west and the south-central regions, respectively. The catchments of GRRH and PGH hospitals are endemic areas for rabies, whereas rabies has not been reported in dogs or other domestic animals in Thimphu (catchments area for JDWNRH) for at least 18 years (Tenzin. et al., 2010a). Although rabies is not prevalent in the interior north of Bhutan, anti-rabies vaccination is normally administered to dog bite patients visiting hospitals for treatment due to the presence of rabies in south Bhutan (Tenzin. et al., 2011a).

In this survey, all dog bite victims who visited the injection section of these three hospitals to receive anti-rabies vaccine injections were interviewed using a pre-tested structured questionnaire designed to obtain information about the epidemiology of dog bite and bite-victim's knowledge about rabies. The questionnaire (Appendix 4) included closed questions about the demographics of the victims, circumstances of bite incidents, body parts injured and the degree of injury, ownership of biting animals, the level of knowledge about rabies, and post bite home treatment (washing of bite wound) prior to visiting the hospital for medical treatment. The interviews were conducted by the staff nurse of the respective hospitals providing PEP rabies vaccination, after the patients were prescribed rabies vaccine by the clinician on duty. The survey was conducted from 18 February 2009 to 8 February 2010 at PGH, 16 February to 20 September 2009 at JDWNRH, and from 11 February to 4 December 2009 at GRRH.

Ethics statement

The purpose of the study was explained to each individual and they were informed that participation was voluntary and data collected were confidential. The participants who agreed to be interviewed were made to sign a consent form. The study was approved by the Ethics Committee of the Human Research and Epidemiology Unit, Ministry of Health, Bhutan (reference No.RESEARCH/PROPOSAL/08/8636).

10.2.2. Data analysis

The questionnaire data were entered into a database (EpiInfo version 3.5.1). Descriptive analyses of the data were performed using a statistical software package (SPSS version 11.5, SPSS Inc, Chicago IL). Bhutan population census data from 2005 (NSB, 2005) were used to determine the population at-risk in the three hospital catchment areas. Dog bite incidence was calculated for each hospital catchment area and was expressed as the number of bite cases per 100,000 population at-risk. The initial plan to conduct the survey in each hospital for one year had to be modified due to logistical reasons. Therefore, because the survey period was variable between the three study areas (<12 months), the annual incidence for each hospital catchment area was estimated and was expressed as number of bite cases per 100,000 population at-risk per year.

The relationship between the number of bite cases and population density of the three hospital catchment areas according to age group was examined using the Spearman rank correlation test. Chi-square tests were used to compare the difference in proportions of dog bites between gender, age group and other variables. To make meaningful comparisons between age groups, observed frequencies of bites for various age groups were compared with expected frequencies calculated from the 2005 Bhutan census data (NSB, 2005). For other variables, equal expected frequencies were assumed between groups. The variables of interest – such as occupation of the victims, ownership of dog involved in the bite incident, circumstances of bite incidents and knowledge about rabies – were compared among the three hospitals using Chi-square tests. A p-value of <0.05 was considered statistically significant.

In addition, standardized morbidity ratios (SMRs) were calculated for gender and age categories to examine whether there was any significant difference between the observed and the expected bite incidents in each category. This was expressed as: SMR = observed

frequencies ÷ expected frequencies. The expected frequencies for each category were calculated using the 2005 catchment area census population for each hospital (NSB, 2005).

10.2.3. Modeling human rabies deaths

We estimated human rabies deaths in the two hospital areas (Phuentsholing and Gelephu) of south Bhutan by using dog bite data and constructing a decision tree model developed by (Cleaveland et al., 2002). Dog bite data from JDWNRH (interior west Bhutan, described in the descriptive analyses in this paper) was excluded from this model to avoid biased estimates since no rabies cases have been reported (either in dogs and humans) in that hospital region for at least 18 years.

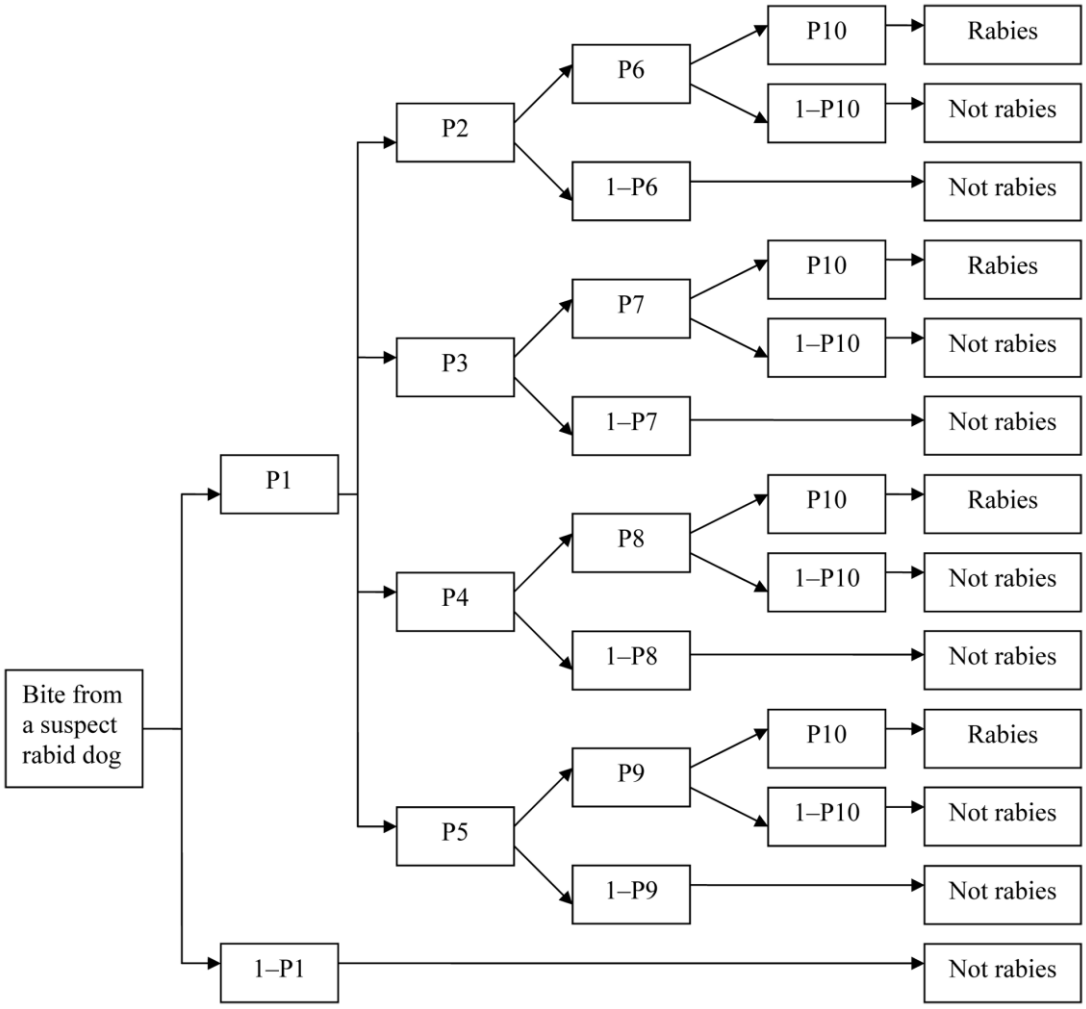

Figure 10.1: Decision tree model outlining the probability of rabies deaths. The model is adapted from Cleaveland et al., 2002. Probabilities (P1–P10) are defined in Table 4 and described in the methods section. The probability calculated represents the probability of death following the bite of a suspect rabid dog.

Table 10.1: Model parameters, probability distributions and data sources used in the prediction of human deaths from rabies in Phuentsholing and Gelephu areas of south Bhutan from dog bite survey data.

Parameter	Description	Probability and distribution	Data source
P1	Probability of a suspected rabid dog being confirmed rabid on laboratory diagnosis (33/46)	Binomial: p=0.720; n=46	Field data, (Tenzin. et al., 2011b)
P2	Bite injury to the head or neck	Point estimate: (11/193) =0.057	Field data
P3	Bite injury to the hand or arm	Point estimate: (41/193) = 0.212	Field data
P4	Bite injury to the trunk	Point estimate: (3/193) = 0.016	Field data
P5	Bite injury to the leg or foot	Point estimate: (137/193) = 0.715	Field data
P6	Probability of developing rabies following a bite injury to the head by a rabid dog	Triangular: minimum = 0.30, mode = 0.45, maximum = 0.60	(Cleaveland et al., 2002; Fe`vre et al., 2005; Knobel et al., 2005)
P7	Probability of developing rabies following a bite injury to the hand or arm by a rabid dog	Triangular: minimum = 0.15, mode = 0.28, maximum = 0.40	(Cleaveland et al., 2002; Fe`vre et al., 2005; Knobel et al., 2005)
P8	Probability of developing rabies following a bite injury to the trunk by a rabid dog	Triangular: minimum = 0.00, mode = 0.05, maximum = 0.10	(Cleaveland et al., 2002; Fe`vre et al., 2005; Knobel et al., 2005)
P9	Probability of developing rabies following a bite injury to the leg or foot by a rabid dog	Triangular: minimum = 0.00, mode = 0.05, maximum = 0.10	(Cleaveland et al., 2002; Fe`vre et al., 2005; Knobel et al., 2005)
P10	Probability of an individual receiving post exposure treatment if bitten by a suspected rabid dog (see methods)	Triangular: minimum = 0.80, mode = 0.90, maximum = 0.95	(Tenzin. et al., 2011a)

The decision tree model consists of 10 probability steps *(P1 to P10)* (Figure 10.1 and Table 10.1). The first step *P1* is the rabies recognition probability (the proportion of suspected rabid dog bites that are, in fact, rabid) (Cleaveland et al., 2002). In our survey dog bite victims had no knowledge about the status of the biting dog (whether rabid or not) and no biting dogs were traced back to observe their rabies status. Therefore, the disease status of the biting dogs was unknown. However, we used the proportion positive to rabies virus (by florescence antibody test) of rabies suspected dogs in these two areas for the period 1996 to May 2011. A total of 46 dog brain samples were collected from these two hospital areas and submitted to the Veterinary Laboratories in Bhutan for rabies virus confirmation. Of these, 33 (72%) samples were positive for rabies virus by Fluorescent antibody test. Therefore, the rabies recognition probability (*P1*) in dogs was

estimated to be 72% for this analysis (Table 10.1). In addition, a sensitivity analysis was conducted on *P1* using different rabies recognition probabilities to explore the impact on the final model output (Figure 10.2). The probability of rabies recognition was 68% (17/25) in Tanzania (Cleaveland et al., 2002), 51% (43/85) in Kenya (Kitala et al., 2000), 42% to 77% in Uganda (Fe`vre et al., 2005), and between 38% to 50% in Asia and 64% in Africa (Knobel et al., 2005).

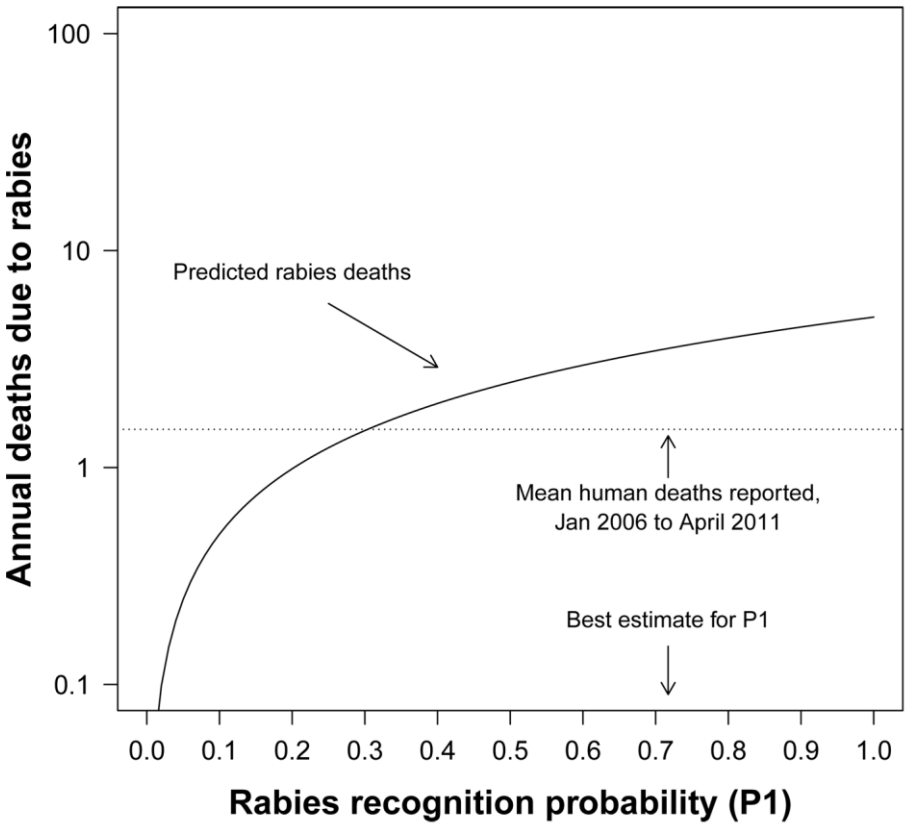

Figure 10.2: Predicted annual human deaths from rabies. Deaths are predicted in Phuentsholing and Gelephu areas of south Bhutan in relation to rabies recognition probability (*P1*) and mean number of human rabies deaths reported, adapted from Cleaveland et al., 2002.

For the *P2*−*P5* probability steps, the dog bite injury data were classified according to the distribution of bites on different body parts: head/neck (*P2*); hand/arms (*P3*), trunk (*P4*), and legs/thigh (*P5*) (Figure 10.1 and Table 10.1); and the age group of the victims: 0−4; 5−9; 10−14, and >15 years of age (Table 10.2). The point estimates (proportion of bites) on each body part and according to each age group were then calculated using the dog

bite data (Tables 10.1 and 10.2). The probability (*P6−P9*) of developing rabies following the bite of a rabid dog to the head (*P6*), arms (*P7*), trunk (*P8*) and legs (*P9*) were 45%, 28%, 5% and 5%, respectively (Cleaveland et al., 2002; Fe`vre et al., 2005; Knobel et al., 2005) (Table 10.1).

Table 10.2: Distribution of bite injuries on the body according to age group of dog bite patients in Phuentsholing and Gelephu hospital areas of south Bhutan. In the case of multiple bites, the site of the most severe bite is given. The row proportions (point estimate) are calculated for each group

Age group (years)	Head/neck	Hand/arms	Trunk	Legs/thigh	Total
0−4	6	3	1	13	23
Point estimate	0.261	0.130	0.043	0.565	1
5−9	2	11	1	35	49
Point estimate	0.041	0.224	0.020	0.714	1
10−14	1	3	1	28	33
Point estimate	0.030	0.091	0.030	0.848	1
> 15	2	24	0	62	88
Point estimate	0.023	0.273	0.000	0.705	1

The last step, the probability of receiving post exposure treatment (*P10*) was determined on the basis of previously published data (Tenzin et al., 2011a). There is a very high probability of people receiving PEP in these areas of Bhutan due to endemicity of rabies, rabies vaccine being freely available in the hospitals, easy accessibility of these hospitals (centrally located in the towns) and due to free medical services. Our previous study (Tenzin. et al., 2011a) on the use of PEP in Bhutan showed that large doses of anti-rabies vaccination were freely provided to all patients with dog bite injuries (even in the interior of Bhutan where rabies is not present) and also to all the WHO categories of exposure (I, II and III) (WHO, 2010). To account for this in the model, we used the following as the probability of receiving PEP following a bite from a suspected rabid dog in these two study areas: minimum=80%, most likely=90% and maximum=95% (Table 10.1).

The probability of dying of rabies following a bite from a suspected rabid dog was then calculated from the probability parameters using the formula (Cleaveland et al., 2002) *Pdeath (probability of death) = P1 × ((P2 x P6) + (P3 x P7) + (P4 x P8) + (P5 x P9)) × (1−P10).* Then the total number of deaths (Tdeath) caused by rabies per year in these two hospital region were calculated using the formula (Cleaveland et al., 2002) *Tdeath = (I ×*

Q × *Pdeath/100 000)*, in which '*I*' is the incidence of suspected rabid dog bites per 100,000 population at risk per year and '*Q*' is the total population at-risk (n=47721) which was based on the 2005 population and housing census data of Bhutan (NSB, 2005). The confidence limits for the total number of deaths from rabies were calculated by assigning the probability distribution to the inputs parameters (Cleaveland et al., 2002) and running Monte Carlo simulations for 10,000 iterations using R software (version 2.12.0 (210-10-15), R Development Core Team, http://www.r-project.org) (Appendix 4). The mean and the 95% confidence interval were estimated. The total number of human deaths due to rabies was also estimated in each age category of dog bite victims on the basis of the bite injury distribution on the body (Table 10.3).

Table 10.3: Annual predicted death counts and incidence rate from rabies for different age groups in Phuentsholing and Gelephu areas of south Bhutan, calculated using the decision tree model.

Age group	Annual predicted death counts from rabies in humans (95% Confidence interval)	Predicted deaths from rabies in humans/100,000/year (95% Confidence interval)
0–4 years	0.35 (0.20−0.55)	7.43 (4.15–11.62)
5–9 years	0.48 (0.26−0.77)	9.16 (4.90–14.72)
10–14 years	0.23 (0.11−0.38)	4.58 (2.25–7.70)
> 15 years	0.88 (0.47−1.42)	2.69 (1.43–4.34)

10.3. Results

A total of 339 patients were interviewed at the three hospitals. Fifteen questionnaires were excluded from the analyses because two were incomplete and 13 were related to cat (n = 8) and other animal bite (n = 5) injuries. The final analysis was undertaken on 324 dog bite questionnaires, but not all questions were completed and so the sample size differed for each question analyzed.

10.3.1. Bite incidence

A total of 131, 100 and 93 dog bite victims were reported to JDWNRH, GRRH and PGH, respectively, for post bite rabies vaccination during 2009–2010. The incidence of bites differed significantly (P<0.001) between the hospital areas: 869.8 (95% CI,

722.8−1022.5), 293.8 (240.9−358.2) and 284.8 (251.2−323.0) per 100,000 population per year in Gelephu, Phuentsholing and Thimphu, respectively.

10.3.2. Age and gender of the victims

There were significantly more bite cases in males (201/324, 62%) than females (123/324, 38%) (χ^2 = 18.78; P<0.001). Males were 1.15 times (95% CI 1.00–1.32) more likely to report dog bites than expected, compared to females (P=0.053) (Table 10.4). The median age of all bite cases was 17.5 years and the modal age was 6 years (mean 21.2 years; range: <1 to 80 years). There were significant differences in the proportion of bite victims between various age categories (χ^2=73; P<0.001). Approximately two-thirds of the bite cases were reported in people <25 years of age. However, those in the age groups 5−9 years were the most common victims of dog bites (74/324; 23%) and were 2.3 times (95% CI 1.83–2.88) more likely to report dog bites than expected, compared to adults or other age categories (P<0.001) (Table 10.4). There was also significant correlation between dog bite incidents and population density in the three hospital catchment areas according to age group (r_s = 0.77; P = 0.005). Figure 10.3 illustrates the age and gender distribution of dog bites and shows that the incidence of dog bites was greater amongst children aged 5−9 years and greater in males than females across all age groups.

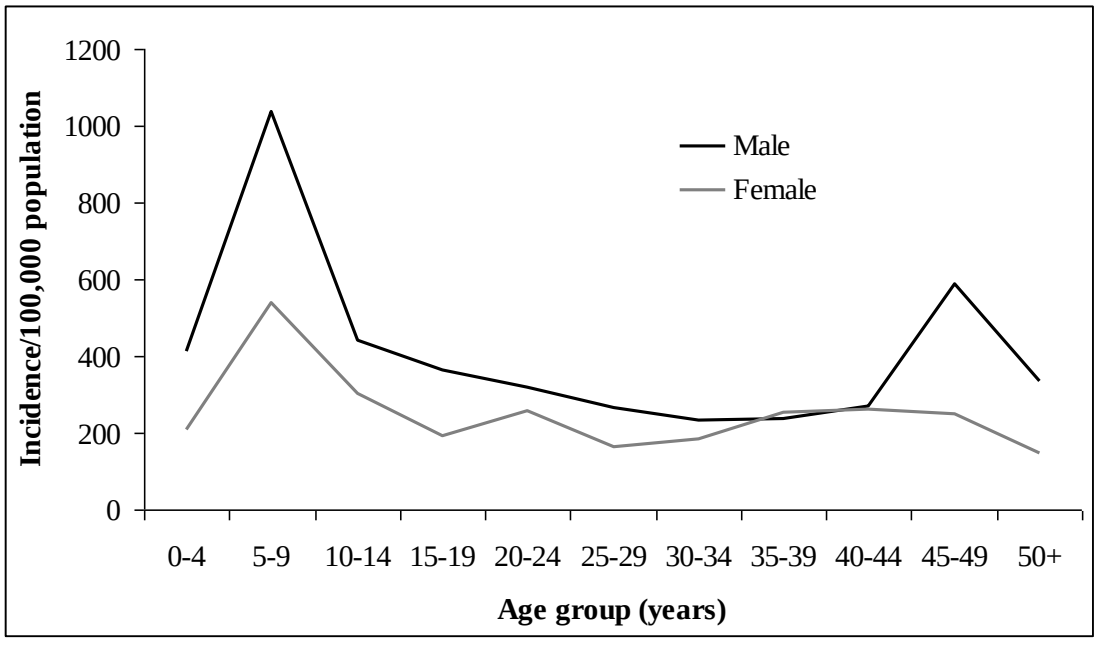

Figure 10.3: Annual incidence of dog bites/100,000 population classified by age and gender. Data is based on a survey of dog bite victims attending three hospitals (Jigme Dorji Wangchuk National Referral hospital, Phuentsholing General hospital, Gelephu Regional Referral hospital) in Bhutan, 2009–2010

Table 10.4: Standardized morbidity ratio of reported dog bite incidents according to gender and age of victims in three hospital catchment areas (Jigme Dorji Wangchuk National Referral hospital, Phuentsholing General hospital, Gelephu Regional Referral hospital) in Bhutan, 2009–2010.

Variables/categories	N	Percent	SMR*	95% Confidence interval	*P*-value
Gender					
Female	123	38	0.83	0.69–0.98	0.030
Male	201	62	1.15	1.00–1.31	0.053
Age group (years)					
0–4	31	10	0.97	0.67–1.35	0.882
5–9	74	23	2.31	1.83–2.88	<0.001
10–14	40	12	1.14	0.83–1.54	0.393
15–19	27	8	0.73	0.50–1.04	0.089
> 20	152	47	0.80	0.68–0.94	0.005

N=number of reported dog bite victims in each group.
*SMR >1 means that they are more likely to report bites than expected, a value <1 means that they are less likely to report bites than expected and a value of 1 means that they are equally likely to reported than expected. Children in age group 5–9 are 2.3 times more likely to report dog bites than expected comparing to other age groups.

10.3.3. Occupation of the victims

A significant difference (χ^2=138.44; P<0.001) in the proportion of bites was observed between the various occupational groups of the victims. School children were the most common (45%) victims of dog bites. There were also significant differences among the three hospital catchment areas with respect to the number of bite cases among the various occupational groups (χ^2= 39.83; P<0.001), with school children reporting more bites than other occupational groups in JDWNRH, PGH and GRRH (Table 10.5).

10.3.4. Ownership of dogs involved in the bites

The victims were predominantly bitten by stray dogs (231/324; 71%), rather than by owned dogs (93/324; 29%) (χ^2 = 58.77; P<0.001). Of the owned dogs, 71 cases were bitten by a neighbor's dog. A significant difference among the three hospital catchment areas was observed with respect to the bite incidents by owned and stray dogs (χ^2=6.124; P=0.047), with the largest difference (19 versus 81%) occurring in PGH (Table 10.5).

Table 10.5: Comparison of occupation and other responses to a questionnaire of dog bite victims attending three hospitals (Jigme Dorji Wangchuk National Referral hospital, Phuentsholing General hospital, Gelephu Regional Referral hospital) in Bhutan, 2009–2010

Variables/categories	Hospital			Total (%)	*P*-value
	JDWNRH (N, %)	PGH (N, %)	GRRH (N, %)		
Occupation					<0.001
Housewives/businessman	19 (14)	13 (14)	2 (2)	34 (10)	
Employees	42 (32)	16 (17)	9 (9)	67 (21)	
Farmers	11 (8)	7 (7)	16 (16)	34 (10)	
Preschool children	12 (9)	17 (18)	14 (14)	43 (13)	
School children	47 (36)	40 (43)	59 (59)	146 (45)	
Ownership of dogs involved in the bites					0.047
Own dogs	40 (31)	18 (19)	35 (35)	93 (29)	
Stray dogs	91 (69)	75 (81)	65 (65)	231(71)	
Circumstances of bites (was the bite provoked?)					0.183
Yes	26 (20)	19 (23)	10 (12)	55 (19)	
No	104 (80)	64 (77)	72 (88)	240 (81)	
Availability of biting dog for observation					0.052
Yes	45 (35)	19 (21)	35 (36)	99 (31)	
No	85 (65)	71 (79)	63 (64)	219 (69)	

10.3.5. Circumstances of the bite incident

Most bites were reported to be unprovoked (240/295, 81%) rather than provoked (55/295, 19%) (χ^2=116.02; P<0.001). However, there were no significant differences between the circumstances of bites incidents reported in the three hospitals (χ^2=3.39; P=0.183) (Table 10.5).

10.3.6. Anatomical site of bite and injury type

Most (90%) dog bites were inflicted on the extremities with 73% on the legs and 18% on the hand/arms. There was a significant difference between the bite incidents and the anatomic sites (χ^2=412.07; P<0.001). However, a majority of the bites were single bite injuries (218/324, 67%). There was also a significant difference between the severity and anatomic locations of bite wounds (χ^2=15.18; P<0.019) (Figure 10.4). Figure 10.5

illustrates the anatomic location of dog bite wounds according to the age group of the victim. The lower extremities (leg/thigh) were the most common site of bite in all age groups and no significant difference was observed between these two age groups (0–24 versus ≥25 years) with respect to different anatomic bite sites (χ^2=4.05; P=0.212). However, no case of a fatal dog bite injury was reported during the study period.

10.3.7. Status of dogs involved in the bite and availability for observation

Of the 320 respondents, a majority (189, 59%) of the victims mentioned that the disease (rabies) status of the dogs involved in the bite incidents was unknown, 101 victims (32%) mentioned that the biting dog looked normal, and 30 victims (9%) mentioned that the biting dogs were suspected of rabies. However, a majority of the victims responded that the biting dog was not available for observation (219/318, 69%). Three respondents mentioned that the dog involved in the bite was killed. There was only borderline significant differences among the three hospital catchments areas with respect to the response of the victims that the dog was unavailable for observation (χ^2 = 5.911; P=0.052).

10.3.8. Seasonal distribution of bites

Dog bite incidents were reported throughout the year with more bite incidents during the spring months (March–May) (129/324; 40%) followed by winter months (December–February) (90/324; 28%) and autumn (September–November) (72/324; 22%). The reported incidents were lowest during the summer months (June–August) (33/324; 10%). There were significant differences in the proportions of bite incidents between the seasons (χ^2 = 58.88; P<0.001).

10.3.9. Previous history of dog bite

A previous history of dog bite was reported by 40 victims: 33 persons were bitten twice, five persons were bitten three times, and two persons were bitten four times.

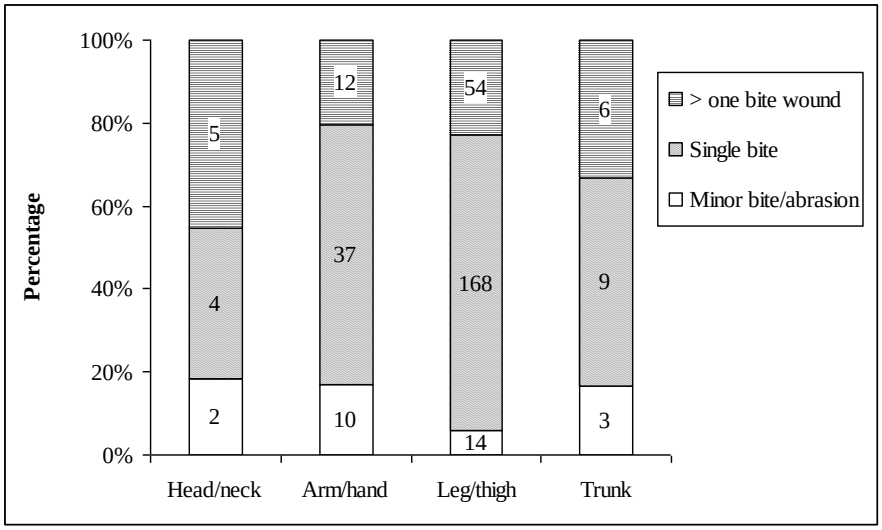

Figure 10.4: Anatomic location and severity of dog bite wounds. Data is derived from victims attending three hospitals (Jigme Dorji Wangchuk National Referral hospital, Phuentsholing General hospital, Gelephu Regional Referral hospital) in Bhutan, 2009–2010 (the number in the figure indicates the number of bite victims).

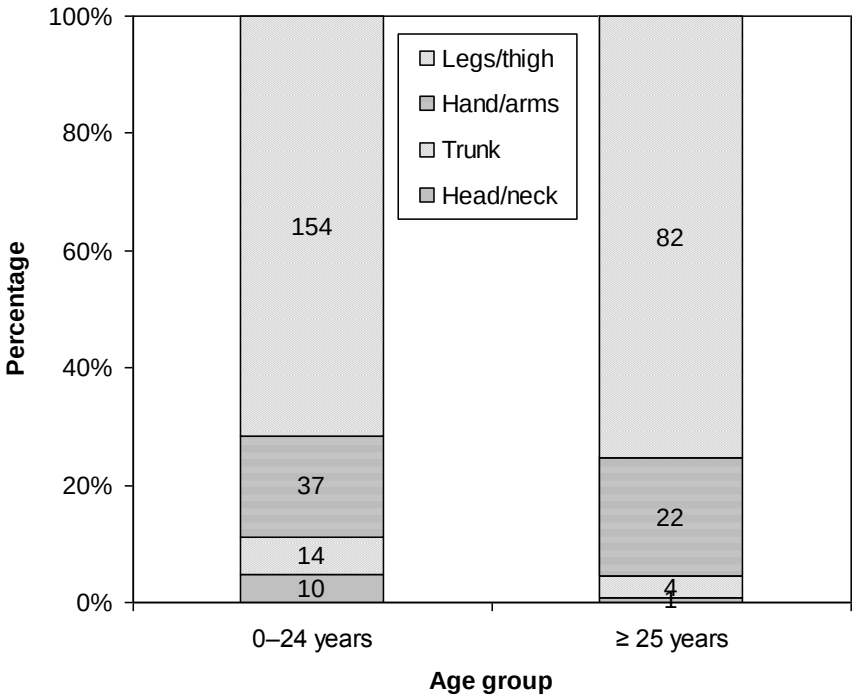

Figure 10.5: Anatomic location of dog bite wounds according to age group. Data is derived from victims attending three hospitals (Jigme Dorji Wangchuk National Referral hospital, Phuentsholing General hospital, Gelephu Regional Referral hospital) in Bhutan, 2009–2010 (the number in the figure indicates the number of bite victims). Age was categorized into the two groups indicated.

Table 10.6: Comparison of knowledge and practices about rabies prevention based on the response of dog bite victims attending three hospital areas (Jigme Dorji Wangchuk National Referral hospital, Phuentsholing General hospital, Gelephu Regional Referral hospital) in Bhutan, 2009–2010.

Variables/categories	Hospital catchment areas JDWNRH (N, %)	PGH (N, %)	GRRH (N, %)	Total (%)	*P*- value
Had heard of rabies					<0.001
Yes	89 (69)	78 (91)	96 (96)	263 (83)	
No	40 (31)	8 (9)	4 (4)	52 (17)	
Believe that regular vaccination of dogs can prevent rabies					<0.001
Yes	116 (91)	69 (88)	92 (93)	277 (91)	
No	12 (9)	9 (12)	7 (7)	28 (9)	
Had washed dog bite wound with soap and water					<0.001
Yes	72 (59)	27 (29)	73 (74)	172 (55)	
No	50 (41)	65 (71)	26 (26)	141 (45)	

10.3.10. Knowledge about rabies

Of the 318 respondents, a majority (263, 83 %) of the victims or the guardian/parents of the victims (in minor cases) was aware of and had heard about the fatality of rabies. There was a significant relationship between the hospital catchment areas with respect to knowledge about rabies (χ^2 = 34.26; P<0.001). The proportion of bite victims from the rabies endemic areas reported to two hospitals (GRRH and PGH) were more aware of rabies compared to victims reported to JDWNRH. A majority (277/305, 91%) of the victims were also aware that rabies can be prevented and controlled by regular vaccination of dogs (χ^2=203.28; P<0.001). However, no significant difference was observed among the three hospitals with respect to the dog bite victims' knowledge that rabies can be prevented by vaccination of dogs (χ^2 = 1.054; P=0.590) (Table 10.6).

10.3.11. Bite wound washing at home

Of the 313 respondents, 141 victims (45%) had washed their bite wound with soap and water at home before presenting to the hospital. However, there were no significant

differences in the proportions of those who washed and those that did not wash the bite wound at home ($\chi^2 = 3.07$; P=0.08) (Table 10.6).

10.3.12. Rabies post exposure prophylaxis

All dog bite victims were given rabies post exposure prophylaxis (vaccine) on the first day (day 0) of their visit to the hospital, irrespective of the epidemiological likelihood of the implicated dog being rabid and the local epidemiology of rabies. The patients were believed to have been advised to complete the course (5-doses) since Bhutan follows the 5-dose intramuscular regimen (WHO, 2010). However, no post exposure vaccine course data (subsequent vaccine series dada) were collected for our analysis since our earlier study on PEP vaccine use had provided important information about the epidemiologic characteristics of rabies post exposure prophylaxis in Bhutan (Tenzin. et al., 2011a).

10.3.13. The prediction of human rabies death

From 2006 to April 2011, there were eight reported human deaths due to rabies in Gelephu (n=4) and Phuentsholing (n=4) hospital areas, which accounted for a cumulative incidence of 16.76 deaths/100,000 population. The mean annual number of reported human deaths due to rabies (from 2006 to April 2011) in these two areas were 1.5 (95% 0.75–3.00), equivalent to an annual incidence of 3.14 (95% CI: 1.57–6.29) per 100,000 population. Based on our dog bite survey data, the model predicted 2.23 (95% CI: 1.20–3.59) deaths per year, equivalent to an annual incidence of 4.67 (95% CI: 2.53–7.53) deaths/100,000 population in Gelephu and Phuensholing areas of south Bhutan. Table 10.3 summarizes the predicted death distribution by age group in these two hospital catchment areas (Gelephu and Phuesholing), and shows that annual human predicted deaths from rabies per 100,000 population were greater for ages <15 years. In the absence of any post exposure treatment, the 223 bite incidents would result in a total of 19.24 (95% CI: 13.69–25.14) deaths per year in these two areas of Bhutan, equivalent to an annual incidence of 40.31 (95% CI: 28.70–52.68)/100,000 populations. Figure 10.3 shows the predicted annual human deaths due to rabies in the two hospital areas (Gelephu and Phuentsholing) of south Bhutan in relation to different rabies recognition probabilities (*P1*) and the mean number of deaths reported between 2006 and April 2011.

10.4. Discussion

The annual incidence of dog bites was higher in the GRRH catchment area than in the other two hospital catchment areas. This difference could be explained by the geographical location of the individual study areas, population density and the local epidemiology of rabies. It is important to note that the GRRH catchment area is located in the south-central area of Bhutan, which includes towns adjacent to Indian towns across the international border, and has experienced frequent rabies outbreaks (Tenzin. et al., 2011b). It is likely that the dog bite victims (irrespective of the disease status of biting dogs – whether rabies suspect or normal healthy dogs or pet dogs) might have reported to the GRRH for medical treatment because of a fear of rabies. In addition, high dog population density and trans-border movement of dogs (particularly stray dogs in such border towns) could be another reason for the high incidence of dog bites. A recent media report indicates that about 500 people visited GRRH for dog bite injuries treatment during 2010 (Bhutantimes, 2011) which greatly exceeds the number we recorded in our study. Reported dog bite incidents have been increasing, as have human deaths from rabies infection (Kuensel, 2009, 2011b).

The risk factors for human dog bites identified in this study are very similar to those of other studies conducted elsewhere, mostly in developed countries (Overall and Love, 2001). For instance, dog bite injuries were more common in children, particularly those aged 5−9 years, and more common in males than females. A previous study on post exposure rabies events in humans in Bhutan also showed that PEP were provided more often to younger age groups and to males (Tenzin. et al., 2011a). In general, our results are in agreement with those from several studies conducted both in developed (Sinclair and Zhou, 1995; Sacks et al., 1996a; Weiss et al., 1998; Overall and Love, 2001; Gichrist et al., 2003; Wake et al., 2006; Daniels et al., 2008; Rosado et al., 2009) and developing (Pancharoen et al., 2001; Khokhar et al., 2003; Sriaroon et al., 2006; Sudarshan et al., 2006; Georges and Adesiyun, 2008; Hossain et al., 2011) countries. Increased dog bite incidents in children is considered a behavioral risk because of their extreme curiosity, lack of inhibition, limited knowledge and experience about dog behavior, and inability to protect themselves from an attack (Berzon et al., 1972; Sinclair and Zhou, 1995; Sacks et al., 1996a; Overall and Love, 2001; Daniels et al., 2008). It has also been suggested that bites in children are more likely to be reported than in adults because of more parental

concern towards children or the severity of their injuries (Sacks et al., 1996a). However, it is also believed that children in developing countries do not report minor bites or scratches to their parents, which increases the risk of rabies infection (Dodet et al.,2010).

Animal bites usually occur as a result of provocation by the victims during play and by abusing/teasing the animal, repeated irritation or as unprovoked bites in which people are attacked (Overall and Love, 2001; Reisner et al., 2007). Our results show that a majority of the dog bites occurred as an unprovoked bite (76%) in which people were attacked and, mostly by stray dogs (68%). This suggests that the presence of a high density of dogs on the street (commonly seen in developing countries, including Bhutan) (Pancharoen et al., 2001; Khokhar et al., 2003; Villa et al., 2010) is a risk factor for increased reports of dog bites incidents in Bhutan. It has been suggested that human behaviors not generally regarded as provocative can frighten dogs or may be misinterpreted by some dogs as an invasion of their territory and may incite an attack (Overall and Love, 2001). It is also important to note that the high number of unprovoked bites in this study may be due to biased opinions given by the victims. However, rabid dogs (in rabies endemic countries) would be aggressive and bite people indiscriminately without any provocation.

Dog bite injuries to the lower extremities were more common (72%) than to other body parts, irrespective of the age of the victim in this study. This result is in contrast to some other studies in which more bite injuries were reported to the head, neck and face (Sacks et al., 1996a; Weiss et al., 1998; Overall and Love, 2001; Feldman et al., 2004; Lang and Klassen, 2005; Schalamon et al., 2006; Daniels et al., 2008; Rosado et al., 2009). However, this difference may be explained by the ownership of the biting dogs, the physical environment of the bite incidents and the study areas. In the developed world, pet dogs (owned dogs or neighbors' dogs) – which are known to the victim – are most commonly involved in bite incidents to the head, neck and face. This may be due to the short stature of children and playful interaction with pets – kissing, hugging and petting (Szpakowski et al., 1989; Sacks et al., 1996a; Overall and Love, 2001; Lang and Klassen, 2005; Schalamon et al., 2006; Rosado et al., 2009). Our study showed that people were more commonly bitten by stray dogs and bites occurred more commonly to the lower extremities. Similarly, some other dog bite studies in developing countries have shown that stray dogs were commonly involved in bites to the extremities (Pancharoen et al.,

2001; Khokhar et al., 2003; Sudarshan et al., 2006), which is in agreement with our findings. It is also likely that the victims (e.g. children) would have used a hand or leg to abuse/tease the dogs or to separate fighting dogs or defending dog attacks, resulting in more bites on the extremities (Morton, 1973; Rosado et al., 2009). Rabid dog bites to the upper body and extremities (head, neck, arm, hand) are more dangerous than bites to the lower extremities. The median risk of death following rabid dog bites to the head, hand, trunk and legs have been reported to be 45%, 28%, 5% and 5%, respectively (Cleaveland et al., 2002; Fe`vre et al., 2005; Knobel et al., 2005).

Our study showed that dog bite incidents occurred throughout the year, with increased cases from late winter to mid-spring (February through April). It is difficult to correlate factors that might explain this peak during this period in Bhutan. However, there is a possible bias in estimated annual dog bite incidence in this study because the survey could not be conducted for one full year within two of the survey areas due to logistical constraints, and dogs bites might have seasonal variability. Studies in developed countries have reported that most dog bite incidents occur during the spring and summer months (Sacks et al., 1996a; Ostanello et al., 2005; Keuster et al., 2006; Schalamon et al., 2006; Daniels et al., 2008). Such observations have been explained by behavioral changes: more interaction between pets and children during the warmer months with less parental supervision, thus increasing the risk of bite incidents (Keuster et al., 2006). In Thailand, reports of dog bite incidents in children increased during the months of March–May and October, the period of school vacation (Sriaroon et al., 2006).

Understanding people's level of knowledge about dog bites and the risk of potential zoonotic disease transmission – particularly rabies – is important for planning an awareness education program. In this study, the majority (81%) of dog bite victims (or the parents/guardian of minors) were aware of rabies, which is in agreement with the results from some other studies in Asia (Singh and Choudhary, 2005; Ichhpujani et al., 2006; Matibag et al., 2007; Matibag et al., 2008). However, the respondents that reported to two hospitals (GRRH and PGH) in south Bhutan were more aware of rabies than respondents who reported to JDWNRH in Thimphu. This difference is expected because the south is an endemic region for rabies with frequent reports of outbreaks; the people might have previously seen rabies cases in dogs and farm animals, or might have heard about rabies

from family, friends or the news media (Tenzin. et al., 2011b). The interior of Bhutan is free of rabies and people may not be aware of the disease. On the contrary, most victims (52%) reportedly did not wash their wound with soap and water at home before visiting the hospital for medical treatment. This finding suggests that a proper health educational program on rabies and wound care at home (WHO, 1996; 2010) is required. Cleaning and flushing of the bite wound with soap and water immediately after being bitten is one of the most important steps recommended by the WHO. This procedure will remove much of the rabies virus from the wound and may considerably reduce the risk of contacting rabies (if the biting dog is infected with rabies) (Rupprecht and Gibbons, 2004; WHO, 2010).

Predicted human deaths due to rabies from the decision tree model were almost the same as the annual mean human rabies deaths reported in these two study areas, indicating that there is no serious under-reporting of rabies in Bhutan. The fatal nature of the disease (with classic rabies symptoms), availability of free medical services and accessibility to the hospitals might be the main reasons for good reporting of human rabies deaths in Bhutan, but some extent of under reporting of dog bites may be possible. The model also predicted that in the absence of any post-exposure treatment, the annual dog bite counts of 223 would result in a total of 19.24 (95% CI: 13.69–25.14) deaths per year in these two areas of Bhutan, which is equivalent to an annual incidence of 40.31 (95% CI: 28.70–52.68) deaths per 100,000 population. Therefore, human rabies PEP is important for rabies prevention in Bhutan. On the basis of laboratory examination of submitted samples in these two areas, a rabies recognition rate of 72% was used in this study. However, the proportion of the victims bitten by a confirmed rabid dog is largely unknown since tracing of the source of biting dogs and confirmation of rabies is not usually done. Active surveillance of bite injuries and tracing of the biting dogs would provide clear information about the public health hazard of rabies. Nevertheless, with high recognition probability of rabies in dogs in these areas, it is important to make people aware of the danger of rabies and encourage reporting to hospitals for post bite treatment. It is important to note that we have estimated human deaths from rabies in two areas of south Bhutan that are endemic for rabies. Accordingly, the human population at-risk for canine rabies was also assumed to be the number of people living within these two hospital catchments areas. We did not include the entire population of Bhutan in order to avoid bias estimates, since rabies cases have not been reported in the interior of Bhutan.

In conclusion, this study has provided important information about human dog bites, risk factors and the burden of rabies in Bhutan. The presence of large numbers of stray dogs is a public health issue in Bhutan. Intervention measures should include public educational programs on dog behavior, dog-child interaction, and the importance of responsible dog ownership, particularly in children (Chapman et al., 2000; AVMA, 2001). Lessons on dog behavior, the risk of dog bites, bite wound management (e.g. washing with soap and water) and rabies can also be integrated into the elementary school curriculum to educate children on the public health hazard of dog bites (Dodet, 2010). In a randomized control trial of an educational intervention for the prevention of dog bites in children in Australia, (Chapman et al. 2000) demonstrated that children who had been educated and provided information on ways to approach dogs displayed appreciably greater precautionary behaviors than children that did not receive any awareness education on dog behaviors and intervention. Therefore, dog bite preventive education is important in children. Similarly, enforcement of regulations for licensing of dogs and rabies vaccination, stray dog population management and animal birth control programs are important to reduce the bite incidents and post bite treatment cost (AVMA, 2001; Villalbi et al., 2010). One study in Spain has shown a significant decline in hospitalizations caused by dog bites after enactment of stricter regulations on dog ownership (Villalbi et al., 2010). This suggests that a regulatory approach may also help in reducing dog bite injuries in addition to other educational programs. Continuing surveillance of dog bites is necessary to detect trends and evaluate the effect of prevention efforts. For this, a national dog bite database and reporting system implemented through local primary health care centers may be appropriate for the surveillance and monitoring of dog bite incidents in Bhutan.

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CHAPTER 11

11. COMMUNITY-BASED STUDY ON KNOWLEDGE, ATTITUDES AND PERCEPTION OF RABIES IN GELEPHU, SOUTH−CENTRAL BHUTAN

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CHAPTER 11

Community-based study on knowledge, attitudes and perception of rabies in Gelephu, South−Central Bhutan

Abstract

Community knowledge, attitudes and practices are important both for prevention of human deaths due to rabies and for control of the disease in animals. This study was a cross-sectional survey investigating the level of community knowledge as well as attitudes and perceptions about rabies in Gelephu, south central Bhutan, a region endemic for rabies. A total of 615 household respondents were interviewed, of which 224 (36%) were males and 391 (64%) were females. The majority of the respondents had high level of knowledge, and attitude and perception of rabies, and has a positive attitude towards the prevention and control of rabies. Multivariable logistic regression model showed that better knowledge about rabies was predicted by gender, educational level and dog ownership status of the respondents, whilsthealth-seeking behaviors of animal bite injuries were predicted by dog ownership status, presence of children in the household and occupation of the respondents. The majority of the respondents believed that stray dogs are a problem in the community and felt that it was important to control the dog population in Gelephu. These findings also indicate that there exists a knowledge gap about rabies in the community that could be improved by creating an awareness education programme.

Keywords: Knowledge, Attitude, Perception, Rabies, Cross-sectional survey, Bhutan

11.1. Introduction

Rabies is an invariably fatal zoonotic disease, but can be prevented by avoiding contact with rabid animals and by immediate post exposure treatment. WHO guidelines on rabies post-exposure prophylaxis (PEP) recommend three important aspects of the treatment immediately following exposure to rabid animals: thorough washing of the bite wound with water and soap or detergent, or water alone; administration of rabies vaccine; and infiltration of rabies immunoglobulin into and around the wound (WHO, 1992; 2010). However, in reality people in developing countries, particularly the poor sections of society, may not receive these life-saving treatments either because the PEP treatment is expensive and not readily available or because people may not visit the hospital for treatment owing to lack of knowledge about rabies (Kayali et al., 2003; Knobel et al., 2005; Hampson et al., 2008).

Understanding community knowledge, attitudes and perceptions of rabies is important because of their influence on post-exposure treatment seeking behavior (Matibag et al., 2008) and because community support is essential for rabies prevention and control programme (Kayali et al., 2003). Some studies have been conducted to understand knowledge, attitude and practices for rabies in India (Agarvval and Reddaiah, 2003; Singh and Choudhary, 2005; Ichhpujani et al., 2006), Sri Lanka (Matibag et al., 2007; Matibag et al., 2008; Matibag et al., 2009), and in North America (McGuill et al., 1997; Goodwin et al., 2002). These studies demonstrated a high level of people's awareness regarding rabies. A few other studies have also reported about knowledge and perception of rabies risk among travelers travelling in rabies-endemic countries (Altmann et al., 2009; Piyaphanee et al., 2010). However, these studies have also found that people apply chilli and turmeric powder, lime, kerosene oil, herbal paste or salt on the dog bite wound, or perform folk remedies at home rather than seeking conventional treatment from health facilities (Agarvval and Reddaiah, 2003; Singh and Choudhary, 2005; Ichhpujani et al., 2006; Sudarshan et al., 2006; Matibag et al., 2008).

Rabies is endemic in south Bhutan (an area that borders India) and results in sporadic human deaths (approximately 0.28 deaths per100000 population per year) following rabid dog bites (Tenzin. et al., 2011a). Although rabies PEP is given free of charge to dog bite victims, some people fail to receive PEP owing to a lack of awareness about rabies (Tenzin. et al., 2011b). To our knowledge, no detailed study has been conducted to

understand the community level of knowledge, attitudes and practices for rabies prevention and treatment among the general population in Bhutan. A limited hospital-based dog bites survey in Bhutan showed that 80% of the interviewed dog bite victims had heard of rabies, whilst only 45% had washed their bite wound at home before visiting the hospitals (Tenzin et al., 2011c).

The objective of this study was to understand the knowledge, attitudes and perception of rabies and rabies control measures in the community of Gelephu, south central Bhutan. Gelephu was chosen for this study because this sub-district is endemic for rabies and has had frequent outbreaks (Tenzin et al., 2011a). There have been four human deaths due to rabies reported in Gelephu during the period from 2008 to May 2011 (Kuensel, 2009; Bhutantimes, 2011). It is expected that the information from this study will be useful for planning an awareness education program in Gelephu and elsewhere in Bhutan.

11.2. Materials and methods

11.2.1. Study area

This survey was conducted in Gelephu, a small sub-district (area 53.6 km^2) located in the south central Bhutan district of Sarpang (Figure 11.1). Gelephu is one of the main entry points into Bhutan from India and is also one of the commercial centres in south Bhutan. Administratively the sub-district is divided into two main areas: municipal (urban) areas located close to the Indian border town of Dathgari in Assam; and semi-urban areas (Pemathang, Lekithang, Pelrithang, Dzomlingthang) located within 2−5 km of the municipal boundary (Figure11.1). There were 2685 households and 11418 inhabitants in Gelephu according to the 2005 national population and housing census of Bhutan (NSB, 2005). The sub-district has one medical hospital located in the centre of the town, which can be easily accessed by the community.

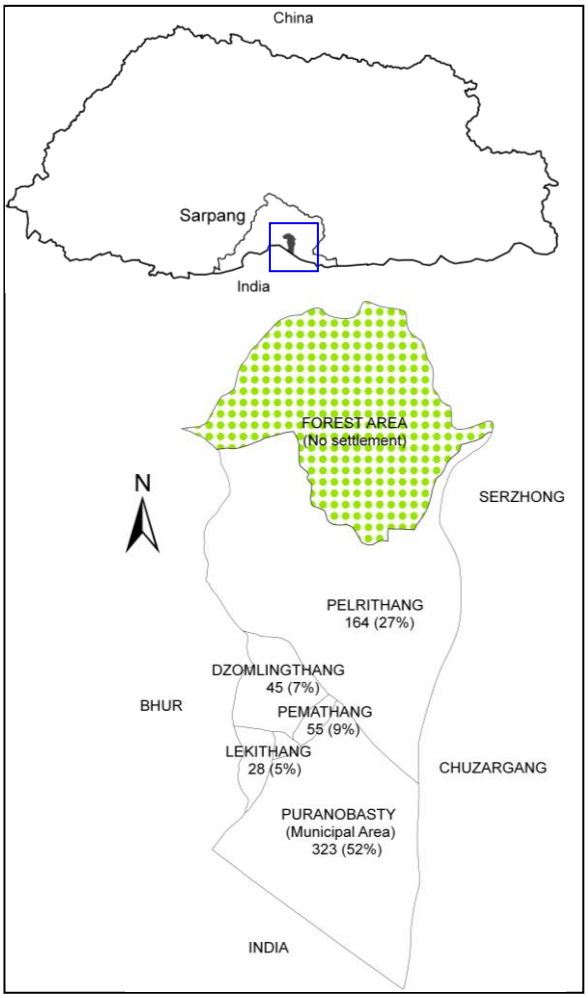

Figure 11.1: Bhutan map showing the administrative boundary of Sarpang District and Gelephu sub-district. The number and percentage of households interviewed are shown for each administrative area in Gelephu.

11.2.2. Study design

11.2.2.1. Sample size

Assuming approximately 3000 households in Gelephu during 2010, a target sample size of 788 was calculated to estimate the proportion of respondents of households or families who would have knowledge, attitude and perceptions of rabies with 95% confidence and a 3% error rate, assuming that the expected proportion of respondents that have knowledge of rabies was 50% (after applying finite population correction).

11.2.2.2. Questionnaire design

A questionnaire was designed for this study, partly adapted from similar studies conducted elsewhere (Matibag et al., 2007; Matibag et al., 2009; Bingham et al., 2010) ,consisting of closed and a few open questions. The questionnaire consisted of four parts (see Appendix 5): items regarding the respondent and socio-demographic information (age, sex, education level, occupation, religion, ethnicity, place of living, number of people in the household, number of children in the household, ownership status of pets and farm animals); questions related to the knowledge and perception of rabies; questions related to attitudes and perception of rabies and its control activities; and questions on pet care practices (asked only of dog owners). The questionnaire was piloted with five people prior to the actual survey and was modified to improve clarity and interpretation.

11.2.2.3. Sampling procedure

Owing to the lack of a proper sampling frame, a door-to-door survey was conducted using a rolling sample method (in which the first selected household provides information about the next available household in the area or within the building) until the target number of household respondents was interviewed in the study area. However, it was ensured that a representative sample was selected from all locations (both in urban and semi-urban areas). One adult person (>18 years of age) from each selected household/family was interviewed face-to-face. The selected person was informed about the purpose of the study and that participation was voluntary and data collected were confidential. Written informed consent was obtained from the participants. The interview was carried out between February and April 2010 and was administered in local and national language, but the answers were recorded in English.

11.2.3. Data management and analysis

Data were entered into a database developed in Epi Info™ (V.3.5.3. http://www.cdc.gov/epiinfo) (CDC, Atlanta, Georgia, USA). Data cleaning, management and analysis were carried out using Microsoft Excel (Microsoft Corp., Redmond, Washington, USA) and SPSS software V.16 (SPSS Inc. Chicago, Illinois, USA).

11.2.3.1. Descriptive statistics

Descriptive statistics were calculated for each variable of interest (see Table 11.1). Bivariate analyses were performed using χ^2 or Fisher's exact tests to compare the responses to the questions related to the knowledge, attitude and perception of rabies between the respondents from urban and semi-urban areas as well as between dog owners and non-dog owners. A p-value <0.05 was considered statistically significant.

11.2.3.2. Factors associated with community knowledge and perception of rabies

Respondents were asked eight questions on knowledge and perception of rabies (see Table 11.2 and Figure 11.2), which resulted into a response of either 'yes' (have knowledge of rabies) or 'no' (do not have knowledge of rabies). The number of questions for which the respondent gave positive responses were counted and this score was then categorised based on the median (0 = score index ≤ 6 and 1 = score index > 6). A binary logistic regression model was constructed to evaluate the association of this outcome variable with demographic and socio-demographic variables (listed in Table 11.1). Initially, univariate logistic regression analyses were conducted to evaluate the associations between the various potential explanatory variables and the dependent variable. Those explanatory variables with a likelihood-ratio p-value <0.25 were included for further evaluation in the multivariable logistic regression models. The selected variables were tested for collinearity in pairs by calculating Spearman's rank correlation coefficient (ρ). Amongst highly correlated pairs of variables (ρ>|0.70|), only the variable most strongly associated with the outcome was retained for further analysis. A multivariable logistic regression model was constructed, using a manual forward stepwise selection approach. Variables with $P<0.05$ were considered significant. The fit of each model was assessed using the Hosmer-Lemeshow goodness-of-fit test.

11.2.3.3. Factors associated with the community attitude of reporting animal bite injuries to the hospital for treatment

A perception index (similar to that described above) was created based on the seven questions asked of the respondents about their attitude and perception of reporting animal bite injuries to the hospital for treatment (see Table 11.3 and Figure 11.3). The number of positive responses were counted and this score categorized into a binary variable with

score index value ≤ 6 coded as 0 and score index value > 6 coded as 1 (outcome variable). Then, binary logistic regression (both univariable and multivariable) models were constructed using the same explanatory variables (demographic and socio-demographic variables (listed in Table 11.1) as described above. The fit of the model was assessed using the Hosmer-Lemeshow goodness-of-fit test.

11.3. Result

11.3.1. Respondent demographic and sociodemographic characteristics

A total of 615 respondents (one per household) were interviewed in the survey. Table 11.1 shows the demographic and socio-demographic characteristics of the respondents. The median age of the respondents was 33 years (mean 35.8 years; range 18–85 years).

Table 11.1: Characteristics of household respondents in a study of knowledge, attitude and perception of rabies in Gelephu, south−central Bhutan, during 2010 (n=615).

Variable/category	n (%)	Variable/category	n (%)
Gender		Location	
Female	391 (64)	Municipal (urban)	323 (53)
Male	224 (36)	Pelrithang (semi-urban)	164 (27)
Age (years)		Lekithang (semi-urban)	28 (5)
18−29	202 (33)	Pemathang (semi-urban)	55 (9)
30−41	242 (39)	Dzomlingthang (semi-urban)	45 (7)
≥42	171 (28)	Dog ownership	
Educational level		Yes	146 (24)
No education	338 (57)	No	469 (76)
Primary	105 (18)	Cat ownership	
High school	107 (18)	Yes	118 (19)
Secondary	29 (5)	No	489 (81)
University	16 (3)	Missing data	8
Missing data	20	Cattle ownership	
Occupation		Yes	180 (30)
Farmer	172 (28)	No	427 (70)
Dependent/housewife	214 (35)	Missing data	8
Businessman	83 (13)	Goat ownership	
Student	22 (4)	Yes	98 (16)
Employee	124 (20)	No	509 (84)
Religion		Missing data	8
Buddhist	406 (66)	Horse ownership	
Hindu	199 (32)	Yes	17 (3)
Other	10 (2)	No	587 (97)

Variable/category	n (%)	Variable/category	n (%)
Ethnicity [a]		Missing data	11
Lhotsham	224 (36)	Pig ownership	
Kheng/Bumthap	110 (18)	Yes	55 (9)
Sharchop	237 (39)	No	549 (91)
Ngalong	44 (7)	Missing data	11
No. of persons in household		Poultry ownership	
1	5 (1)	Yes	150 (25)
2	45 (7)	No	457 (75)
3	61 (10)	Missing data	8
4	133 (22)		
5	140 (23)		
6	106 (17)		
≥7	125 (20)		
No. of children in household			
0	89 (15)		
1	76 (13)		
2	145 (24)		
3	151 (25)		
≥4	142 (23)		
Missing data	12		

[a] People from different parts of Bhutan who were permanently or temporarily settled in Gelephu during the time of survey in 2010.

11.3.2. Community knowledge and perception of rabies

Table 11.2 includes bivariate analyses of the respondents' knowledge and perception of rabies and rabies control measures in Gelephu. In total, 89.6% of the respondents had heard of rabies. Of those respondents who had heard of rabies, the majority believed that rabies is a dangerous and fatal disease; that rabies can be transmitted by dogs and cats, that itcould be prevented by regular vaccination of dogs and believed that there are no locally available methods of treatment for bite wounds and rabies. Only 55.6% (229/412) of the respondents believed that rabies can be confirmed by a laboratory test. The majority of respondents were also aware that animal bite wounds should be washed with soap and water (Table 11.2).

There were significant differences between dog owners and non-dog owners with respect to the awareness of rabies (P=0.026), knowledge that rabies can be transmitted by dogs (P=0.044), and that there are no locally available methods of treatment for dog bites and rabies (P=0.001). Similarly, there was a significant difference (P=0.019) between the response of the participants from urban and semi-urban areas with respect to the belief

that rabies can be confirmed by laboratory tests (Table 11.2). Figure 11.2 summarizes the percentage of respondents that have knowledge and perception of rabies in Gelephu.

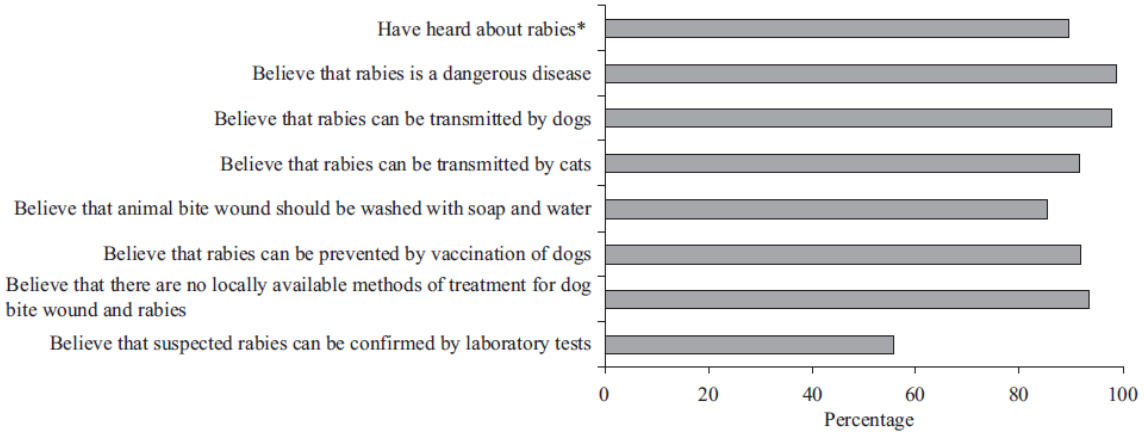

Figure 11.2: Community knowledge and perception of rabies in Gelephu, during 2010. * The response of the first question, 'Have you heard of rabies? – (yes/no)', was based on responses from 615 participants, whilst the percentage of responses to the remaining seven questions (except the washing of bite wound question) were based on those who answered 'yes' to the first question.

Table 11.2: Descriptive and bivariate χ^2 analyses of responses to questions related to the knowledge and perception of rabies, comparing dog owners with non-dog owners as well as respondents living in urban and semi-urban areas in Gelephu, Bhutan during 2010.

Variable/category	n(%)[a]	Dog ownership status [n (%)]		*P*-value	Respondent's area of living [n (%)]		*P*-value
		Yes	No		Urban	Semi-urban	
Have heard of rabies				0.026			0.154
Yes	551 (89.6)	138 (94.5)	413 (88.1)		284 (87.9)	267 (91.5)	
No	64 (10.4)	8 (5.5)	56 (11.9)		39 (12.1)	25 (8.5)	
Believe that rabies is a fatal disease				0.499			0.777
Yes	526 (98.7)	134 (99.3)	392 (98.5)		272 (98.9)	254 (98.4)	
No	7 (1.3)	1 (0.7)	6 (1.5)		3 (1.1)	4 (1.6)	
Believe that rabies can be transmitted by dogs				0.044*			0.265
Yes	508 (97.7)	129 (100)	379 (96.9)		256 (96.9)	252 (98.4)	
No	12 (2.3)	0 (0)	12 (3.1)		8 (3.1)	4 (1.6)	
Believe that rabies can be transmitted by cats				0.703			0.966
Yes	380 (91.6)	98 (92.4)	282 (91.3)		186 (91.6)	194 (91.5)	
No	35 (8.4)	8 (7.6)	27 (8.7)		17 (8.4)	18 (8.5)	
							0.993

Believe that bite wound should be washed with soap and water							
Yes	493 (85.4)	120 (82.2)	373 (86.5)	0.198	258 (85.4)	235 (85.5)	
No	84 (14.6)	26 (17.8)	58 (13.5)		44 (14.6)	40 (14.5)	
Believe that rabies outbreaks can be prevented by vaccination of dogs				0.257			0.125
Yes	458 (91.8)	127 (94.1)	331 (90.9)		236 (93.6)	222 (89.8)	
No	41 (8.2)	8 (5.9)	33 (9.1)		16 (6.4)	25 (10.1)	
Believe that suspected rabies can be confirmed by laboratory tests				0.106			0.019
Yes	229 (55.6)	69 (50.0)	160 (58.4)		96 (49.5)	133 (61.0)	
No	183 (44.4)	69 (50.0)	114 (41.6)		98 (50.5)	85 (39.0)	
Believe that there are no locally available methods of treatment for dog bite and rabies				0.001			0.077
Yes	461 (93.3)	99 (86.1)	362 (95.5)		241 (95.3)	220 (91.3)	
No	33 (6.7)	16 (13.9)	17 (4.5)		12 (4.7)	21 (8.7)	

[a] The response to the first question 'Have you heard of rabies? − (yes/no)' was based on the responses from 615 participants, whilst responses to the remaining seven questions (except the question about animal bite wound washing) were based on those who answered 'yes' to the first question (owing to missing data, the total numbers do not sum exactly). * Fisher's exact test.

11.3.3. Factors associated with knowledge and perception of rabies

Shortlisted demographic and socio-demographic variables associated with community knowledge and perception of rabies based on univariable analyses (P<0.25) are shown in Table 11.3. When adjusted for other variables in the final multivariable model, male respondents (OR= 1.47; 95% CI: 1.02–2.12), the respondents with some education (up to high school level) (OR= 1.74; 95% CI: 1.10–2.74) and dog owners had high level of knowledge and perception of rabies (OR= 1.48; 95% CI: 1.01–2.18) (Table 11.4). The model fit the data adequately (Hosmer-Lemeshow goodness-of-fit test P=0.520).

Table 11.3: Univariable analyses of the demographic and socio-demographic characteristics of the respondents associated with the community knowledge and perception of rabies in Gelephu, Bhutan during 2010.

Variable/category	***b***	***SE***	***P-value***	**OR (95% CI)**
Location				
Semi-urban	0	-	-	1
Urban	0.200	0.162	0.216	1.22 (0.89–1.68)
Gender				
Female	0	-	-	1
Male	0.564	0.169	0.001	1.76 (1.26–2.45)
No. persons in the household				
≤ 5	0	-	-	1
> 5	0.382	0.167	0.022	1.46 (1.05–2.03)
Education level			0.007*	
No education	0	0	0	1
Primary	0.541	0.225	0.016	1.72 (1.11–2.67)
High school	0.652	0.224	0.004	1.92 (1.24–2.98)
Above secondary	0.002	0.324	0.994	1.00 (0.53–1.89)
Occupation			0.022*	
Farmers	0	-	-	1
Dependants/housewife	-0.566	0.208	0.006	0.57 (0.38–0.85)
Businessman	-0.288	0.268	0.284	0.75 (0.44–1.27)
Student	0.490	0.469	0.296	1.63 (0.65–4.09)
Employee	-0.070	0.236	0.767	0.93 (0.59–1.48)
Dog ownership status				
No	0	-	-	1
Yes	0.426	0.190	0.025	1.53 (1.05–2.22)

Variable/category	*b*	*SE*	*P-value*	OR (95 % CI)
Cat ownership status				
No	0	-	-	1
Yes	0.498	0.207	0.016	1.64 (1.09–2.46)
Cattle ownership status				
No	0	-	-	1
Yes	0.475	0.179	0.008	1.61 (1.13–2.28)
Goat ownership status				
No	0	-	-	1
Yes	0.340	0.221	0.125	1.40 (0.91–2.16)
Pig ownership status				
No	0	-	-	1
Yes	0.629	0.288	0.029	1.87 (1.06–3.29)
Poultry ownership status				
No	0	-	-	1
Yes	0.349	0.189	0.065	1.42 (0.98–2.05)

* Log likelihood ratio test p value.

Table 11.4: Final multivariable logistic regression model of factors associated with community knowledge and perception of rabies in Gelephu, Bhutan during 2010

Variable	*b*	*SE*	*P*-value	OR (95 % CI)
Constant	-0.590	0.126	-	-
Gender				
Female	0	-	-	1
Male	0.386	0.186	0.038	1.47 (1.02–2.12)
Education level			0.042*	
No education	0	-	-	1
Primary	0.394	0.233	0.090	1.48 (0.94–2.34)
High school	0.553	0.232	0.017	1.74 (1.10–2.74)
Above secondary	-0.159	0.332	0.632	0.85 (0.44–1.64)
Dog ownership status				
No	0	-	-	1
Yes	0.389	0.199	0.050	1.48 (1.01–2.18)

Likelihood ratio χ^2 test = 4.49; $P<0.001$; Hosmer-Lemeshow goodness-of-fit test = 5.19; P=0.520; * Likelihood ratio test p-value.

11.3.4. Community attitudes and perception of rabies

Table 11.5 includes the bivariate analyses of the respondents' attitude and perceptions of rabies and rabies control programme in Gelephu. The majority (range: 84−92%) of the

respondents reported that they would report to the hospital for treatment if bitten by stray dogs, owned dogs, stray cats, owned cats, wild animals, were scratched by stray dogs, or were bitten by dogs in other countries (see Table 11.5 and Figure 11.3). Moreover, 98.8% of the respondents mentioned that they would report to the authorities if there is a suspected outbreak of rabies in the community and 61.0% of the respondents believed that stray dogs are a problem in the community. Almost all of the respondents believed that it was important to control both the dog population (99.7%) and also would support a dog rabies control programme (99.5%) in Gelephu. There were significant differences between the dog owners and non-owners as well as the responses of the respondents from urban and semi-urban areas with respect to their attitude and perception of rabies (Table 11.5).

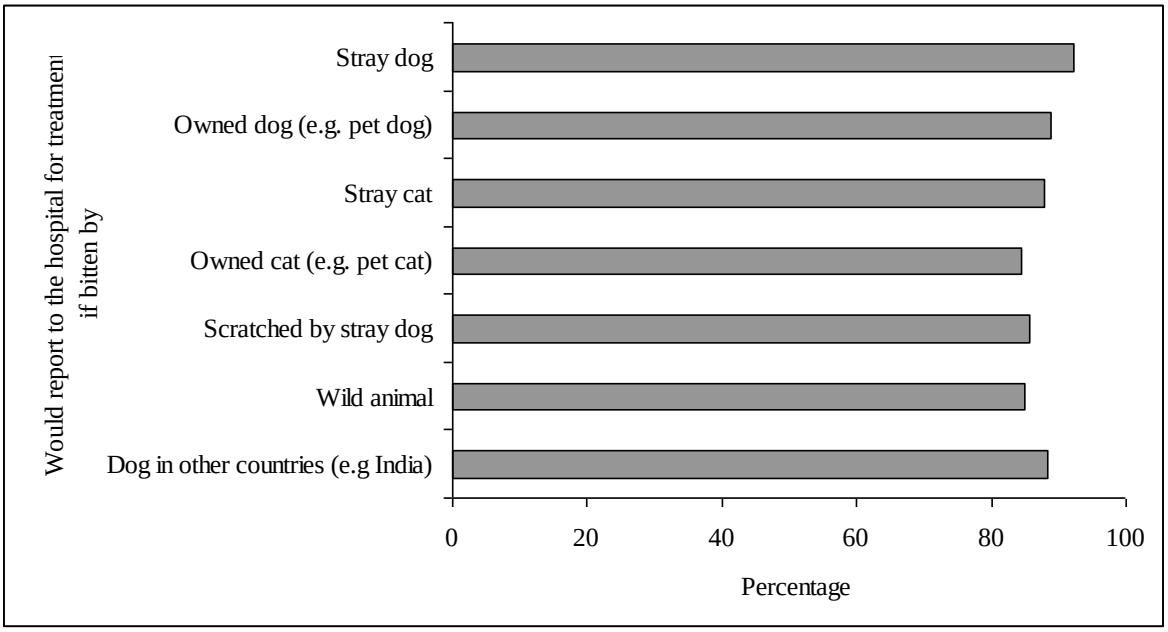

Figure 11.3: Community attitude and perception of reporting animal bite injuries to the hospital for treatment in Gelephu, Bhutan, during 2010.

Table 11.5: Descriptive and bivariate χ^2 analyses of responses to questions related to the community attitude and perception of rabies, comparing responses between dog owner and non-dog owner as well asrespondents' area of living (urban and semi-urban areas) in Gelephu, Bhutan during 2010.

Variable/category		Dog ownership status of respondents [n (%)]		*P*-value	Respondent,s area of living [n (%)]		*P*-value
	n(%)[a]	Yes	No		Urban	Semi-urban	
Would report to hospital for treatment if bitten by stray dog				0.049			0.001
Yes	565 (92.2)	129 (88.4)	436 (93.4)		307 (95.6)	258 (88.4)	
No	48 (7.8)	17 (11.6)	31 (6.6)		14 (4.4)	34 (11.6)	
Would report to hospital for treatment if bitten by owned dog (e.g. pet dog)				< 0.001			<0.001
Yes	537 (88.9)	116 (79.4)	421 (91.9)		296 (94.0)	241 (83.4)	
No	67 (11.1)	30 (20.6)	37 (8.1)		19 (6.0)	48 (16.6)	
Would report to hospital for treatment if bitten by stray cat				0.001			<0.001
Yes	525 (87.9)	110 (79.7)	415 (90.4)		294 (93.6)	231 (81.6)	
No	72 (12.1)	28 (20.3)	44 (9.6)		20 (6.4)	52 (18.4)	
Would report to hospital for treatment if bitten by owned cat (e.g. pet cat)				< 0.001			0.001
Yes	507 (84.4)	103 (73.0)	404 (87.8)		284 (89.0)	223 (79.0)	
No	94 (15.6)	38 (27.0)	56 (12.2)		35 (11.0)	59 (21.0)	

Would report to hospital for treatment if scratched by stray dog				<0.001		<0.001
Yes	517 (85.7)	102 (72.9)	415 (89.6)		291 (91.8)	226(79.0)
No	86 (14.3)	38 (27.1)	48 (10.4)		26 (8.2)	60 (21.0)
Would report to hospital for treatment if bitten by wild animal				< 0.001		<0.001
Yes	496 (84.9)	96 (72.2)	400 (88.7)		280 (90.6)	216 (78.5)
No	88 (15.1)	37 (27.8)	51 (11.3)		29 (9.4)	59 (21.5)
Would report to hospital for treatment if bitten by dogs in other countries (e.g. India)				0.002		0.001
Yes	533 (88.4)	117 (81.3)	416 (90.6)		293 (92.4)	240 (83.9)
No	70 (11.6)	27 (18.7)	43 (9.4)		24 (7.6)	46 (16.1)
Would report to authorities if there is suspected rabies outbreak in the community				0.36*		0.055*
Yes	561 (98.8)	129 (100)	432 (98.4)		300 (99.7)	261 (97.7)
No	7 (1.2)	0 (0)	7 (1.6)		1 (0.3)	6 (2.3)
Would kill stray dog if rabies is suspected				0.002		0.851
Yes	227 (42.8)	72 (54.1)	155 (39.0)		115 (42.4)	112 (43.2)
No	303 (57.2)	61 (45.9)	242 (61.0)		156 (57.6)	147 (56.8)

Is stray dog a problem in your community?				0.048		0.477
Yes	364 (61.0)	96 (68.1)	268 (58.8)		186 (59.6)	178 (62.5)
No	233 (39.0)	45 (31.9)	188 (41.2)		126 (40.4)	107 (37.5)
Believe it is important to control dog population in Gelephu				0.100*		0.500*
Yes	601 (99.7)	140 (100)	461 (99.6)		315 (99.4)	286 (100)
No	2 (0.3)	0 (0)	2 (0.4)		2 (0.6)	0 (0)
Do you support rabies control campaign?				0.100*		0.250*
Yes	600 (99.5)	140 (100)	460 (99.3)		313 (99.0)	287 (100)
No	3 (0.5)	0 (0)	3 (0.7)		3 (1.0)	0 (0)
What methods do you believe is appropriate to control dog population				0.221		0.334
Sterilization	333 (54.1)	87 (59.6)	246 (52.4)		183 (56.6)	150 (51.1)
Impounding	82 (13.3)	14 (9.6)	68 (14.5)		38 (11.8)	44 (15.1)
Sterilization & impounding	167 (27.2)	40 (27.4)	127 (27.1)		88 (27.2)	79 (27.2)
Killing	33 (5.4)	5 (3.4)	28 (6.0)		14 (4.4)	19 (6.6)

[a] Owing to missing data, the total numbers do not sum exactly. * Fisher's exact test.

11.3.5. Factors associated with the community attitude and perception of reporting animal bite injuries to the hospital

Various demographic and sociodemographic variables were found to be significantly (P<0.25) associated with the community attitude and perception of reporting animal bite injuries to the hospital for treatment in univariable analyses (see Table 11.6). When adjusted for other variables in the final multivariable model, respondents who owned dogs were less likely (OR = 0.51; 95% CI: 0.33–0.78) to report animal bite injuries to the hospital than those who did not own dogs. Respondents who had children in their house (OR = 2.27; 95% CI: 1.36–3.78), respondents who were dependent or housewives (OR = 7.08; 95% CI: 3.83–13.09); businessman (OR = 1.95; 95% CI: 1.07–3.55) and employee in the government or private organization (OR = 1.95; 95% CI: 1.15–3.30) were more likely to report animal bite injuries to the hospital for treatment (see Table 11.7). The model fitted the data well (Hosmer-Lemeshow goodness-of-fit test P=0.950).

Table 11.6: Univariable analyses of demographic and sociodemographic variables associated with community attitude and perception of reporting animal bite injuries to the hospital for treatment in Gelephu, Bhutan during 2010.

Variable/category	*b*	*SE*	*P*-value	OR (95% CI)
Location				
Semi-urban	0	-	-	1
Urban	-0.701	0.191	<0.001	0.496 (0.34–0.72)
Gender				
Female	0	-	-	1
Male	-0.828	0.191	<0.001	0.437 (0.30–0.63)
Age class			<0.001	
18–29	0	-	-	1
30–41	0.224	0.240	0.351	1.252 (0.78–2.00)
> 42	-0.843	0.233	<0.001	0.430 (0.27–0.68)
Occupation			<0.001	
Farmers	0	-	-	1
Dependant/housewives	2.211	0.302	<0.001	9.125 (5.05–16.50)
Businessman	0.715	0.293	0.015	2.045 (1.15–3.63)
Student	-0.122	0.455	0.788	0.885 (0.36–2.16)
Employee	0.794	0.258	0.002	2.212 (1.33–3.67)
Religion				
Buddhism	0	-	-	1
Hinduism	-0.960	0.195	<0.001	0.383 (0.26–0.56)

Variable/category	*b*	*SE*	*P*-value	OR (95% CI)
Ethnicity			<0.001	
Lhotsham	0	-	-	1
Kheng/Bumthap	0.604	0.264	0.022	1.830 (1.09–3.07)
Sharchop	1.218	0.232	<0.001	3.382 (2.15–5.33)
Ngalong	0.530	0.375	0.157	1.699 (0.81–3.54)
Presence of children in the household				
No	0	-	-	1
Yes	0.868	0.241	<0.001	2.383 (1.49–3.82)
No. persons in the household				
≤ 5	0	-	-	1
> 5	-0.687	0.190	<0.001	0.503 (0.35–0.73)
Dog ownership status				
No	0	-	-	1
Yes	-1.092	0.204	<0.001	0.335 (0.22–0.50)
Cat ownership status				
No	0	-	-	1
Yes	-0.946	0.219	<0.001	0.388 (0.25–0.59)
Cattle ownership status				
No	0	-	-	1
Yes	-1.580	0.202	<0.001	0.206 (0.14–0.31)

Table 11.7: Final logistic regression model of factors associated with community attitude and perception of reporting animal bite injury to the hospital for treatment in Gelephu, Bhutan during 2010.

Variable/category	*b*	*SE*	*P*-value	OR (95% CI)
Constant	-0.090	0.278		
Dog ownership status				
No	0	-	-	1
Yes	-0.679	0.224	0.002	0.51 (0.33–0.78)
Presence of children in the household				
No	0	-	-	1
Yes	0.820	0.261	0.002	2.27 (1.36–3.78)
Occupation			0.001*	
Farmers	0	-	-	1
Dependent/housewives	1.958	0.314	0.000	7.08 (3.83–13.09)
Businessman	0.669	0.305	0.028	1.95 (1.07–3.55)
Student	-0.211	0.467	0.652	0.81 (0.32–2.02)
Employee	0.669	0.268	0.012	1.95 (1.15–3.30)

Likelihood ratio test, P<0.001, Hosmer-Lemeshow goodness-of-fit test = 2.175; P=0.95
* Likelihood ratio test p value

11.4. Discussion

This cross-sectional study was conducted to understand the community knowledge, attitudes and perception of rabies and to investigate factors influencing their knowledge and perceptions about rabies. This is the first study conducted to understand the public health hazard of rabies in Gelephu, which is endemic for canine rabies. It provided valuable information on which to build a rabies awareness education programme.

It is important to note that like any other observational study, the study design used had some limitations. First, the required sample size could not be achieved due to logistical reasons and time constraints, so that the precision of our estimates might have been reduced. However, the sample size was estimated assuming 50% prevalence of knowledge/perceptions (worst-case scenario), but in fact about 80% of the sample had correct knowledge and perceptions about rabies. This means that the effective precision of our estimates is likely better than what we planned for because a sample size of only 556 is required to estimate a proportion of 80% with 3% precision. Second, households were not randomly selected due to lack of a proper sampling frame. However, it was ensured that a representative sample of households was selected and interviewed from all locations both within urban and semi-urban areas. Third, the sample of people interviewed from the household was those found at home during the visit. Since women (particularly dependents/housewives) are more commonly present at home than men, the number of female respondents in this survey was more than that of males (64% vs 36%). Finally, only adults were interviewed: those younger than 18 years of age were excluded due to ethical issues. We acknowledge that those who were not interviewed may have different knowledge, attitude and perception of rabies. In view of the above issues, the study results should be interpreted with a degree of caution.

Findings of this study indicate that rabies is an important public health problem in Gelephu and the community awareness, knowledge and perception of rabies was high among the respondents. Respondents who owned dog(s) were more likely to have knowledge of rabies, but no significant difference in knowledge was observed between respondents from urban and semi-urban areas. The high level of awareness among the respondents may be due to endemicity of rabies and frequent reports of rabies outbreaks in Gelephu combined with an annual rabies control campaign, and from the news media

about rabies outbreaks. Findings from this study are consistent with those from other studies in the neighbouring countries in south Asia that demonstrated a high level of knowledge of rabies and its transmission (Agarvval and Reddaiah, 2003; Sharma, 2005; Ichhpujani et al., 2006; Matibag et al., 2008; Matibag et al., 2009). However, the current study also identified some knowledge gaps: some respondents had not heard of rabies and its transmission (Figure 11.2; Table 11.2), indicating that rabies awareness education is necessary in Gelephu.

Understanding the community attitude and perceptions of treatment-seeking behaviours is important for rabies prevention in humans (Matibag et al., 2008) Immediate PEP is required to neutralize the rabies virus in the wound before it spreads into the central nervous system and brain (Warrell and Warrell, 2004; WHO, 2010). This study showed good treatment-seeking behaviours as a majority of the respondents would report to the hospital for animal bite wound treatment (Table 11.5, Figure 11.3). However, the odds of reporting animal bite wounds to the hospital were higher for owners of dogs, in households with children and in dependent/housewives, businessmen or employees (Table 11.7). These findings are comparable with previous studies reporting that a large number of people visited the hospitals for rabies PEP following dogs bites, touching/feeding of rabid animals and ingestion of meat and dairy products derived from rabid animals in Bhutan (Tenzin. et al., 2011b). Similarly, treatment records from the hospital also revealed that during the period 2000–2010, more than 3000 people visited the Gelephu hospital following dog bites and contact with rabid animals to receive PEP vaccine (Bhutantimes, 2011; Tenzin. et al., 2011b). This evidence supports the current finding that the people in Gelephu have good health-seeking behaviours. However, it is to be noted that human deaths due to rabies have occurred in Gelephu and in the south Bhutan region, especially in children (Kuensel, 2009, 2010, 2011). Of the 12 reported human rabies deaths in Bhutan between 2006 and June 2011, 9(75%) occurred in children (<15 years of age) who did not receive post exposure prophylaxis. It is possible that children would often interact with dogs resulting in dog bite injuries, but probably do not report the incident to their parents or to the hospital owing to lack of awareness of rabies (Dodet et al., 2010). Furthermore, studies in other countries have shown that children are more often bitten on the head and neck, which carries a much higher risk than bites to other parts of the body (Pancharoen et al., 2001; Cleaveland et al., 2002; Knobel et al.,

2005). Further studies should be conducted to confirm this proposition, and if found to be correct, awareness education should be planned targeting children.

The study results shows that male respondents have better knowledge about rabies but they are less likely to report animal bite cases to the hospital. This is not surprising because it is well documented that compared to women, men in general have limited contacts with physicians and seek less healthcare services (Mansfield et al., 2003; Galdas et al., 2005; Smith et al., 2006). It is assumed that several factors might be involved in men's decisions, including masculine ideologies regarding seeking help when faced with illness or problems (Galdas et al., 2005; Smith et al., 2006). This is comparable to field observations that of the 12 human rabies deaths in Bhutan (from January 2006–April 2011), 11 (92%) were males but most (75%) were children under 15 years of age.

The current results also indicate that the attitudes of the respondents were positive: the majority mentioned that they would report suspected rabies outbreaks in the community to the appropriate authorities for investigation. The majority of respondents also believed that stray dogs are a public health problem in the community and would support a dog population control programme. Community support of, and participation in a rabies control programme is important in order to achieve good coverage of vaccination (>70 %). This is necessary to break the chain of infection and to prevent the maintenance of rabies in the dog population (WHO, 1992; Coleman and Dye, 1996).

In conclusion, this study has shown that the community level of knowledge, attitude and perception of rabies is high in Gelephu and that people have positive attitudes towards the prevention and control programmes. However, there are some knowledge gaps in the community regarding rabies: some respondents had not heard of rabies and the risk of transmission from all warm-blooded animals (see Figure 11.2 and Table 11.2), whilst some would not report animal bites injuries to the hospital for treatment (see Figure 11.3 and Table 11.5). Therefore, rabies awareness education within the community is necessary on the following areas: the danger of rabies and mode of transmission to humans; the importance and usefulness of washing the animal bite wound with plenty of soap and water; the importance of seeking health facilities following animal bites injuries

or exposures to suspected/rabid animals; and providing community support and participation for dog rabies control programme.

Authors' contributions

All authors contributed to the concept and design of the study; BDR, C, ST, KT, PU and KS implemented the field survey; T, NKD and MPW analysed the data, all authors interpreted the analysed data, T drafted the manuscript. All authors contributed critically to revising the manuscript and read and approved the final version.T is guarantor of the paper.

Conflicts of interest: None declared

Ethical approval

This study was approved by the Research Ethics Board of Health, Bhutan. Written informed consent was obtained from the participants prior to the interview.

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CHAPTER 12

12. HUMAN AND ANIMAL RABIES PREVENTION AND CONTROL COST IN BHUTAN, 2001−2008: THE COST−BENEFIT OF DOG RABIES ELIMINATION

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CHAPTER 12

Human and animal rabies prevention and control cost in Bhutan, 2001−2008: The cost−benefit of dog rabies elimination

Abstract

The objective of this study was to estimate the cost of various interventions and to quantify the economic impacts of rabies in Bhutan. Cost–benefit of dog rabies elimination versus human post-exposure treatment (PET) cost was also assessed. The average direct medical cost of human post-exposure treatment (using rabies vaccine only) was estimated to be Nu. 1615 (US$ 35.65) per 5-dose Essen regimen per patient. The cost would increase to Nu. 2497 (US$ 55.13) and Nu. 19,633 (US$ 433.41) per patient when one dose of either equine rabies immunoglobulin (ERIG) or human rabies immunoglobulin (HRIG) is administered, respectively. The societal cost (direct medical and indirect patient expenses) per patient was estimated to be Nu. 2019 (US$ 45), Nu. 2901 (US$ 64) and Nu. 20,037 (US$ 442) using vaccine only, vaccine with ERIG and vaccine with HRIG, respectively. The average cost per dog vaccination and sterilization were estimated to be Nu. 75 (US$ 1.66) and Nu. 288 (US$ 6.36), respectively. The total direct cost of rabies and various interventions between 2001 and 2008 was estimated to be Nu. 46.95 million (US$ 1.03 million). The direct cost for intensified human PET was estimated to be Nu. 5.85 million (US$ 0.11 million) per year with a cumulated estimated costs of Nu. 35.10 million (US$ 0.70 million) while the cost of mass dog vaccination with at least 70% coverage is estimated to be approximately Nu. 10.31 million (US$ 0.21 million) at the end of 6 years. The combined cost of mass dog vaccination and human PET was estimated to be greater than the cost of human PET alone during the first 2 years of the campaign, and then would be lower than human PET cost alone after the 5th year of the campaign. The total cumulated cost of the combined strategy was estimated to be Nu. 34.14 million (US$ 0.73 million) and would be lower than the cumulated cost of human PET alone (Nu. 35.10 million, US$ 0.77 million) at the end of 6 years. Rabies

represents a substantial economic impact to the Bhutanese society. Well-planned and implemented mass dog vaccination would result in elimination of rabies reservoirs in the domestic dog population and would eliminate human rabies cases. It would also reduce the recurrent expenditure on human post-exposure treatment.

Keywords: rabies, human post-exposure treatment, cost-benefit analysis, Bhutan

12.1. Introduction

An estimated 70,000 people worldwide die of rabies annually despite the existence of post exposure vaccine for victims of rabid-animal bites injuries since 1885 (Hampson et al., 2011). Most of these deaths occur in developing countries of Asia and Africa where domestic dogs are the principal reservoir and a vector for human rabies transmission, and rabies control programs are inadequate (Knobel et al., 2005; Cleaveland et al., 2006; Hampson et al., 2011). Although human rabies can be prevented by post-exposure treatment (PET), it does not eliminate the main source of infection. Only vaccination of dogs and elimination of rabies virus in animal reservoirs can prevent further transmission to humans (Bogel and Meslin, 1990; Zinsstag et al., 2009). This is demonstrated by the successful eradication of canine rabies in the industrialized and some developing countries that were once endemic for canine rabies (Wells, 1954; Bogel and Meslin, 1990; Cleaveland et al., 2003; Belotto et al., 2005; Cleaveland et al., 2006; Takahashi-Omoe et al., 2008). Those countries that are free from canine rabies enjoy direct economic savings by discontinuing animal and human rabies prevention and control programs (Fishbein et al., 1991; Cleaveland et al., 2006).

Human post-exposure treatment costs and dog rabies control program costs are the greatest factors determining the societal cost of rabies in any rabies endemic country (Meltzer and Rupprecht, 1998a). Moreover, the expenses borne by the patients for PET represents the bulk of expenditure, accounting for nearly half the total cost of rabies in countries in which health services are not subsidized (Knobel et al., 2005). Many studies on the public health burden of rabies, the cost of rabies control programs, and the cost−benefit of rabies elimination have

been undertaken (Bogel and Meslin, 1990; Fishbein et al., 1991; Wilde et al., 1999; Goswami et al., 2005; Knobel et al., 2005; Kayali et al., 2006). Cost-effectiveness studies of rabies control strategies in rabies endemic countries have demonstrated that dog rabies elimination is more cost-effective than the intensified use of tissue culture vaccine in humans (Bogel and Meslin, 1990; Fishbein et al., 1991; Zinsstag et al., 2009). It has also been estimated that just 10% of costs currently used to treat people bitten by potentially rabid dogs would be sufficient to eradicate dog rabies in the world and thereby prevent almost all human rabies cases (Vallat, 2011).

Canine rabies is endemic in areas of south Bhutan bordering India and results in spillover infection of both domestic livestock and sporadic cases in humans (Tenzin. et al., 2011a). Animal bite victims reporting to hospitals are given PET free of charge (Tenzin. et al., 2011b). Rabies and its intervention activities cause financial costs to society in the form of PET cost, the dog vaccination and dog population control program, surveillance cost, control measures cost and livestock losses due to rabies (Tenzin et al., 2010). Although the economic burden of rabies may be significant, very little is known about the real costs of these various interventions and financial losses in Bhutan, since no detailed study of the impact of rabies has been carried out so far. Therefore, the main objectives of this study were:

1. to present a cost description of the rabies control program in Bhutan by estimating the unit cost of post exposure treatment in human, cost per dog vaccination and sterilization, and cost of animal rabies surveillance activities;
2. to estimate the overall financial burden of rabies during the period 2001−2008, and
3. to estimate the cost−benefit of dog rabies elimination versus human post exposure treatment cost.

12.2. Materials and methods

12.2.1. Human rabies post-exposure treatment

The direct medical costs and indirect patient cost of human post-exposure treatment were calculated in this analysis (Meltzer and Rupprecht, 1998a; Knobel et al., 2005). The cost components included in estimating human rabies PET cost are illustrated in Figure 12.1 and described below:

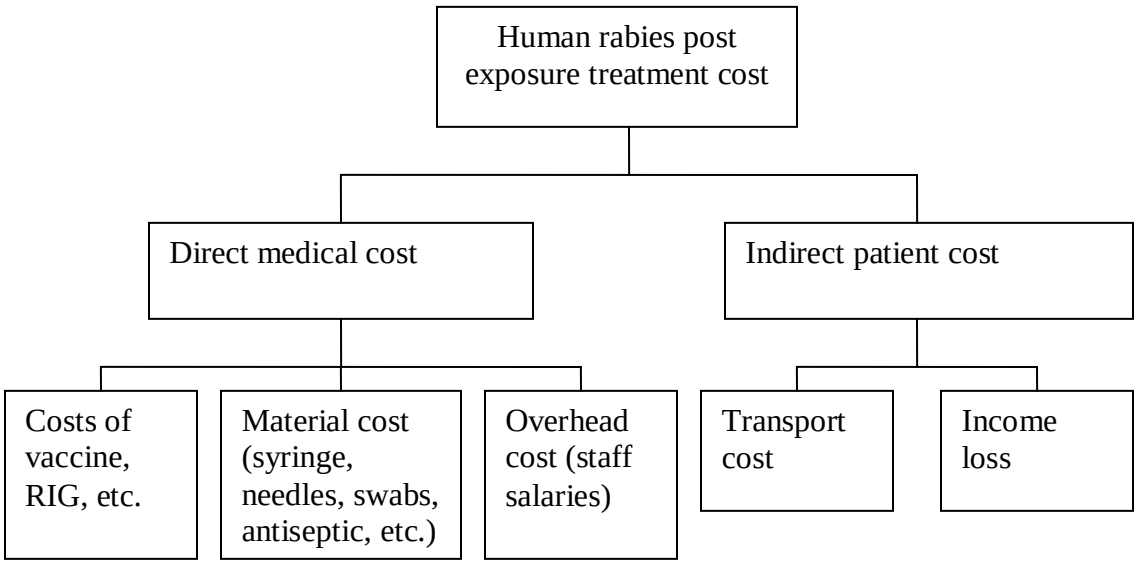

Figure 12.1: Schematic representation of estimating the cost of human rabies post-exposure treatment for rabies in Bhutan (direct medical cost plus indirect patient cost = societal cost); RIG: rabies immunoglobulin.

12.2.1.1. The direct medical cost

The direct medical costs included the cost of cell-culture rabies vaccine, rabies immunoglobulin, material costs, and overhead/operating cost (including staff salaries). These costs are fully borne by the government and are provided free of charge to any patients in Bhutan. The overhead cost included the cost associated with doctor consultation time and staff nurse time (wound dressing and vaccine injection) calculated based on the monthly salaries of the staff and the time taken per patient. Details of cost calculations are summarized in Table 12.1. The cost associated with the administration of antibiotics or tetanus toxoids, vaccine storage cost (including staff salaries, cost of refrigerator, electricity

charge) and the vaccine delivery expenses (including vehicle, fuel cost) to the medical centers were not included in this analysis due to a lack of data. Rabies immunoglobulin is very rarely used in Bhutan because of the high cost. However, we compared the estimated direct medical cost of PET using vaccine only and vaccine with rabies immunoglobulin (ERIG or HRIG)) (see Table 12.1 for detail cost estimation).

Table 12.1: Direct medical cost of post-exposure treatment and data sources in Bhutan

Parameters	Estimates		Source
	Nu.	US$	
PET patient numbers			
No. of doses of HDCV imported from January 2001 to December 2008: 51,113 doses			MoH
No. of PET cases from 2001 to 2008: 10,223 patients[a]			Calculated
No. of PET visit per patient to the health centre : 5			Essen 5-dose regimen
No.of injections per patient : 5			Essen 5-dose regimen
Costs			
Vaccine cost per dose (HDCV)	305	6.73	MoH
Material costs per injection (antiseptic, swabs, needle, syringes, etc.)	1.31	0.03	MoH
Material costs per patient wound dressing (antiseptic, cotton, bandages, etc.)	10	0.22	MoH
One time doctor consultation cost per patient to approve PET[b]	14	0.31	Calculated
Overhead cost per vaccine injection[c]	9	0.19	Calculated
Overhead cost per patient for one time wound dressing[c]	17	0.38	Calculated
Cost per patient for first time visit to the health center[d]	356	7.86	Calculated
Cost per patient for subsequent visit to the health centers (per visit)[e]	315	6.95	Calculated
Total cost per patient for one course of PET (5 times PET visit)	1615	35.65	Calculated
Rabies immunoglobulin cost			
ERIG cost per 5 ml vial containing 1500 IU	432	9.54	MoH
ERIG cost per dose (require 2 vials for 50 kg body weight) at 40IU/kg body weight (max 3000IU)	864	19.07	Calculated
HRIG cost per 2 ml vial containing 300IU	6000	132.45	MoH
HRIG cost per dose (require 3 vials for 50 kg body weight) at 20IU/kg body weight (max 1500IU)	18,000	397.35	Calculated
Material costs per injection (antiseptic, swabs, needle, syringes, etc.)	1.31	0.03	MoH
[c]Overhead cost for one injection	17	0.38	Calculated
Cost for one dose of ERIG injection	882	19.48	Calculated
Cost for one dose of HRIG injection	18,018	397.76	Calculated
Total direct cost per patient (5-dose regimen)			
Cost of vaccine, one dose of ERIG, material and overhead cost	2497	55.13	Calculated
Cost of vaccine, one dose of HRIG, material and overhead cost	19,633	433.41	Calculated

PET = post exposure treatment; HDCV = human diploid cell vaccine; ERIG = equine rabies immunoglobulin; HRIG = human rabies immunoglobulin; Nu.= Bhutanese currency Ngultrum (1 US$ =46.3 Nu, exchange rate in 2006). MoH= Ministry of Health
[a] No. of vaccine doses imported divided by 5, assuming each person received 5-doses of Essen Regimen. [b]Cost = (no. of minutes per patient consultation × daily wage) ÷ (no. of working hours per day × 60), assuming only one time consultation of patient with doctor during first visit to the hospital to approve post exposure treatment. The patient can directly visit the injection section during subsequent injection course. [c]Cost = no. of minutes taken for vaccine injection/wound dressing/rabies immunoglobulin administration per patient × daily wage) ÷ (no. of working hours per day × 60). [d]*Cost per patient for first time visit to the health center* : This is a summary cost of (vaccine cost per dose, material cost per injection, material cost per patient for wound dressing, one time doctor consultation cost, overhead cost per vaccine injection and overhead cost per patient for one time wound dressing). [e]*Cost per patient for subsequent visit to the health centers (per visit*): This is also a summary cost but excludes the cost of doctor consultation, material cost for wound dressing and overhead cost of wound dressing assuming these are not required in the subsequent visits (patient can directly visit the injection section).

Since an accurate estimate of the number of persons that received PET was not available, we estimated the total persons that received PET from the total doses of rabies vaccine imported by the Ministry of Health and distributed to various hospitals in the country between 2001 and 2008 (n=51,113 doses). We assumed all doses imported and distributed yearly to the medical centers would have been used and that each animal-bite victim or other mode of

exposures/contacts would have received 5-doses of vaccine, so that an estimated 10,223 patients (51,113/5) would have been treated from 2001 to 2008) (see Table 12.1). The Ministry of Health in Bhutan imports human diploid cell rabies vaccine and follows the 5-dose Essen regimen of intramuscular administration (Tenzin. et al., 2011b). The wastage of rabies vaccine stocks is probably negligible since it is available as 1-dose vial, so wastage was not accounted for in the analysis. A sensitivity analysis was also carried out by varying the PEP doses per patient (for example, 4 doses per patient) assuming that not all patients would have completed the full course schedule (Tenzin. et al., 2011b) and also by recalculating the cost using the lower confidence limits of the estimated PET patients.

The future expected direct medical cost was also estimated based on the most recent three years (2009–2011) rabies vaccine imports and distribution data in Bhutan. A total of 51,600 doses of rabies vaccine were imported and distributed to health centers between 2009 and 2011. Assuming all patients received 5-doses, an estimated 10,323 patients (51,600/5) would have been treated with these vaccines during 2009 and 2011 (annual average of 3440 patients). From this baseline data, we estimated annual future direct costs of human PET with vaccine only or vaccine with rabies immunoglobulin (ERIG or HRIG) assuming it would be necessary to provide rabies immunoglobulin to about 10% of the annual total number of patients (see Table 12.1 for cost calculation).

12.2.1.2. The indirect patient cost

The indirect cost or the patient expenses included the loss of income and transport cost incurred by the patients for travel to and from the medical hospital to receive each post exposure treatment (Knobel et al., 2005; Kayali et al., 2006; Zinsstag et al., 2009). Loss of income is the opportunity cost of labor lost by the patient whilst visiting the hospital for PET, and was calculated based on the per-capital Gross National Income of Bhutan for the year 2006 (Ngultrum. 39,639; US$ 875) (NSB, 2007). We assumed that each patient would require an average of 3 hours of his/her work time to visit health centers and return to home or work (including waiting time at the hospital). We also assumed that all children under 16 years of age would have been accompanied by an adult (40% of the total visits would be accompanied by an adult) (Knobel et al., 2005). Therefore, the transport costs was calculated

for both patients and the accompanying adults (n=14,312) whilst the income loss was estimated for the accompanying adults (n=4089) and the PET patients, but not children (n=6134) since children below 16 years of age was assumed to have no income.

The input data for estimating the indirect cost is shown in Table 12.2. Finally, the societal cost per person was estimated for one full course (5-dose Essen regimen and also for 4-dose per patient) of PET per patient (societal cost = direct medical cost + indirect patient cost) (see Table 12.2).

Table 12.2: Indirect (patient) costs of post exposure treatment and data sources in Bhutan

	Estimates			
Parameters	**No.**	**Nu.**	**US$**	**Source**
PET patients numbers				
No. of PET patients from 2001 to 2008 (from Table 12.1)	10,223			
Proportion of visits accompanied by an adult (for children <16 years of age)	0.4			Knobel et al., 2005
No. of accompanying adult (from 2001 to 2008) [a]	4089			Calculated
Total no. of PET patients visits (patients plus accompanying adults)	14,312			Calculated
Transport costs				
Transport costs per person per visit (average taxi/bus fare)		40	0.88	Pers. commun
Transport costs per person for 5 times visit		200	4.42	Calculated
Income loss				
Estimated no. of hours lost per person per PET visit	3			
Equivalent no. of working days lost per person per PET visit[b]	0.38			Calculated
Per capita Gross National Income for Bhutan (year 2006)		39,639	875	NSB, 2007
Daily per capita Gross National Income[c]		109	2.40	Calculated
Income loss per person per PET visit[d]		41	0.90	Calculated
Income loss per person for 5 times PET visits		204	4.50	Calculated
Total indirect patient cost (transport cost plus income loss)				
Total indirect patient cost per person per visit[e]		81	1.78	Calculated
Total indirect patient cost per person for 5 times visit		404	8.91	Calculated
Societal cost of PET				
Societal cost per 5 times visit with vaccine injection only		2019	45	Calculated
Societal cost per 5 times visit with vaccine plus ERIG		2901	64	Calculated
Societal cost per 5 times visit with vaccine plus HRIG		20,037	442	Calculated

[a] No. of accompanying adults: 40/100×10,223 = 4089; [b] No. of hrs. lost per person per PET visit ÷ No. of hrs per day: 3/8 = 0.38; [c] Per capita GNI ÷ No. of days in one year: 39,639/365 = 109; [d] Income loss per person per PET visit: equivalent no. of working days lost per person per PET visit × daily per capita income: 0.38 × 109 = 41; [e] Total indirect cost per person per visit: transport cost plus income loss (40 + 41 = 81).

Societal cost =direct medical cost (from Table 12.1) + indirect patient cost (from this table)

12.2.2. Rabies control in domestic dogs

12.2.2.1. Dog vaccination cost

Cost analysis was carried out from the public sector perspective (direct medical cost) with all the expenditures incurred for the campaign (including the cost of vaccine, material costs, daily operating costs and the campaign organization cost) being borne by the Government of Bhutan. These costings were included in the analysis to estimate the unit cost of dog vaccination (recurring costs) (see Table 12.3 for input cost details). The capital costs were not included in the costing analysis since they remain as a permanent infrastructure of the government (although large costs would have been incurred initially by the government). In addition, the public awareness cost and the vaccine storage and delivery expenses to various livestock centres were not included in this analysis due to lack of data. Dog vaccination cost was calculated in a campaign setting assuming that a 3 staff team would vaccinate about 100 dogs per day (the throughput would depend on dog accessibility) (Kinley Dorjee, personal communication).

The overall expenses of mass vaccination of dogs for the period 2001−2008 were then calculated using the unit cost estimated per dog vaccination to understand the economic burden of rabies in Bhutan. We calculated the cost per dog vaccinated for stray and pet dog separately since stray dog vaccination would require additional expenditure than pet dog vaccination. In Bhutan until 2008, people that captured stray dogs on the street and brought them to the vaccination points were given a dog catching incentive of Nu. 30 per dog (US$ 0.61).

We have assumed that all stray dogs captured and vaccinated were paid incentives. We estimated the total expenses based on the assumption that 60% of the vaccinated dogs would be stray dogs and the rest (40%) would be pet dogs since mass dog vaccination campaigns have been mostly targeted at stray dogs in Bhutan.

The income loss incurred by dog owners for bringing dogs to the veterinary centres or vaccination point was estimated. We assumed that a dog owner would require about 2 hours

for bringing their dogs to the vaccination point and to return home (including waiting time). However, the time taken would depend on the number of dogs at the vaccination centre. The income loss was calculated using the daily per capita gross national income of Bhutan (year 2006) as described above (see Table 12.3).

12.2.2.2. Animal birth control (ABC) cost

The total number of dogs sterilized (male dogs: 39,502; female dogs: 29,321) in Bhutan between 2001 and 2008 was retrieved from the Veterinary Information System database and from the campaign reports. The costs of sterilization for male and female dogs were then estimated separately since the cost of surgical intervention was expected to be slightly different in each category. We assumed that the direct cost per dog sterilized for pet and stray was same since the captured stray dog were vaccinated and also sterilized at the same time of the campaign. The cost of capturing stray dog was included under dog vaccination cost (as described above). We have included only the recurring costs (cost of anesthetics, antibiotics, analgesics, antiseptic, suture materials, needle and syringes, staff salaries and per diem, refreshment) for the unit cost estimation per dog sterilization. Cost estimation was made assuming a reasonably organized campaign setting in the field with minimum basic facilities (as occurred in Bhutan up to 2008) assuming that a 3 staff team would sterilize about 25 dogs per day (the throughput would depend on dog accessibility) (Kinley Dorjee, personal communication and personnel observation) (see Table 12.3 for input costing data). The capital costs (surgical instruments and other infrastructure costs) were not included in the analysis. The indirect cost (income loss) of pet dog sterilization was estimated based on a daily per capita income (year 2006) assuming a minimum of 3 hours is required to spend at the campaign site till the dog partially recovers from general anesthesia. More waiting time may be required if there are large numbers of dogs at the campaign site. The indirect cost (income loss) for both vaccination and sterilization can also be negligible if the dogs are brought by the children who do not have any income.

12.2.3. Disease surveillance cost

The total number of laboratory tests (n=234 tests) performed to confirm animal rabies between 2001 and 2008 at the veterinary laboratories in Bhutan were retrieved from the laboratory database. We estimated only the recurring cost per test (Fluorescent Antibody Test) by including the cost of the laboratory consumables (rabies conjugates, reagents and other materials required for the tests) and the operating cost (including staff salaries) (see Table 12.4 for input data). The capital cost (such as cost of FAT microscope, laboratory equipments) was not included in the analysis since the government is expected to have the diagnostic facility in place for disease surveillance. Other costs related to rabies outbreak investigation and outbreak control costs were not included due to lack of accurate data.

Table 12.3: Direct medical and indirect cost of dog vaccination against rabies and dog sterilization in Bhutan

Parameters	Estimates			Source
	No.	Nu.	US$	
I. Dog vaccination				
Dog numbers				
No. of doses of tissue culture rabies vaccine imported and distributed to all livestock centers (2001− 2008)	106,790			VPC data, NCAH
Estimated no. of dog vaccinated (assuming all vaccine doses were used, one dose per dog)	106,790			Calculated
Estimated no. of stray dog vaccinated (assuming 60% of the total dogs were stray)	64,074			Calculated
Estimated No. pet dog vaccinated (assuming 40% of the total dogs were pets)	42,716			Calculated
Vaccination Cost				
Vaccine cost per dose		20	0.44	VPC data, NCAH
Material and overhead cost per dog vaccination[a]		25	0.55	Calculated
Dog capturing cost per stray dog[b]		30	0.66	K. Dorjee, pers. commun
Estimated cost per stray dog vaccination		75	1.66	Calculated
Estimated cost per pet dog vaccination		45	0.99	Calculated
Income loss for dog owners				
Estimated no. of hours lost per person for bringing pet dog to the vaccination point	2			K. Dorjee, pers. commun
Equivalent no. of working days lost per person per dog	0.25			Calculated
Per capita Gross National Income for Bhutan (year 2006)		39,639	875	NSB, 2007
Daily per capita Gross National Income		109	2.40	Calculated
Income loss per person per dog		27	0.60	Calculated
Societal cost per pet dog vaccination (direct cost plus income loss)		72	1.59	Calculated
II. Dog sterilization				
Dog numbers				
Total no. of dog sterilized in Bhutan from 2001 to 2008	68,823			VIS, NCAH, field campaign data
No. of male dog castrated	39,502			VIS, NCAH, field campaign data
No. of female dog spayed	29,321			VIS, NCAH, field campaign data
Sterilization cost				
Cost per male dog castration (includes drugs, materials and overhead cost) [c]		281	6.19	Calculated, pers observation, drugs procurement data
Cost per female dog spaying (includes drugs, materials and overhead cost) [d]		296	6.52	Calculated, pers observation, drugs procurement data
Average cost per dog sterilization		288	6.36	Calculated
Income loss for dog owners				
No. of hours lost per person per pet dog for sterilization	3			
Equivalent no. of working days lost per person per dog	0.38			Calculated
Per capita Gross National Income for Bhutan (year 2006)		39,639	875	NSB, 2007
Daily per capita Gross National Income		109	2.40	Calculated
Income loss per person per dog		41	0.90	Calculated
Societal cost per pet dog sterilization (direct cost plus income loss)		329	7.26	Calculated

VPC = vaccine production centre, NCAH=National Centre for Animal Health, NSB =National Statistical Bureau, VIS=Veterinary Information System. [a] Includes staff salaries, per diem, refreshment, and overhead cost for 3 person team, assuming each team would vaccinate 100 dogs per day in campaign setting. [b] Payment of dog catching incentives per dog capture and brought to the vaccination point. [c,d] This cost excludes dog catching cost since it was included under vaccination cost. **Note:** Anesthetic, antibiotic and analgesic costs were estimated for 10kg body weight and would vary the cost with different body weight of the dogs.

12.2.4. Livestock losses cost

The cost of livestock losses between 2001 and 2008 were estimated based on the average cost per head of live animals (cattle, horses, pigs and goats) (Tenzin et al., 2010). The age, sex and breed of cattle that have died of rabies were taken into account for cost estimation since the unit cost would be different for each category. From this, the average cost per head of cattle was estimated (Nu. 11,132, US$ 245.72) and used for analysis (see Table 12.4 for input costing data). The future losses of income (e.g. milk yield loss, meat loss, future calf loss) were not included in the analysis.

The cost estimates in this analysis were based on Bhutan currency Ngultrum (Nu.) and then converted to US dollars (US$) at the exchange rate of 1US$ = Nu. 45.3 (year 2006) while the future cost were estimated at the exchange rate of 1US$ = Nu. 50 (year 2012). The input data were initially managed in a Microsoft excels spreadsheet (Table 12.1, 12.2, 12.3 and 12.4) and then analysed using R software (R Development Core Team) (see Appendix 6 for R-code). The uncertainty of the parameter estimates in the model was accounted for by including estimates as triangular distributions with the maxima and minima set as ± 10% of each parameter's values and then run for 10,000 iterations in R (Knobel et al., 2005). The minimum, maximum, median cost and the 5th and 95th percentile were (see Table 12.5). The sensitivity analysis was also performed to investigate the influence of model parameters, particularly those not supported by sufficient information, and to examine model assumptions as described in each section above.

Table 12.4: Costing data of rabies diagnostic test and livestock loss cost in Bhutan

	Estimates			
Parameters	**No.**	**Nu.**	**US$**	**Source**
Laboratory test cost				
No. of FAT done (2001−2008)	234			NCAH/RVL data
Cost per test (includes reagent & overhead cost)		1,246	27.51	Calculated
Livestock cost				
No. of cattle death and the average cost per cattle [a]	287	11,131	245.72	Tenzin et al., 2010
No. of horses death and the average cost per horse	14	25,000	551.88	
No. of pig death and the average cost per pig	9	10,000	220.75	
No. of goat death and the average cost per goat	5	2,500	55.19	

FAT=Fluorescent Antibody Test; NCAH= National Centre for Animal Health; RVL=Regional Veterinary Laboratory.
[a] Average cost of cattle, calculated based on cost of animal at different age, sex, breed of cattle.

12.2.5. Cost−benefit of dog rabies elimination versus human PET costs

Intensified post exposure treatment can prevent human deaths and may lower the human mortality level, but cannot eliminate rabies incidence completely (Bogel and Meslin, 1990; Zinsstag et al., 2009). However, mass dog vaccination results in a rapid decrease in both dog and human rabies incidence, as has been demonstrated in other studies in Sri Lanka, Philippines, Tunisia, Ecuador, Tanzania and Thailand (Bogel and Meslin, 1990; Miranda and Miranda, 1997; Cleaveland et al., 2003; Kamoltham et al., 2003; Kumarapeli and Awerbuch-Friedlander, 2009; Atienza, 2011; Harischandra, 2011). In this study, we compared the cost of PET in humans with the cost of mass dog vaccination in Bhutan using a 6 years time frame rabies elimination program, based on a rabies control guidelines model designed by Bogel and Meslin (Bogel and Meslin, 1990).

The following parameters and assumptions were used for the model.

I. Dog parameters

1. Dog population in Bhutan was assumed to be approximately 50,000 but is expected to be underestimated since no actual population counting has been undertaken. For our analysis, we used a figure of 77,314 based on an assumed dog: human ratio in Asia of 1:9 (Knobel et al., 2005). From this, the dog population in the south and east of Bhutan that shares a border with India (at risk for rabies occurrence) was assumed to be 34,365 dogs (and remaining 42,949 dogs in the interior Bhutan). The dog population was assumed to stabilize over the years due to an ongoing sterilization program (discussed in detail under discussion) (MoA, 2009).

2. Mass dog vaccination program

The mass dog vaccination program was simulated based on a 6 years time frame. During the first year, 70% of the total dog population in the whole country (n=54,119 dogs) was assumed to be covered under the mass vaccination program. In year 2, 60% of the dog population in the rabies risk areas (south and east Bhutan sharing a border with India) and 10% of the dog population in the interior of Bhutan (especially pups, grown up and unvaccinated dogs in the previous year) to be covered under the campaign. There has been no indigenous cases of rabies in dogs, other domestic animals, wildlife or humans during the last

20 years in interior Bhutan, but mass vaccination with the WHO recommended coverage (>70%) would be necessary to declare rabies free zones. Active surveillance and sero-monitoring studies of vaccinated dogs needs to be undertaken after the initiation of each campaign to examine the sero-conversion rate and the level of protective antibody against rabies virus in the dog population (Bogel and Meslin, 1990).

During years 3 and 4, 60% of the total dog population in those districts that share a border with India (south and east Bhutan that have high risk areas for rabies occurrence) and 10% in the interior of Bhutan (especially in major town areas) are to be covered under mass vaccination program. Active searching of residual foci and elimination has to be undertaken in border areas during this phase. It is expected (best case scenario) that the rabies incidences would decreased or become zero in domestic animals in the south of Bhutan after the initiation of mass dog vaccination (provided there is no incursion from across the border) based on what has been demonstrated in other countries (Tanzania, Equador, Sri Lanka, Thailand, Philippines and Latin America) (Bogel and Meslin, 1990; Miranda and Miranda, 1997; Kamoltham et al., 2003; Belloto et al., 2005; Cleaveland et al., 2003; Kumarapeli and Awerbuch-Friedlander, 2009; Atienza, 2011; Harischandra, 2011). In year 5 and 6, 50% vaccination coverage of the dog population in the border areas and 10% coverage in the interior Bhutan would need to be undertaken to maintain rabies free zones. This would ensure rabies free zones in the interior of Bhutan and an immunity belt in the border areas is maintained. This model was considered in a best case scenario. Cost per dog vaccination was assumed to be Nu. 60 (US$ 1.2), an average cost for stray and pet dog throughout the years. Mass dog vaccination scenarios were simulated with coverage levels of at least 75% in every two years (year 1, 3 and 5) and 25% coverage in intermediate years (year 2, 4 and 6), assuming that a protective antibody titer would be maintained for at least two years.

II. Human PET parameters:

We assumed that there would be 3440 PET cases per year based on average cases from the period 2009 to 2011, and this number of patients is expected to remain stable (or may increase) if the rabies vaccine is administered at the existing rate in the medical hospitals and if the interior of Bhutan is not declared a rabies free zone. For our analysis, we assumed that

the annual expenditure on PET would be high in the first year of the mass dog vaccination campaign due to public awareness of rabies from the vaccination program, and then the human PET cost would progressively reduce by about 15% after the first mass dog vaccination campaign, then by about 35% after the second campaign, and 55%, 65%, and 80% (best case scenario) after the subsequent campaign due to mass dog vaccination (with 70% dog vaccination coverage) and elimination of rabies infection foci (there can also be drastic reduction of expenditure on human PET). Most PET cases are reported in the interior of Bhutan (especially in the capital city, Thimphu) due to high incidence of stray dog bites, but not by rabid dogs (Tenzin. et al., 2011b). Cost of PET including vaccine and overhead cost for 5-dose Essen regimen is Nu. 1615, and assumed to remain the same throughout the year. We assumed that ERIG was administered to only 10% of the total PET cases in high risk areas that have category III exposures. Cost for one dose of ERIG (max 3000 IU) and the overhead cost is estimated at Nu. 882. A sensitivity analysis was performed to estimate the human PET cost assuming that the PET cost decreases by only 5% and 10% annually despite 70% dog vaccination coverage. The above hypothetical model was prepared in Microsoft excel using the above input parameters and the expected cost for human PET alone, cost for combined dog vaccination and human PET, and their cumulated cost over the years of the campaign was calculated. The cost estimates in this analysis were based on Bhutan currency Ngultrum (Nu.) and then converted to US dollars (US$) at the exchange rate of 1US$ = Nu.50 (year 2012).

12.3. Results

12.3.1. Human post exposure treatment

The average direct medical cost of human PET (including the cost of cell culture rabies vaccine, material cost and overhead cost) was estimated to be Nu. 1615 (US$ 35.65) per course (5-times visit). The combined direct medical cost of vaccine and one dose of ERIG or HRIG (for a 50kg body weight patient) for one course of PET were estimated to be Nu. 2497 (US$ 55.13) and Nu. 19,633 (US$ 433.41), respectively. The indirect patient cost (income

loss plus transport cost) was estimated to be Nu. 404 (US$ 8.91) for one course of PET. The societal cost (direct medical cost plus indirect patient cost) per course of PET without rabies immunoglobulin was estimated to be Nu. 2019 (US$ 45) per patient, and would increase to Nu.2901 (US$ 64) and Nu. 20,037 (US$ 442) when one dose of ERIG or HRIG is given to the patient (for ~50kg body weight), respectively (see Table 12.1, 12.2 and 12.5).

During the period from 2001 to 2008, an estimated 10,223 patients were treated with rabies vaccine injection alone (assuming each patient received 5-dose vaccine course). The total direct medical expenses (without rabies immunoglobulin) were estimated to be Nu. 16.49 million (US$ 0.36 million) and a societal cost of Nu. 21.45 million (US$ 0.47 million) (Table 12.5). The estimated direct medical cost was Nu.15.22 million (US$ 0.34 million) when the lower confidence limits of the total PET cases were considered. If each patients received 4-dose vaccine course (instead of 5-dose), the direct medical cost is estimated to be Nu. 16.62 million with a societal cost of Nu.20.56 million.

During the period from 2009 to 2011 (as of July 2011), 51,600 doses of HDCV were imported and used in Bhutan to treat about 10,320 patients, assuming each patient received 5-dose vaccine course (annual average of 3440 patients). The direct medical cost (without rabies immunoglobulin) within these three years was estimated to be Nu. 16.57 million (US$ 0.36 million), which had slightly exceeded the estimated expenditures incurred during the previous eight years (2001−2008). If the annual PET cases remain same (at 3440 per year) in the future, the direct medical cost with vaccine alone (plus overhead cost) is estimated to be Nu. 5.55 million (US$ 0.11 million) per year. If 10 % of the estimated 3440 patients (annual PET cases) are to be given ERIG in the future, the total direct medical cost (vaccine plus ERIG) for PET is estimated to be Nu. 5.83 million (US$ 0.12 million) per year, and Nu. 11.74 million (US$ 0.23 million) when HRIG is given. The annual direct vaccine (HDCV) cost and estimated direct medical cost (from 1994 to July 2011) is shown in Figure 12.2, illustrating a rapid increase of estimated expenditure for human PET from the year 2006.

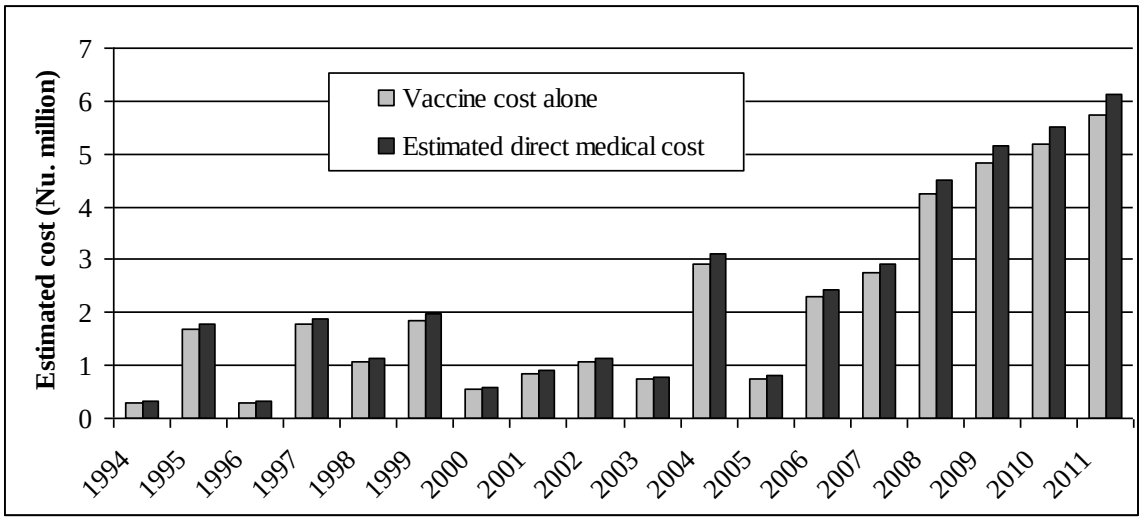

Figure 12.2: The vaccine cost and estimated annual direct medical cost for human post-exposure treatment for rabies in Bhutan from 1994 to July 2011 (in million Ngultrum). The number of patients treated with PET for each year was estimated based on yearly imports and use of human diploid cell rabies vaccine (HDCV) doses assuming that each patient received 5-doses of vaccine course. The annual direct medical cost = no. PET cases per year × Nu. 1615 (see Table 1). The vaccine cost = no. of annual doses of vaccine procured × Nu.305 (see Table 12.1).

Table 12.5: Estimated direct medical costs and societal costs due to rabies in Bhutan from 2001 to 2008

	Cost estimate in million Ngultrum		
Category	**Median cost[b]**	**Minimum[c]**	**Maximum[c]**
PET cost in humans			
Direct medical cost [a]	16.49 (15.00−18.06)	13.76	19.64
Indirect (patient cost)	4.93 (4.47 −5.45)	4.11	5.97
Income loss	2.08 (1.89−2.29)	1.72	2.47
Transport cost	2.84 (2.50−3.24)	2.31	3.49
Societal cost(direct medical + indirect cost)	21.45 (19.67−23.30)	18.06	25.02
Dog vaccination cost (direct cost)	6.72 (6.23−7.22)	5.75	7.80
Stray dog	4.80 (4.31−5.26)	3.96	5.71
Pet dog	1.92 (1.74−2.10)	1.59	2.29
Indirect cost			
Income loss	1.16 (1.08−1.24)	1.04	1.27
Societal cost for dog vaccination (stray + pet)	7.88 (7.39−8.39)	6.88	9.07
Dog sterilization cost	19.76 (18.44−21.11)	17.02	22.45
Male	11.08 (10.05−12.17)	9.15	13.19
Female	8.66 (7.87−9.50)	7.18	10.26
Livestock loss cost	3.64 (3.34−3.95)	3.07	4.26
Cattle loss cost	3.19 (2.89−3.50)	2.63	3.78
Horses loss cost	0.35 (0.31−0.38)	0.28	0.42
Pigs loss cost	0.09 (0.08−0.10)	0.07	0.10
Goats loss cost	0.01 (0.009−0.01)	0.008	0.01
Laboratory test cost	0.29 (0.26−0.32)	0.24	0.35
Total direct cost	46.95 (44.80−49.06)	42.15	51.23

[a] Direct medical costs included the cost of vaccine, material cost, and overhead cost (rabies immunoglobulin cost was not included in the analysis since it was not administered to patients).
[b] Median cost and the figures in parentheses are the 5th and 95th percentiles of output probability distributions
[c] Minimum and maximum cost of output probability distributions.

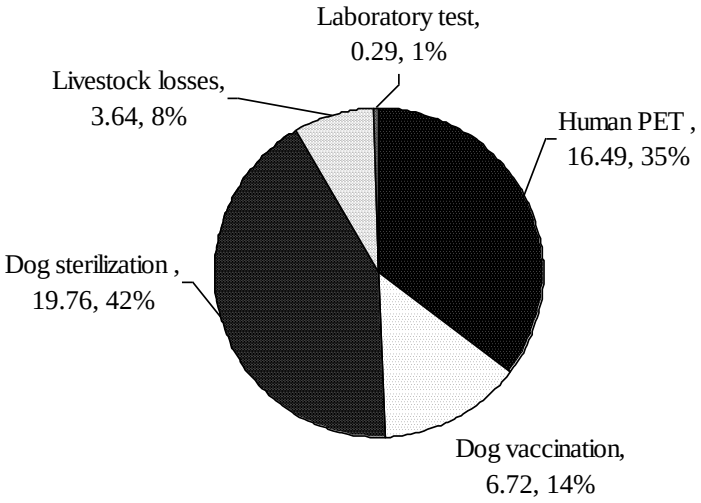

Figure 12.3: Total estimated direct cost of human post-exposure treatment for rabies, dog rabies vaccination and sterilization, laboratory test and livestock losses cost (in million Ngultrum) in Bhutan between 2001 and 2008.

12.3.2. Dog vaccination and animal birth control cost

The average direct medical cost per dog vaccinated is estimated to be Nu. 75 (US$ 1.66) and Nu. 45 (US$ 0.99) for stray and pet dogs, respectively. The indirect cost per pet dog vaccination was estimated to be Nu. 27 and a societal cost of Nu. 72 (US$ 1.59) using the per capital Gross National Income for the year 2006. During the period from 2001 to 2008, a total of 106,790 dogs were estimated to have been vaccinated in Bhutan based on the vaccine imports and distribution data. The direct medical costs were estimated to be Nu. 6.72 million (US$ 0.15 million) (see Table 12.3 and 12.5) whilst the cost of vaccine alone was estimated to be Nu. 2.13 million (US$ 0.05 million). When the direct cost was recalculated using the lower confidence limits of the vaccinated dog population (to account for the variability of vaccinated dogs), the total direct cost from 2001 to 2008 was estimated to be Nu. 6.21 million (US$ 0.14 million).

The average direct medical cost per dog sterilized was estimated to be Nu. 281 (US$ 6.19) for male and Nu. 296 (US$ 6.52) for female dog, with an overall average cost of Nu. 288 (US$ 6.36) per dog. This cost excludes the capturing cost for stray as it was included under vaccination cost as described in Section 12.2.2.2 above since the captured stray dog were vaccinated and also sterilized at the same time of the campaign. During the period from 2001 to 2008, a total of 68,823 dogs were sterilized, and the direct medical costs were estimated to be Nu. 19.77 million (US$ 0.43 million) (see Table 12.3 and 12.5). When the direct cost was recalculated using the lower confidence limits of the sterilized dog population (to account for the variability of sterilized dogs), the total direct cost from 2001 to 2008 was estimated to be Nu. 17.46 million (US$ 0.38 million). Assuming that about 3 hours is required for dog owners to bring dogs and wait until sterilization is done and the dog recovers from anesthesia, an average societal cost per pet dog sterilization is estimated to be Nu. 329 (US$ 7.26) (Table 12.3).

12.3.3. Disease surveillance and livestock losses cost

The average cost per laboratory test to confirm rabies using fluorescent antibody test was estimated to be Nu. 1246 (US$ 27.51). The total cost for confirming 234 samples using

laboratory testing from 2001 to 2008 was estimated to be Nu. 0.29 million (US$ 0.006 million) (see Table 12.4 and 12.5). This cost would vary by using different diagnostic procedure such as rapid antigen detection kit. The total cost of direct loss of livestock (cattle, horses, pigs and goats) due to rabies from 2001 to 2008 was estimated to be Nu. 3.64 million (US$ 0.08 million) (see Table 12.4 and 12.5).

The overall direct cost for the human PET, dog vaccination and animal birth control, laboratory test, and livestock losses during the period 2001−2008 was estimated to be Nu. 46.95 million (US$ 1.03 million) (Table 12.5). Figure 12.3 shows the distribution of direct medical cost.

12.3.4. Benefit-cost of dog rabies elimination versus human PET cost

- If the human PET (vaccine plus ERIG at 10% of total PET cases) is provided to any animal bite victims at the existing rate, the total direct medical cost is estimated to be Nu. 5.85 million (US$ 0.12 million) per year and this annual expense is estimated to remain stable (may increase) over the years with a cumulated cost of Nu. 35.10 million (US$ 0.70 million) at the end of 6 years (Figure 12.4). If HRIG is given instead of ERIG, the estimated annual cost would increase to Nu. 11.75 million (US$ 0.23 million) with a cumulated cost of Nu.70.50 million (US$ 1.41million) at the end of 6 years. However the rabies incidence in humans would remain stable at about 2 cases per year based on the existing incidence (would increase if there is no control program in the dog population).
- If mass dog vaccination is implemented (70% coverage of the entire dog population in the country in year 1, 60% coverage in the rabies risk areas plus 10% coverage in the interior areas of Bhutan in year 2, 3 and 4, and 50% coverage in rabies risk areas and 10% coverage in interior Bhutan in year 5 and 6), the total cost in the first year was estimated to be Nu. 3.49 million (US$ 0.07 million) with gradual decrease of the cost with a cumulated cost of Nu. 10.31 million (US$ 0.21 million) at the end of 6 years (see Figure 12.4).
- Dog rabies incidence is expected to reduce drastically or become zero within 2 to 3 years of the campaign (assuming best case scenario) based on field data in other

rabies endemic countries, provided there is no incursion of rabid dogs from across the border in South Bhutan.

- If both mass dog vaccination and human post exposure treatment are implemented through a "One Health Approach", the combined cost is estimated to be greater than the cost of human PET alone during the first 2 years of the campaign, and would become lower than human PET cost alone after the 3rd year of the campaign provided there is a gradual reduction of PET administration after the mass dog vaccination (see Figure 12.4).
- The cumulated cost of the combined strategy (human PET cost + dog vaccination cost) would be greater than the human PET cost alone up to the 4th year of the program, and then would be lower than human PET cost alone after the 5th year of the campaign (Figure 12.5). The total cumulated cost of the combined strategy is estimated to be Nu. 30.79 million (US$ 0.61 million) which would be lower than the cumulated cost of the human PET cost alone (if intensified PET is done) at the end of 6 years (Nu.35.10 million, US$ 0.70 million) (see Figure 12.5).
- If mass dog vaccination is implemented with 75% coverage in every two years and 25% coverage in intermediate years (of the estimated 77,314 dogs in the entire country), then the cumulated cost of mass dog vaccination would be Nu. 13.91 million (US$ 0.28 million) at the end of 6 years.
- If the human PET administration is reduced by only 5% and 10% annually despite implementation of mass dog vaccination, the total direct medical cost expenses during 6 years would be Nu.33.68 million and 32.22 million respectively.

- Therefore, the benefit of mass dog vaccination and elimination of rabies in the animal reservoir would accrue from the saving of expenditures on human PET, dog vaccination, human rabies treatment cost and benefits of additional earnings of humans and from livestock whose death would be prevented by elimination of rabies in dogs (not quantified in this analysis).

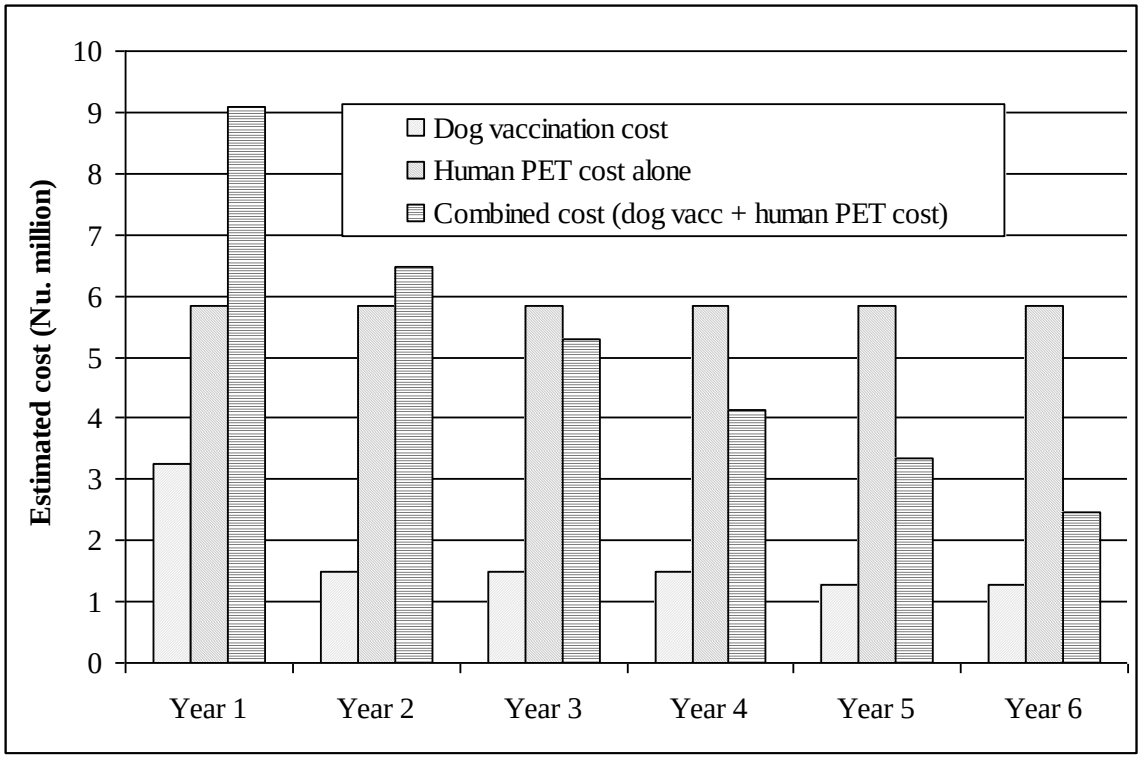

Figure 12.4: Estimated cost of dog rabies elimination, human post-exposure treatment alone and combined cost (dog vaccination and human post-exposure treatment) program for a 6 years time frame (in million Ngultrum).

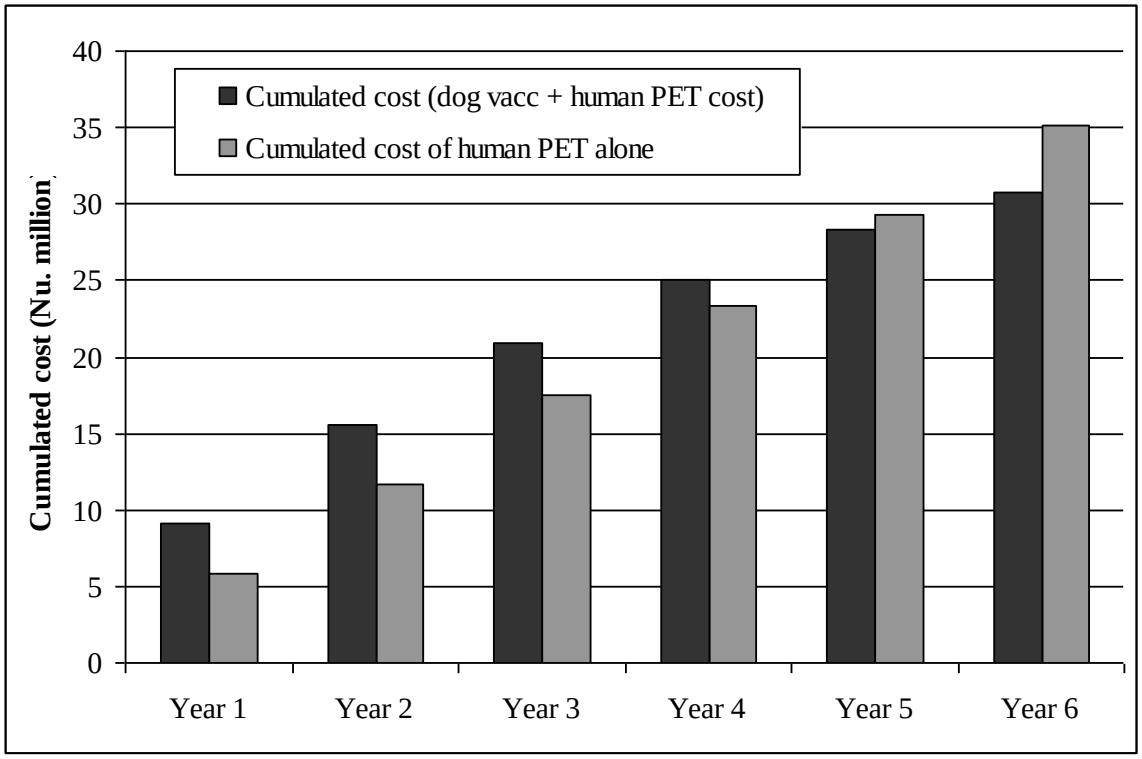

Figure 12.5: Cumulated cost of combined dog rabies vaccination and human post-exposure treatment cost versus human post-exposure treatment cost alone (in million Ngultrum)

12.4. Discussion

In this study we describe the results of the first attempt to quantify the public health burden and economic impact of rabies in Bhutan. The key findings from this study are the estimation of the direct medical and indirect cost per human PET, cost per dog vaccination and sterilization, surveillance cost, cost of livestock losses, cost−benefit of dog rabies elimination versus human heath and public health finances, and the overall cost of rabies and its intervention activities in Bhutan. However, the assumptions made to estimate the costs of various interventions in this study must be examined cautiously.

Our result demonstrates that a substantial amount of direct medical expenses for human PET has been incurred by the Government in Bhutan over the last 8 years (2001−2008). Interestingly, the combined expenditures for the past eight years were about the same as the total expenditure incurred during less than 3 years (2009–July 2011). This indicates that the

cost of human PET has substantially increased in recent years representing a significant financial burden on the public health budget. However, this increasing trend of PET cases and its associated cost may not reflect the true human exposure to rabies (true exposure to confirmed rabid dogs) in Bhutan. The rapid increase in the costs may be due to overused or inappropriate administration of PET, as has been demonstrated in previous studies: the medical hospital provides rabies vaccine injection to almost all dog bite victims irrespective of the nature of exposure (Tenzin. et al., 2011b). Although the rabies incidence is zero in the interior of Bhutan (but dog bites are widespread due to increased numbers of stray dogs), the bite from any dog is still considered a rabies risk that results in continued usage of many human PET doses, which are provided free of charge by the government medical hospitals. Therefore, most of the PET may have been provided as precautionary measures due to heightened concern and anxiety of the people rather than in response to a true exposure, thus increasing the costs (McCombie, 1989; BBS, 2010). Similar issues of overused or inappropriate use of PET is also a major concern in the United States and has been discussed by many authors in their studies (Currier et al., 1975; Helmick, 1983; CDC, 1995; Bell, 1996; Noah et al., 1996; Auslander and Kaelin, 1997; Krebs et al., 1998; Blanton et al., 2005; O'Bell et al., 2006; Christian et al., 2009). However, the cost of human PET can be greatly reduced if improved evaluations of risk and need for PET is done by the physician following WHO guidelines and also through close coordination between veterinary and public health officials (McCombie, 1989; Meltzer and Rupprecht, 1998a; Rupprecht and Gibbons, 2004; Dubnov et al., 2006; WHO, 2010b). Our analysis also shows that the indirect patient expenses accounted for only a small fraction (23%) of the total societal cost for rabies related treatment because of free medical services in Bhutan (see Table 12.5). This is in contrast to other rabies endemic countries, where patient-borne expenses form the bulk of the expenditure: the patient has to buy the biological (vaccine, rabies serum), pay for the treatment expenses (doctor and nurse consultation and administration fee), and bear other indirect expenses of transport cost and income loss (Bogel and Meslin, 1990; Fishbein et al., 1991; Knobel et al., 2005; Zinsstag et al., 2009). This resulted in more human rabies death since appropriate postexposure prophylaxis is not always available and affordable in resource limited countries (Knobel et al., 2005; Zinsstag et al., 2009).

The estimated cost per dog vaccination is very low compared to the cost of human PET. Our estimated direct cost per dog vaccination (US$ 0.66−1.66) is comparable with findings from other countries: US$ 1.30 in Asia and Africa (Bogel and Meslin, 1990; Knobel et al., 2005); US$ 1.19−4.27 in the Philippines (Fishbein et al., 1991); US$ 1.30 in Thailand and Tunisia (Bogel and Meslin, 1990), US$ 2.70 in Malawi (Edelsten, 1995), US$1.70 in Tanzania (Cleaveland et al., 2006), and US$ 2.14 in Chad (Zinsstag et al., 2007), but the average cost of dog vaccination could vary in a range of different rural and urban settings depending on the accessibility of dogs, and the level of community participation during the campaign. In Bhutan, mass vaccination is mainly targeted towards the stray dog population in the street. Although most dogs are accessible for vaccination, it is often difficult to capture those dogs and bring them to the vaccination point. The time and resources spent capturing stray dogs would increase costs of vaccination (Meltzer and Rupprecht, 1998b). Well planned campaigns and community participation would reduce the costs and increase vaccination coverage, as has been demonstrated elsewhere (Kayali et al., 2003; Kayali et al., 2006). Similarly, the direct cost per dog surgical sterilization (US$ 6.19–6.62) was estimated in a fairly organized campaign setting in a field situation conducted between 2001 and 2008. Our estimated cost would have been underestimated when compared with well organized campaigns. For instance, the cost of surgical sterilization would vary greatly, mainly due to differences in staff salaries and efficiency of the surgical team, availability of existing facilities, quality of suture materials used, differences in drug and anesthetic costs, body weight of dogs (would influence the anesthetic and drug requirement) and also depending on the number of animals being sterilized (for example, significant savings can be made in high-throughput systems). The World Society for the Protection of Animals project has estimated the costs per dog sterilization of approximately US$ 6–25 (WHO, 2010a). The Blueprint for Rabies Prevention and Control had estimated with a range from US$ 3 to15 with an average of US$ 7.50 for the medicines and consumables, and the full costs (including veterinarians and veterinary support staff, clinic running costs, all medicines and consumables) ranged from US$ 10 to 52, with an average of US$ 30 per dog sterilization (http://www.rabiesblueprint.com). Although surgical sterilization is an effective permanent method for dog birth control in the long run, it is expensive. Sterilization may be required for many years and is difficult to sustain in a resource limited country. In addition, the

sterilization of dogs in one or more areas may divert resources away from the priority of mass vaccination, which is required for elimination of rabies in dogs. Nevertheless, ABC programs have been recommended to control dog population (rather than killing of dogs) on ethical ground and it has been reported that properly conducted ABC programs are expected to decrease the dog population, with stabilization occurring 5–7 years after implementation (Leney and Remfry, 2000). For example, in an organized pilot campaign in India (Jaipur city), about 24,986 neighborhood dogs (about 65% coverage) were captured, sterilized, vaccinated and released between November 1994 and December 2002 (Reece and Chawla, 2006). As a result, the dog population declined by 28%, and human cases of rabies declined to zero in the programme area but increased in other areas where the campaign was not organized (Reece and Chawla, 2006). Similarly in another intensive field study in Jodhpur (India), population size and demographics of stray dogs were measured before and after implementation of an ABC program between 2005 and 2007 (Totton et al., 2010). About 62–87% of the free-roaming dog population were surgically sterilized and vaccinated for rabies in the five survey areas of Jodhpur by 2007, which resulted in a drastic decline of the dog population (Totton et al., 2010). However, a population demographic model predicted that at the current level of sterilization (62−87%), the dog population would decrease by 69% and reach stability after 13–18 years, suggesting it would require long term planning and can be very expensive (Totton et al., 2010).

In Bhutan, mass dog sterilization was used as a strategy to control dog population since the early 1990s after shooting and poisoning of dog was abandoned on cultural grounds. Mass dog sterilization has been intensified throughout Bhutan (since 2009) in collaboration with the Human Society International and will continue untill 2015. Although it is expensive, this project is expected to sterilize and vaccinate more than 70% of the dog population in the country with an objective to stabilize the dog population to a manageable level (MoA, 2009). However, the impact of this program is yet to be assessed in terms of the demography of the street dog population, dog bite incidents in humans, prevalence of rabies in both human and animals and the cost of the program.

This study demonstrates that rabies associated cost was a substantial financial burden to the government in the form of direct medical cost for PET in humans, rabies control program in

dogs, disease surveillance, and also to the people in the form of livestock and production losses, income loss and transport costs. However, our estimates would have been under estimated since our analysis did not include the cost associated with disease outbreak investigation, outbreak response costs, and other recurring and capital costs (as explained in Section 12.2.). Improved record keeping of all the expenses and documentation would provide reliable information about the impact of rabies and its associated costs.

Cost−benefit analysis of mass dog vaccination versus human PET costs over a 6 year period indicate that the use of intensified human PET alone would incur huge direct medical costs to the government if the annual trend of PET remain stable (or increase) over the years in the absence of a mass dog vaccination program (Figures 12.4 and 12.5). If both mass dog vaccination and human PET are implemented through a "One Health Approach" program program by sharing resources and proving financial assistance by the public health to the veterinary services (Vallat 2011; Zinsstag et al., 2007) the recurring cost of human PET would decrease progressively and save the scare resources for the public health finances in the country (in the best case scenario). Our analysis also shows that the cumulated cost of PET alone would be greater than the combined cost (human PET plus dog vaccination cost) after 6 years of rabies elimination program implementation (Figure 12.4 and 12.5). Mass dog vaccination is expected to eliminate rabies infection foci within 2−3 years of effective program implementation, as has been demonstrated in other rabies endemic countries (Tanzania, Thailand, Sri Lanka, Equador, Philippines) (Wells, 1954; Bogel and Meslin, 1990; Miranda and Miranda, 1997; Meltzer and Rupprecht, 1998b; Belotto et al., 2005; Cleaveland et al., 2003; Kumarapeli and Awerbuch-Friedlander, 2009). For instance, in Tanzania, dog bites injuries have declined by 51% after the first campaign and, by 90% and 92% after the second and third dog vaccination campaign, respectively (Cleaveland et al., 2003). Therefore, human public health should support/fund the veterinary services for mass dog vaccination since the elimination of rabies at the reservoir species (dogs) is the only ultimate strategy to prevent human rabies deaths and also reduce recurring cost of human PET.

The major limitation of this study is the assumptions that we made to estimate the cost for each intervention program (as discussed in each section above). Nevertheless, our study provides baseline information about the cost of various interventions that can be used as a guideline for estimating the economic impacts of rabies and for control program planning. It is important to make periodic estimates of the monetary costs of various interventions for economic assessment of the burden of rabies and to assess expenditure and achievement of the public health program for policy discussion. The recurrent cost of human PET may be reduced drastically, if rabies control is directed towards animal reservoir (Zinsstag et al., 2009; Zinsstag et a., 2007; Vallat, 2011) or by introducing cost-effective intradermal administration of rabies vaccine in Bhutan. Rabies elimination is achievable in Bhutan by maintaining an immunity belt area in south Bhutan bordering India and by instituting an active surveillance system to monitor cross-border incursion of all suspect rabid animals and confirm by a laboratory test.

Conflict of interest statement: there was no conflict of interest

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CHAPTER 13

13.GENERAL DISCUSSION AND RECOMMENDATIONS

13.1. Introduction

Rabies is a neglected viral zoonosis and occurs worldwide but is highly endemic in resource limited countries (Knobel et al., 2005; Fooks, 2005). Globally, the main reservoir of rabies is the domestic dog which is responsible for almost 99% of fatal rabies cases in humans (Knobel et al., 2005). Canine rabies is endemic in Bhutan and poses a public health risk and causes economic losses to society. Therefore, this study was conducted to better understand the epidemiology of rabies occurrence in animals and humans and estimate the cost of various rabies interventions measures in humans and animals.

To understand the epidemiology of rabies in animals, animal rabies surveillance data (1996 to 2009) were analysed using Geographic Information System, time series analyses, logistic regression and spatial statistics (Chapters 3, 4, 5, 6 and 7). In addition, molecular and phylogenetic analyses were conducted to identify the rabies virus strain circulating in dog population (Chapter 8). To understand the epidemiology of rabies in humans, the dog bites in humans and the community knowledge and perception of rabies were studied using cross-sectional epidemiological studies (Chapters 10 and 11). In addition, the pattern of PEP use in humans was also studied by analysing the retrospective data retrieved from the hospital database (Chapter 9). Finally, the financial impact of rabies in Bhutan was analysed by estimating the unit cost of rabies interventions in human and animals and compared the cost of dog rabies elimination versus human post-exposure prophylaxis cost (Chapter 12).

Key findings from all studies presented in the thesis are discussed in this chapter with the aim to provide guidelines for developing policy for control of rabies in Bhutan.

13.2. Epidemiology of rabies in animals

Analysis of surveillance data identified more reports of rabies cases in dogs and cattle in Bhutan and demonstrated a significant cross-correlation between the reports of rabies in dogs and other domestic animals, wherein the report of cases in dogs predicts cases in other domestic animals (Chapter 3) (Tenzin. et al., 2011a). This finding could be used to enhance educational activities and rabies surveillance in the event of rabies occurrence in dogs. This will help in early detection and prevention of rabies cases in animals and humans.

A significant seasonal variation of reported animal rabies cases was also observed with increased incidences during spring and summer months (Tenzin. et al., 2011a), likely to be associated with the breeding season of dogs, as has been similarly demonstrated in other countries (Malaga et al., 1979; Ezeokoli and Umoh, 1987; Mitmoonpitak et al., 1998; Panichabhongse, 2001). It has been reported that the dog-breeding season is associated with increased contact rates between dogs, leading to frequent fights and increases the risk of virus transmission (Malaga et al., 1979; Ezeokoli and Umoh, 1987; Mitmoonpitak et al., 1998; Panichabhongse, 2001; Hampson et al., 2007). Therefore, mass dog vaccination campaign can be scheduled prior to the dog breeding season. Rabies surveillance should also be enhanced during these seasons.

The trend of reported rabies cases in animals was found to be relatively stable from 1996 to 2005 with geographic distribution limited to southern parts of Bhutan (Chapters 3 and 6), but it increased in 2006 and 2008 (with increased number of cases and area of spread). This was due to re-emergence of rabies in eastern and south west Bhutan, areas that have been previously free from rabies (Chapters 4 and 5) (Tenzin et al., 2010a; Tenzin. et al., 2010b). The study indicated that human activities, road network accessibility (for example, see Appendix 1 and 3), and high stray dog density − played an important role in the spread of rabies. The public education, surveillance and control activities should be targeted in town areas that have high dog density and have high risk of rabies occurrence. Early case detection and immediate implementation of control measures is necessary for preventing spread of disease.

The geopolitical location and human population characteristics − the cross-border movement or translocation of stray dogs and inadequate control activities − may be responsible for increased incidence of rabies in the south border areas of Bhutan that share a porous border with India (Chapters 3, 6 and 7) (Tenzin. et al., 2011a; Tenzin et al., 2012a, b). The phylogenetic analyses also suggest that the rabies virus variants circulating in southern Bhutan originated from a single common ancestor, and were more closely related to the rabies virus strains from India and Arctic-like-1 viruses circulating in South Asia (Chapter 8) (Nadin-Davis et al., 2007; Kuzmin et al., 2008; Tenzin. et al., 2011b; Nadin-Davis et al., 2012). To gain a better appreciation of the transmission dynamic and rabies virus diversity in the region more sampling from Bhutan and the adjoining Indian States of Assam and West Bengal is needed and accordingly carry out TMRCA analysis to confirm the origin of viruses. Since rabies is particularly reported from southern parts of Bhutan along the Bhutan-India border, more resources for rabies control programmes and surveillance should be targeted in these areas. Mass dog vaccination in the border areas would maintain an immunity buffer (*cordon sanitaire*) and prevent the incursion of rabies into interior Bhutan.

13.3. Epidemiology of rabies in humans

In humans, rabies cases were found to be sporadic, mainly reported in the south rabies endemic areas. A total of 15 human rabies deaths was reported between January 2006 and July 2011 (with 5 deaths reported in 2011 alone), equivalent to a cumulative incidence of 2.14 per 100000 populations (Chapter 9) (Tenzin. et al., 2011c). The main cause of human rabies deaths was failure to seek immediate care at the health facilities or negligence on the part of health workers, despite PEP being provided free of charge to patients by the medical hospitals in Bhutan (Kuensel, 2009; 2010; 2011).

Although the number of human rabies deaths was sporadic, there were increased number of dog bite incidents and subsequent rabies PEP administration to the people (Tenzin. et al., 2011c; Tenzin et al., 2011d). Provision of PEP free of charge to patients may be associated with low number of human rabies deaths in Bhutan.

The hospital based survey indicated significant gender and age differences in dog bite incidents: males and children, particularly those aged 5–9 years were more at risk of dog bites than females and other age groups (Chapter 10) (Tenzin et al., 2011d). This is in agreement with findings from studies conducted elsewhere (Overall and Love, 2001; Pancharoen et al., 2001; Rosado et al., 2009). It has been stated that increased dog bite incidents in children is a behavioural risk because of their extreme curiosity, lack of inhibition, limited knowledge and experience about dog behaviour, and inability to protect themselves from an attack (Berzon et al., 1972; Sinclair and Zhou, 1995; Sacks et al., 1996; Daniels et al., 2008).

Analysis of PEP usage in humans also revealed a significant gender and age difference among the PEP recipients in which males received more PEP than females across all age groups, and children – particularly 5–9 years of age − received more rabies PEP than other age groups (Chapter 9) (Tenzin. et al., 2011d). The increased usage of PEP in males and in children has similarly been demonstrated in other studies (Martin et al., 1969; Helmick, 1983; Pancharoen et al., 2001; Sriaroon et al., 2006). This indicates that children and males are at higher risk of rabies exposure and therefore, educational activities should be directed towards this group. Integration of education on rabies and its prevention and control in the 'elementary school' and 'village level non-formal education' curriculum would be an alternative approach to create awareness about rabies to school children and the people (Dodet, 2010).

Post-exposure prophylaxis is effective and safe, but it is expensive and is often used inappropriately even in industrialized countries (Moran et al., 2000; Rupprecht and Gibbons, 2004). This study also showed an inappropriate or unnecessary use of PEP in individuals that have no risk of rabies exposure (such as consumption of dairy product derived from rabid or rabies suspected animals) (Chapter 9) (Tenzin. et al., 2011d); such use can be best prevented by proper risk assessment of the exposure pathways to reduce the use of scare vaccine resources and government expenses (Rupprecht and Gibbons, 2004; Dubnov et al., 2006; WHO, 2010). Although there have been no well-documented reports of human rabies transmission through dairy product consumption (WHO, 2010), we believe that PEP may

have been administered because of the heightened concerns and anxiety of the people rather than in response to a true exposure (McCombie, 1989; Blanton et al., 2005; Kuensel, 2010).

The study also identified a lack of patient compliance to complete the course of PEP (received <5-doses of Essen Regimen) (Chapter 9) (Tenzin. et al., 2011d). However, the results suggest that patients with animal bite injury were less likely to receive an incomplete vaccine course than non-bite recipients, and also patients presenting to hospitals in rabies endemic or outbreak areas were less likely to receive an incomplete course than in rabies free areas, thus reducing the chance of vaccination failures (Chapter 9) (Tenzin. et al., 2011d).

The health seeking behaviour of people for PEP following dog bite incidents and other mode of exposures was confirmed by a community-based KAP study in one of the rabies endemic area in south central Bhutan (an area endemic for rabies) (Chapter 11) (Tenzin et al., 2012c). The study showed that a majority of the interviewed respondents had heard of rabies, had a positive attitude towards the prevention and control of rabies, and had good health seeking behaviours. Studies conducted elsewhere (Agarvval and Reddaiah, 2003; Matibag et al., 2007; Dodet et al., 2008; Bingham et al., 2010) have also shown increased awareness about rabies. However, these findings also indicated the existence of knowledge gaps (knowledge about rabies and importance of wound washing) which could be filled by creating awareness education programmes on: the danger of rabies and mode of transmission to humans and the importance of washing the animal bite wound with soap and water and visiting a hospital following animal bites injuries or exposures to suspected/rabid animals (Tenzin et al., 2012c).

To estimate the true public health burden of rabies and to estimate the under-reporting of human rabies cases, a decision tree model was constructed using the distribution of dog bites on different body parts, the probability of death according to the bite location, and the probability of receiving PEP following bites (Cleaveland et al., 2002). The model predicted 2.23 (95% CI: 1.20−3.59) human deaths from rabies per year, which is equivalent to an annual incidence of 4.67 (95% CI: 2.53–7.53) deaths per 100,000 population in the two southern rabies endemic areas of Phuentsholing and Gelephu (Chapter 10) (Tenzin et al., 2011d). These predicted deaths were comparable with the officially reported human rabies

deaths of 1.5 (95% 0.75–3.00) per year in these areas with an annual incidence of 3.14 deaths (95% CI: 1.57–6.29) per 100,000 population, indicating there is no major underreporting of rabies mortality in Bhutan, unlike the situation in some other rabies endemic countries that have gross under estimates of human rabies mortality by about 75 to 100 times than officially reported (Kitala et al., 2000; Cleaveland et al., 2002; Knobel et al., 2005). Easy accessibility to the hospitals and availability of free PEP provided by the government medical hospitals in Bhutan might be the reasons for people reporting dog bite cases to the hospitals as indicated by increasing usage of PEP in Bhutan (Tenzin. et al., 2011c), although some underreporting of dog bites is possible. The model also predicted 19.24 (95% CI: 13.69–25.14) human rabies deaths per year in these two rabies endemic areas in the absence of PEP, suggesting that PEP is important for preventing human rabies deaths.

13.4. Economic analysis

The final part of this thesis dealt with an economic analysis of rabies interventions in Bhutan, which were quantified by estimating the unit costs of each intervention in both humans and animals (Chapter 12) (Tenzin et al., 2012d). The study showed that rabies and its related intervention has substantial financial impacts on society in the form of PEP cost in humans, dog vaccination and sterilization program costs, livestock losses due to rabies, and epidemiological surveillance and disease control costs. The cost analysis of dog rabies elimination versus human PEP cost indicated that intensified human PEP would incur huge recurring cost every year, while mass dog vaccination would eliminate human rabies cases through elimination of rabies virus in the dog population and thus reduce the recurring cost of human PEP. Therefore, a ‘One Health Approach’ to rabies control in partnership with public health and veterinary services is important if rabies is to be eliminated from Bhutan. Although dog vaccination is labour intensive and poses logistical challenges, cost-effectiveness studies have demonstrated that dog rabies elimination is logical, ethical and more economical than the widespread use of intensified tissue-culture vaccines in humans (Bogel and Meslin, 1990; Zinsstag et al., 2007; Zinsstag et al., 2009). It has been stated by the World Organization for Animal Health (OIE) that just 10% of the total cost currently spent on intensified rabies vaccination in humans would be sufficient to enable the National

Veterinary services throughout the world to eliminate the rabies reservoir in domestic animal populations and prevent rabies cases in humans (Vallat, 2011).

13.5. Conclusions and recommendations

Despite some limitations as discussed in each of the chapters, this study provides useful information about the pattern and risk of rabies occurrence, and the rabies virus strains circulating in Bhutan. In addition, the information generated from the dog bite study and the use of PEP in humans, KAP study and the economic impacts of rabies and its intervention would help in reorienting a rabies control programmes in Bhutan. Considering rabies is a fatal zoonosis and causes serious economic losses to society, the study recommends the following for effective rabies control programmes in Bhutan:

- More resources should be allocated in the highly rabies endemic 'hot spots' in southern Bhutan sharing a border with India. Mass vaccination of dogs in these areas would create an immune buffer (*cordon sanitaria*) and prevent incursion of rabies into interior Bhutan. Mass vaccination of dogs would be a more beneficial, less expensive, logical and ethical way to control rabies in animals than by focussing on the use of intensified post-exposure prophylaxis in humans.
- Rabies elimination is achievable in Bhutan by maintaining an immunity belt area in southern Bhutan bordering India by mass dog vaccination programmes. Therefore, a regionally coordinated rabies control program is necessary at the Bhutan-India border town areas.
- A well-coordinated national rabies control programme through a 'One-Health Approach' is the key towards rabies elimination in Bhutan. Active collaboration and pooling of resources between public health and veterinary services is necessary.
- Epidemiological surveillance of rabies should be improved by the laboratory confirmation of all suspected cases, including human cases, and the data so generated should be shared between the public health and veterinary sectors and also relevant international organizations. Sharing of information about the local epidemiology of

rabies in animals between veterinary and medical professionals can help the clinician to make appropriate decisions for post-exposure prophylaxis in humans.

- Surveillance of rabies should be extended in wildlife to confirm the presence or absence of rabies or rabies related viruses in wildlife in Bhutan.
- More sampling of virus from potential reservoirs from different rabies outbreak areas during different time periods is needed to provide detailed information about rabies virus transmission dynamics in Bhutan.
- Since dog bites are common in Bhutan, continuing surveillance of dog bites is necessary to detect the risk factors, trends and to evaluate the impacts of rabies prevention efforts in dogs. For this, a national dog bite database and reporting system should be maintained at every health centre to asses the impacts of dog vaccination and sterilization program and rabies awareness education.
- An animal exposure risk pathway algorithm should be used by clinicians for deciding PEP in humans to reduce unnecessary use of post-exposure prophylaxis.
- Bhutan should start using the intradermal method of post-exposure prophylaxis to save cost and optimize utilization of vaccine resources. Rabies immunoglobulin should be given to people in the severe exposure category (based on WHO guidelines), particularly in rabies endemic southern Bhutan.
- Public awareness education, especially for children, should be conducted on the prevention of dog bites, the danger of rabies and the mode of transmission to humans; the importance and usefulness of washing the animal bite wound with soap and water and seeking health facilities following animal bites injuries or exposures to suspected/rabid animals.
- School curriculum should include information about careful handling and approaches towards dogs, dog bites, rabies and the importance of washing animal bite wounds and seeking health facilities.
- Since rabies has not been reported in the interior of Bhutan for many years, rabies free zones should be established after conducting active surveillance in the domestic dog population. This would require strengthening of the veterinary surveillance system to maintain the freedom status, and ultimately reduce unnecessary treatment of people for rabies post-exposure prophylaxis.

- Sero-surveillance of vaccinated dogs should be conducted after each campaign to assess protective rabies virus neutralizing antibodies among vaccinated dogs.
- The dog population should be controlled through implementation of regulation, registration, licensing, movement restriction, habitat control, sterilization and through responsible dog ownership.
- It is important to make periodic estimates of the monetary costs of various interventions for economic assessment of the burden of rabies and to assess expenditure and achievement of the public health program for policy discussion.
- Detailed evaluation of the current rabies control program should be conducted to assess the demographics of dog populations, prevalence of rabies neutralizing antibodies in the vaccinated or free-roaming dog population in Bhutan and incidences of animal and human rabies, and the cost of the program with a view to re-orienting future control programs.
- International collaboration is necessary for technical and financial support for sustaining rabies control in Bhutan.

13.6. References

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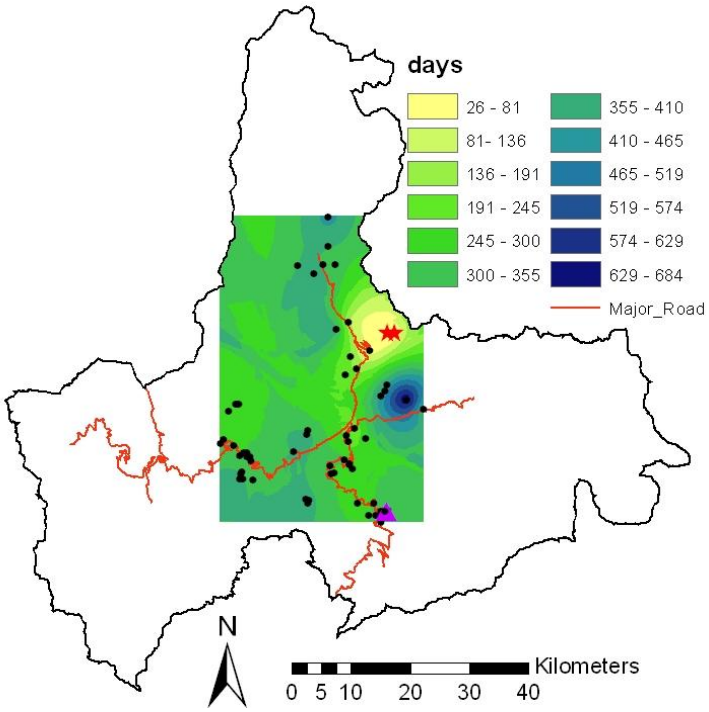
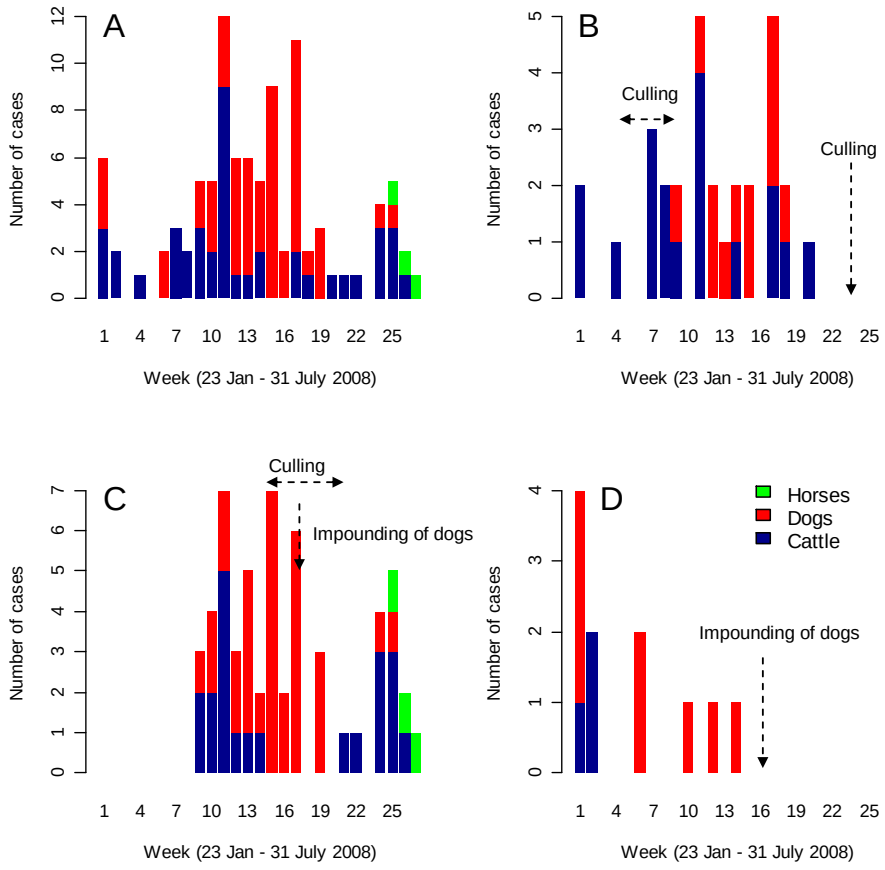
Appendix 1: Rabies outbreak spread in Eastern Bhutan (Chapter 4)



Note: The reported cases were mapped (ArcGIS 9.3; ESRI, Redlands, CA, USA) by using a Bhutan shapefile. A black dot indicates the location of cases in the study area and red line is the road network. Using the date of onset of first case in each case location or village in the study area, the local indicator of spatial autocorrelation (LISA) statistic was used to investigate the local clusters of cases; and identified clusters of early reported (★)(star) and late reported (▲) cases. Variography (VarioWin 2.2) and ordinary kriging (ArcGiS ™ 9.3 Spatial Analyst, ESRI Inc.) were performed to model and produce an interpolated map of the day (since 2 May 2005) of rabies reporting. The interpolated map was then overlaid on the location of cases to identify the area of early occurrence of cases and its subsequent spread during the epidemic. The figure above shows the interpolated day of onset of rabies at each location in eastern Bhutan between May, 2005 and November, 2007. The epidemic started with first occurrence of rabies in the far-east part of the region and then spread rapidly southwards during the next 214 days of the first report. The distribution of cases followed the road network (some road network data not shown on the map), the towns and their surrounding villages.

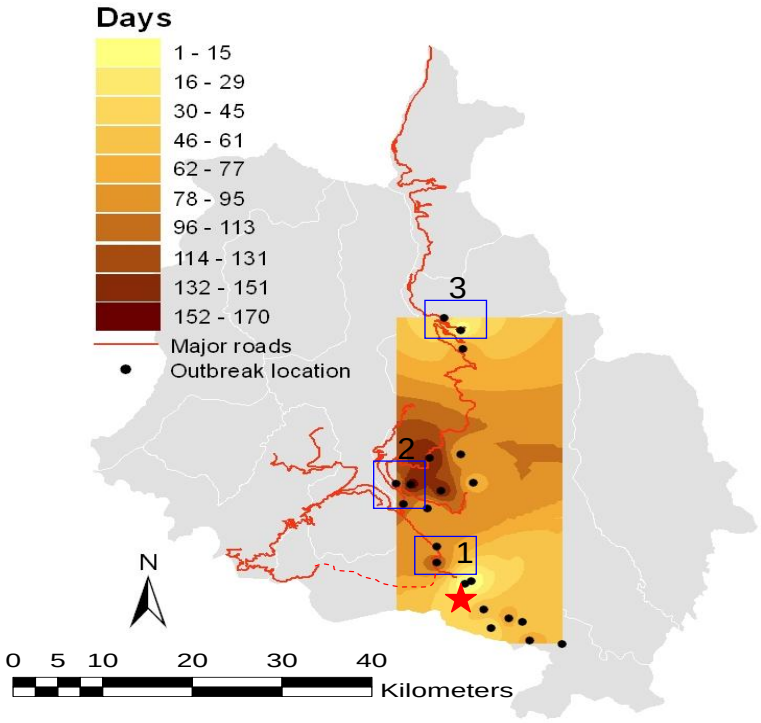
Appendix 2: Epidemic curve of rabies outbreak in Chhukha (Chapter 5)



Note: Figure (A). The overall epidemic curve of rabies outbreak in Chhukha district, January 23−July 31, 2008, and the epidemic curve of rabies reported in Dala **(B)**; Bongo **(C)**, and Bjachho sub-district **(D).** The main intervention measures (culling and impounding of free-roaming dogs) in the outbreak areas are superimposed on the graph. A series of rabies control programs were implemented in the outbreak areas. About 411 unvaccinated free-roaming dogs were culled in Dala (16%) during March and June and in Gedu town (84%) between 7 May and 10 June (weeks 16–20) (Figure **B** and **C**). In addition, 544 free-roaming dogs (Tsimasham and Tsimalakha town area, 227; Gedu town area, 170; Chapcha, 147) were impounded at Tsimasham dog shelter during May 2008 (week 16–17) (Figure **C** and **D**).

Appendix 3: Rabies outbreak spread in south west Bhutan

(Chapter 5)



Note: Interpolated day of rabies outbreak in Chhukha district of Bhutan, 23 January – 31 July 2008.

The reported cases were mapped using a Bhutan shapefile. A black dot indicates the location of cases in the study area. The existence of local clusters of cases was investigated by using the local indicator of autocorrelation (LISA) statistic. To visualize the evolution of the outbreak, we performed interpolation of onset day at each location using Inverse Distance Weighting (Spatial Statistics Tools in ArcGIS 9.3). A significant early cluster of rabies outbreak day identified by LISA statistic is shown on the map (★) (star). Blue colour boxes (□) on the map indicate the location of main towns – Dala (1); Gedu (2), and Tshimasham and Tshimalakha (3). Rabies spread around the index case and south of Dala during the first 30 days, followed by spread towards north of Dala by 45 days, and then to the villages of Bongo sub-district by 60 days. It then spread to the main town of Gedu by 90 days. The distribution of cases followed the road network (some road network data not shown on the map), the towns and their surrounding villages.

Appendix 3: Dog bite in human study questionnaire (Chapter 10)

A hospital-based survey of dog bites in humans (JDWNRH, Gelephu and Phuentsholing): to be administered to dog bite patients visiting the hospital for rabies post-exposure prophylaxis

Date and year of survey: (Date/Month/Year): …**……../……../……**

Hospital name:……Interviewer**: ……..**

(Please tick or circle the answers)

1. Patient age.……years

2. Patient sex:
    a. Male
    b. Female

3. What is your occupation?
    a. Preschool children
    b. Student
    c. Employee (Government/corporate)
    d. Farmers
    e. Business person
    f. Others

4. What animal has bitten you?
    a. Owned dog
    b. Stray dog
    c. Cat
    d. Other animal (Please specify…….)

5. In which part of the body were you bitten?
    a. Face
    b. Head
    c. Hand/arm
    d. Leg/foot
    e. Thigh
    f. Buttock
    g. Other parts (Please specify……..)

6. Was it a provoke bite? (Did you play or disturb the animal)? **Yes [ ] No [ ]**

7. What was the severity of the bite (to be assessed by the interviewer)?
    a. Single bite wound
    b. Multiple bite wound
    c. Only scratches
    d. Others (Please specify……..)

8. With this bite, how many times were you bitten by dogs?.....times

9. In which place/area were you bitten?
    a. At village
    b. At home
    c. At relative's/friend's house
    d. In town area

10. At what time of the day were you bitten?
    a. Early morning
    b. Day time
    c. Evening
    d. Night

11. Is the biting animal available for observation? **Yes [ ] No [ ] Not sure [ ]**

12. Have you heard of rabies and danger to human life? **Yes [ ] No [ ]**

13. What was status of the biting animals (rabid or normal)?
    a. Rabid
    b. Suspected to be rabid
    c. Normal dog
    d. Unknown

14. Did you wash the bite wound with soap & water immediately after you were bitten? **Yes [ ] No[ ]**

15. Did you apply any local medicine to the wound? **Yes [ ] No [ ]**

16. Did you notice or heard of any other humans or animals bitten in that area by the same animal? **Yes [ ] No [ ] Not sure [ ]**

17. During the time of rabies outbreak, will you support killing of stray dogs in the area of outbreak to prevent spread of the disease? **Yes [ ] No [ ] Not sure [ ]**

18. Do you believe that that regular vaccination of dogs can prevent getting rabies in dogs
Yes [ ] No [ ] Not sure [ ]

19. Who suggested or told you to visit the hospital for treatment?
    a. Friends
    b. Public at the site of bite
    c. Parents/children/relatives
    d. Nobody suggested/I came alone

20. Would you like to complete the anti-rabies vaccine injection course? **Yes [ ] No[ ]**

Appendix 4: R code for estimating human rabies deaths (Chapter 10)

```
library(VGAM)
setwd("C:/Data/Temp")

n_iterations = 10000  (# number of iterations)
```

 # P1 is the rabies recognition probability (the proportion of suspected rabid dog that are, in fact, rabid). The disease (rabies) status of the biting dogs was unknown to the patient or hospital staff. However, a total of 46 dog brain samples were examined in Gelephu and Phuentsholing areas between 1996 and April 2011. Of 46, 33 samples were FAT positive to rabies.

```
# Therefore, P1 was calculated using binomial distribution with 10000 iterations as follows:
  size = 46
  P1<-rbinom(n=n_iterations, size=size, prob=33/46)
  P1 = P1/size
  hist(P1, xlim=c(0,1))
```

 # P2 to P5 is the point estimates of dog bites on different body parts (see Table 10.1, Chapter 10).

```
# P2: Of 193 bites, 11 were on head/neck, and so point estimate for P2 =11/193=0.057
# P3: Of 193 bites, 41 were on hand/arm, and so point estimate for P3 =41/193=0.212
# P4: Of 193 bites, 3 were on trunk, and so point estimate for P4 =3/193=0.016
# P5: Of 193 bites, 137 were on leg/foot, and so point estimate for P5 =137/193=0.71
```

Therefore, the R code for P2 to P5 was written as follows:

```
#P2:5 - point estimate of bite locations
  P2=0.057
  P3=0.212
  P4=0.016
  P5=0.71
```

 # P6 to P9 is the probability of developing rabies following a bite injury to head (P6); hand/arm (P7); trunk (P8); and leg/foot (P9) by rabid dog (See Chapter 10, Table 10.1).

```
# Triangular distribution (minimum, mode, maximum) was used to estimate the probability.
```

These probabilities were based on published data and the code for P6 to P9 was written as follows:

```
#P6 -head/neck
  min  = 0.3
  mode = 0.45
  max  = 0.6
  P6<-rtriangle(n=n_iterations, theta=mode, lower=min, upper=max)
  hist(P6, xlim=c(0,1))

#P7 -hand/arm
  min  = 0.15
  mode = 0.275
  max  = 0.4
  P7<-rtriangle(n=n_iterations, theta=mode, lower=min, upper=max)
  hist(P7, xlim=c(0,1))

#P8 -trunk
  min  = 0.00
  mode = 0.05
  max  = 0.1
  P8<-rtriangle(n=n_iterations, theta=mode, lower=min, upper=max)
  hist(P8, xlim=c(0,1))

#P9 -leg/foot
  min  = 0.00
  mode = 0.05
  max  = 0.1
  P9<-rtriangle(n=n_iterations, theta=mode, lower=min, upper=max)
  hist(P9, xlim=c(0,1))
```

#P10 - Probability of an individual receiving post-exposure prophylaxis/treatment following suspected rabid dog bite. A triangular distribution (minimum, mode, maximum) was used for the calculation based on expert opinions.

```
# P10 - Probability of receiving PEP
  min  = 0.80
  mode = 0.90
  max  = 0.95
  P10<-rtriangle(n=n_iterations, theta=mode, lower=min, upper=max)
  hist(P10, xlim=c(0,1))

##############################################

#Probability of dying of rabies following a bite from a suspected rabid dog was calculated
using the following formula:

  Pdeath = P1 * ((P2*P6)+(P3*P7)+(P4*P8)+(P5*P9))*(1-P10)
  summary(Pdeath)
  hist(Pdeath, xlim=c(0,1))

#The following code is to export output table to Excel as csv

output<-cbind(P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, Pdeath)
head(output)
write.csv(output, file = "P_death.csv") #export to excel(as csv)

# to get 95 % confidence intervals of the probability of deaths
q_P1<-round(quantile(P1, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3); q_P1
q_P2<-round(quantile(P2, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3); q_P2
q_P3<-round(quantile(P3, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3); q_P3
q_P4<-round(quantile(P4, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3); q_P4
q_P5<-round(quantile(P5, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3); q_P5
q_P6<-round(quantile(P6, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3); q_P6
q_P7<-round(quantile(P7, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3); q_P7
q_P8<-round(quantile(P8, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3); q_P8
q_P9<-round(quantile(P9, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3); q_P9
q_P10<-round(quantile(P10, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T), 3);
q_P10

q_Pdeath<-round(quantile(Pdeath, probs = c(0, 0.025, 0.25, 0.5, 0.75, 0.975, 1), na.rm = T),
3); q_Pdeath

# To calculate the predicted number of deaths (T_deaths) per year due to rabies
# Tdeath= i * Q * Pdeath/100000
where i means number of bites per 100,000 population at risk (incidence of bites per year); Q
is population at risk; Pdeath per 100,000 is the probability of death per 100,000.
# Incidence of suspected rabid dog bites per 100,000 populations per year
# data for dog bites was collected for about 9 months only and recorded 193 bites in 2
hospital area (97 bites in Pling and 126 bites in Gelephu); but was adjusted or annualized
for 12 months and estimated into 223 bites

    Q = 47721
    n_bites = 223
    i = n_bites / Q * 100000
# Total number of deaths (mean) predicted per year was calculated as follow:
Total_death = i * Q * Pdeath/100000   # Q and 100,000 cancel out at this step and is
equivalent to:
  Total_death = n_bites * Pdeath
  summary(Total_death)   # mean number of deaths predicted

# to get 95% confidence interval for total predicted deaths
Total_death_pc<-Total_death[order(Total_death)]
Total_death_5pc<-Total_death_pc[n_iterations/100*5]
Total_death_95pc<-Total_death_pc[n_iterations/100*95]
mean(Total_death); Total_death_5pc; Total_death_95pc


# to produce histogram of the mean number of deaths and 95% confidence interval
hist(Total_death, xlim=c(0,max(Total_death)*1.1))
text(5, 1200, paste(n_iterations, " iterations"), font=2, cex=1.5)
text(5, 900, paste("mean: ", round(mean(Total_death),2)), font =2)
text(5, 800, paste("median: ", round(median(Total_death),2)), font =2)
text(5, 700, paste("95% CrI: ", round(Total_death_5pc,2), "-", round(Total_death_95pc,2)),
font =2)
savePlot(filename = "Predicted Rabies deaths_Bhutan", type = "pdf")
```

```
# to calculate annual incidence of predicted death per 100,000 population --mean (population adjusted)
 inc = mean(Total_death)/Q*100000
 inc_5pc = Total_death_5pc/Q*100000
 inc_95pc = Total_death_95pc/Q*100000
 inc; inc_5pc; inc_95pc

# to calculate annual incidence of predicted death per 100,000 population --median(population adjusted)
 inc = median(Total_death)/Q*100000
 inc_5pc = Total_death_5pc/Q*100000
 inc_95pc = Total_death_95pc/Q*100000
 inc; inc_5pc; inc_95pc

############################################################

# To carry out sensitivity analysis of P1 - rabies recognition probability
# Note: The values for P1, P10 or P2 to P5 can be changed based on the field data and then carry out sensitivity analysis. For this, have to put different values to the above formulas and then run again.
P1_range<-seq(0.01, 1, by = 0.01)
# write.csv(P1_range, file = "P1_range.csv")      #export to excel(as csv)

#Probability of death from rabies
Pdeath_range = matrix(ncol = length(P1_range), nrow = n_iterations)
dim(Pdeath_range)
for (i in 1:length(P1_range)) {
Pdeath_range[,i] = P1_range[i] * ((P2*P6)+(P3*P7)+(P4*P8)+(P5*P9))*(1-P10)
}
head(Pdeath_range)
  Q = 47721
#Incidence of suspected rabid dog bites per 100,000 population per year
    n_bites = 223
    i = n_bites / Q * 100000
  # Total no. of deaths per year #formula:
 Total_death = n_bites * Pdeath
 Total_death_range = n_bites * Pdeath_range
dim(Total_death_range)
#mean(Total_death_range[,2]) #mean for column 2
y = apply(Total_death_range, 2, mean) #mean for all columns

# sensitivity analysis plot
plot(P1_range, y, log='y', type='l', ylim = c(0.1, 100), xlim=c(0,1),
axes=F, xlab = "Rabies recognition probability (P1)", ylab = "Annual deaths due to rabies",
cex.lab=1.5, font.lab=2); box()
axis(1, at=seq(0, 1, by = 0.1), cex.axis=1.2)
axis(2,  at=c(0.1,  1,  10,  100,  1000,  10000),  labels=c(0.1,  1,  10,  100,  1000,  10000),
cex.axis=1.2, las=1)
text(mean(P1), 0.2,"Best estimate for P1")
arrows(mean(P1), 0.09, mean(P1), 0.15, length = 0.1, angle = 30, code=1)
  abline(h = 1.5, lty='dotted')
  text(mean(P1), 0.75, "Mean human deaths reported,")
  text(mean(P1), 0.55, "Jan 2006 to April 2011")
  arrows(mean(P1), 1.4, mean(P1), 0.9, length = 0.1, angle = 30, code=1)
  text(0.2, 8, "Predicted rabies deaths")#locator(n=2)
  arrows(0.6, 2, 0.25, 5.7, length = 0.1, angle = 30, code=1)
savePlot(filename = "Predicted Rabies deaths_sensitivity analysis_P10_90", type = "pdf")
#############################################################################################
# To calculate human rabies predicted death according to different age group

library(VGAM)
setwd("C:/Data/Temp")

n_iterations = 10000

#P1 - rabies recognition probability as explained above
  size = 46
  P1<-rbinom(n=n_iterations, size=size, prob=33/46)
  P1 = P1/size
```

```
  hist(P1, xlim=c(0,1))
```

#P2:5 - point estimate of bite locations (as explained above)

```
  P2=0.057
  P3=0.212
  P4=0.016
  P5=0.715
```

 # age dependant - point estimates of bite locations. The data were classified into 4 age categories as follows and then calculated the point estimates based on the location of bites (head, hand, trunk, leg) in each age categories (see Chapter 10, Table 10.2). For instance n_age1 is the number of bite cases in age group 1 (0-4 years of age, and so on..)

```
# 0-4 years
  n_age1 = 23
  P2_age1=6/n_age1
  P3_age1=3/n_age1
  P4_age1=1/n_age1
  P5_age1=13/n_age1

# 5-9 years
  n_age2 = 49
  P2_age2=2/n_age2
  P3_age2=11/n_age2
  P4_age2=1/n_age2
  P5_age2=35/n_age2

# 10-14 years
  n_age3 = 33
  P2_age3=1/n_age3
  P3_age3=3/n_age3
  P4_age3=1/n_age3
  P5_age3=28/n_age3

# 15+ years
  n_age4 = 88
  P2_age4=2/n_age4
  P3_age4=24/n_age4
  P4_age4=0/n_age4
  P5_age4=62/n_age4
```

 # Probability of dying from rabies following bite to head(P6), hand(P7); trunk(P8)and leg(P9) as explained above.

```
#P6 -head
  min = 0.3
  mode = 0.45
  max = 0.6
  P6<-rtriangle(n=n_iterations, theta=mode, lower=min, upper=max)
  hist(P6, xlim=c(0,1))
#P7 -hand
  min = 0.15
  mode = 0.275
  max = 0.4
  P7<-rtriangle(n=n_iterations, theta=mode, lower=min, upper=max)
  hist(P7, xlim=c(0,1))
#P8 & P9 -trunk & leg
  min = 0.00
  mode = 0.05
  max = 0.1
  P9<-P8<-rtriangle(n=n_iterations, theta=mode, lower=min, upper=max)
  hist(P8, xlim=c(0,1))

# Probability of receiving post-exposure prophylaxis following dog bite as explained above.

#P10 - PEP
  min = 0.80
  mode = 0.90
  max = 0.95
  P10<-rtriangle(n=n_iterations, theta=mode, lower=min, upper=max)
  hist(P10, xlim=c(0,1))
```

```
################################################################
#Probability of death from rabies - age-wise
 Pdeath_age1 = P1 * ((P2_age1*P6)+(P3_age1*P7)+(P4_age1*P8)+(P5_age1*P9))*(1-P10)
  Pdeath_age2 = P1 * ((P2_age2*P6)+(P3_age2*P7)+(P4_age2*P8)+(P5_age2*P9))*(1-P10)
  Pdeath_age3 = P1 * ((P2_age3*P6)+(P3_age3*P7)+(P4_age3*P8)+(P5_age3*P9))*(1-P10)
  Pdeath_age4 = P1 * ((P2_age4*P6)+(P3_age4*P7)+(P4_age4*P8)+(P5_age4*P9))*(1-P10)
  summary(Pdeath_age1)
  summary(Pdeath_age2)
  summary(Pdeath_age3)
  summary(Pdeath_age4)

par(mfrow = c(2,2))
  hist(Pdeath_age1, xlim=c(0,0.1), main="Ages 0 to 4 years")
  hist(Pdeath_age2, xlim=c(0,0.1), main="Ages 5 to 9 years")
  hist(Pdeath_age3, xlim=c(0,0.1), main="Ages 10 to 14 years")
  hist(Pdeath_age4, xlim=c(0,0.1), main="Ages 15+ years")

# to calculate total death from rabies according to different age categories
# Population at risk - age-wise
    Q = c(4746, 5207, 4962, 32806)

#Incidence of suspected rabid dog bites per 100,000 population per year
    n_bites = c(n_age1, n_age2, n_age3, n_age4)
    i = n_bites / Q * 100000

# Total no. of deaths per year age-wise
#Total_death = i * Q * Pdeath / 100000  # Q and 100,000 cancel out at this step (see formula
in the text Chapter 10)
    Total_death_age1 = n_bites[1] * Pdeath_age1
    Total_death_age2 = n_bites[2] * Pdeath_age2
    Total_death_age3 = n_bites[3] * Pdeath_age3
    Total_death_age4 = n_bites[4] * Pdeath_age4
  summary(Total_death_age1)
  summary(Total_death_age2)
  summary(Total_death_age3)
  summary(Total_death_age4)

# total no. of deaths (age-wise) and 95% confidence interval
Predicted_Tdeath_age1<-round(c(mean(Total_death_age1), quantile(Total_death_age1,  probs =
c(2.5, 97.5)/100)),2)
Predicted_Tdeath_age2<-round(c(mean(Total_death_age2), quantile(Total_death_age2,  probs =
c(2.5, 97.5)/100)),2)
Predicted_Tdeath_age3<-round(c(mean(Total_death_age3), quantile(Total_death_age3,  probs =
c(2.5, 97.5)/100)),2)
Predicted_Tdeath_age4<-round(c(mean(Total_death_age4), quantile(Total_death_age4,  probs =
c(2.5, 97.5)/100)),2)
Predicted_Tdeath<-rbind(Predicted_Tdeath_age1, Predicted_Tdeath_age2, Predicted_Tdeath_age3,
Predicted_Tdeath_age4)
colnames(Predicted_Tdeath)<-c("mean", "LCL", "UCL")
Predicted_Tdeath
sum(Predicted_Tdeath[,1]) # sum total predicted deaths
# to produce histogram of total no. predicted deaths (age-wise)
par(mfrow = c(2,2))
hist(Total_death_age1,  xlim=c(0,max(Total_death_age4)*1.1),  main="Ages  0  to  4  years",
xlab='Predicted deaths')
hist(Total_death_age2,  xlim=c(0,max(Total_death_age4)*1.1),  main="Ages  5  to  9  years",
xlab='Predicted deaths')
hist(Total_death_age3,  xlim=c(0,max(Total_death_age4)*1.1),  main="Ages  10  to  14  years",
xlab='Predicted deaths')
hist(Total_death_age4,    xlim=c(0,max(Total_death_age4)*1.1),    main="Ages    15+    years",
xlab='Predicted deaths')
savePlot(filename = "Predicted Rabies deaths_Bhutan_agedep", type = "pdf")

# to calculate predicted deaths per 100,000 population per year (age-wise)
Total_deaths_means         =         c(mean(Total_death_age1),         mean(Total_death_age2),
mean(Total_death_age3), mean(Total_death_age4))
Total_deaths_medians       =       c(median(Total_death_age1),       median(Total_death_age2),
median(Total_death_age3), median(Total_death_age4))

# predicted incidence of deaths - age wise (population adjusted)
```

```
 inc_mean = Total_deaths_means/Q*100000
 inc_median = Total_deaths_medians/Q*100000
   Total_death_5pc_age1<-quantile(Total_death_age1, probs = 0.05, type=6)
   Total_death_95pc_age1<-quantile(Total_death_age1, probs = 0.95, type=6)
   Total_death_5pc_age2<-quantile(Total_death_age2, probs = 0.05, type=6)
   Total_death_95pc_age2<-quantile(Total_death_age2, probs = 0.95, type=6)
   Total_death_5pc_age3<-quantile(Total_death_age3, probs = 0.05, type=6)
   Total_death_95pc_age3<-quantile(Total_death_age3, probs = 0.95, type=6)
   Total_death_5pc_age4<-quantile(Total_death_age4, probs = 0.05, type=6)
   Total_death_95pc_age4<-quantile(Total_death_age4, probs = 0.95, type=6)
 inc_5pc_age1 = Total_death_5pc_age1/Q[1]*100000
 inc_5pc_age2 = Total_death_5pc_age2/Q[2]*100000
 inc_5pc_age3 = Total_death_5pc_age3/Q[3]*100000
 inc_5pc_age4 = Total_death_5pc_age4/Q[4]*100000
 inc_95pc_age1 = Total_death_95pc_age1/Q[1]*100000
 inc_95pc_age2 = Total_death_95pc_age2/Q[2]*100000
 inc_95pc_age3 = Total_death_95pc_age3/Q[3]*100000
 inc_95pc_age4 = Total_death_95pc_age4/Q[4]*100000

output_mean<-round(inc_mean,2)
output_median<-round(inc_median,2)
output_LCL<-round(c(inc_5pc_age1, inc_5pc_age2, inc_5pc_age3, inc_5pc_age4),2)
output_UCL<-round(c(inc_95pc_age1, inc_95pc_age2, inc_95pc_age3, inc_95pc_age4),2)
inc_agedep<-cbind(output_mean, output_median, output_LCL, output_UCL)
colnames(inc_agedep)<-c("mean", "median", "LCL", "UCL")
rownames(inc_agedep)<-c("0 to 4 years", "5 to 9 years", "10 to 14 years", "15+ years")
inc_agedep
```

Appendix 5: Knowledge, Attitudes and Practice of Rabies study questionnaire

(Chapter 11)

Knowledge, Attitudes and Practice survey of rabies and rabies control measures in Gelephu, Bhutan

KAP Survey No:……Interviewer**:** ……. Date of survey:…….....

Please tick or circle the answer(s) as per response of the participants.

PART I: PROFILE OF RESPONDENTS or PARTICIPANTS

21. Name of location/area where the respondent is living
    a. Puranobusty (municipality)
    b. Lekithang
    c. Pemathang
    d. Pelrithang
    e. Zomlingthang

22. Gender of the respondent
    a. Male
    b. Female

23. Age of the respondent: [ ] years

24. Education level/qualification of the respondent
    a. No education
    b. Primary level
    c. High school level
    d. Secondary level
    e. University level

25. Occupation of the respondent?
    g. Student
    h. Civil servant
    i. Corporate employee
    j. Businessman
    k. Farmers
    l. Dependents/housewives

26. Religion of the respondent**:**
    a. Buddhism
    b. Hinduism
    c. Others

27. Ethnicity of the respondent:
    a. Lhotshampa
    b. Sharchop
    c. Ngalong
    d. Khengpa/Bumthap

28. Number of people in the house (household size)?

	Male	Female
Number of children (less than 18 years old)		
Number of adults (above 18 years old)		

29. Do you own the animals?

	Yes	No
Dogs		
Cats		
Cattle		
Goats		
Horses		
Pigs		
Poultry		

PART II: KNOWLEDGE ABOUT RABIES AND RABIES CONTROL ACTIVITIES

30. Have you heard about rabies? **Yes[ ] No[ ]**

If the answer is No, do not ask further questions in this section, go to Part III.

31. Do you believe that rabies is a dangerous disease (fatal disease)? **Yes[ ] No[ ] Not sure[ ]**

32. Do you believe that rabies can be transmitted to humans from a

	Yes	**No**	**Not sure**
Dog			
Cat			

33. Do you believe that rabies outbreaks can be prevented by regular vaccination of dogs? **Yes[ ] No[ ] Not sure [ ]**

34. Have you heard about rabies cases in:

	Yes	**No**
Humans in Gelephu		
Dogs in Gelephu		
Other farm animals (cattle/goat/pigs) in Gelephu		

35. Do you know that suspected rabies cases have to be confirmed by laboratory test? (take brain sample to the laboratory and do laboratory test) **Yes [ ] No [ ] Not sure [ ]**

36. Are you aware that dog bite wound should be washed with soap and water? **Yes[ ] No[ ] Not sure[ ]**

37. Is there any traditional method of dog bite wound treatment? **Yes [ ] No[ ] Not sure [ ]**

If yes, what kind of methods……..

38. How do you come to know about rabies?

a. Public health officials
b. Veterinary/livestock officials
c. Friends/neighbours
d. News media (Television, radio, news paper)
e. Rabies awareness campaign program

PART III: ATTITUDE AND PRACTICES ABOUT RABIES AND RABIES CONTROL ACTIVITIES

39. Will you report to the hospital for treatment, if,

	Yes	**No**	**Not sure**
You are bitten by stray dog			
You are bitten owned dog (e.g. pet dog)			
You are bitten by stray cat			
You are bitten by owned cat (e.g. pet cat)			
You are scratched by stray dog			
You are bitten by wild animals			
You are bitten by dog in other countries (e.g India)			

40. Will you inform/report to the health/veterinary authorities if there is a suspected rabies outbreak in your area/community? **Yes[ ] No[ ] Not sure[ ]**

41. Will you kill stray dog if rabies is suspected? **Yes[ ] No[ ] Not sure[ ]**

42. Do you advise children to be careful and not to play with stray dogs? **Yes[ ] No[ ]**

43. Stray dogs related

	Yes	**No**	**Not sure**
Is there any stray dog in your home premises?			
Do you feed stray dogs?			
Is stray dog a problem in your community?			

44. Do you believe it is important to control dog population in Gelephu? **Yes[ ] No[ ] Not sure[ ]**

45. Do you support the rabies control campaign: vaccination and sterilization program organized by the livestock and the municipality in your area? **Yes[ ] No[ ]**

46. What do you think would be the most appropriate methods to control dog population in Gelephu?
    a. Operation (sterilization)
    b. Impounding in the dog shelter
    c. Sterilization/impounding
    d. Kill (poison/shoot)
    e. Others (specify)……..

PART IV: DOG OWNERS and THEIR PRACTICES

47. How many dogs do you own? Males [ ] Females [ ]

48. What breed is your dog?
    a. Local
    b. Others (Specify)……

49. What were the sources of your dogs?
    a. I adopted stray dogs
    b. Given by friends/others
    c. Purchased from others
    d. Bred from own dogs

50. What are the purposes of keeping dogs?
    a. As pet
    b. To guard house/properties
    c. Other purposes (please specify)…

51. How do you keep your dogs?
    a. Housed in cages/tie outside
    b. Living inside the house with families
    c. Free to roam around the house compound during day and night
    d. Free to roam around the house compound, village/town
    e. Others (please specify)……

52. What kind of food do you feed your dogs?
    a. Left over food (food that we eat normally)
    b. Others (please specify)…….

53. Dog registration related

	Yes	**No**
Have you registered your dog(s) with the Livestock Extension Centre (LEC), Gelephu?		
If not registered, are you willing to register?		

54. Is your dog(s) vaccinated against rabies (during last year or this year) **Yes [ ] No [ ]**

If your dog is **NOT** vaccinated against rabies, what were the reasons for not vaccinating?.....

55. Do you believe it is important to vaccinate dogs against rabies every year? **Yes [ ] No [ ] Not sure [ ]**

56. Would you like to take your dog to the LEC for vaccination or should LEC organize a vaccination day in your community/village every year?

a. I would like to take my dogs to the LEC for vaccination [ ]
b. LEC should organize a dog vaccination day in our community [ ]
c. Either one of the above procedure is OK [ ]

57. Have you:

	Yes	**No**	**Not sure**
Ever submitted faecal materials to the LEC/RVL for examination?			
Ever de-wormed your dogs (given any medicine against worms)			

Appendix 6: R code for economic analysis (Chapter 12)

Human and animal rabies prevention and control cost in Bhutan

```
library(VGAM)
setwd("C:/Data/Temp")

n_iterations = 10000 # number of iterations

DIRECT MEDICAL COST ESTIMATION FOR PEP IN HUMNAN
(see Chapter 12, Table 12.1 and materials and methods for details)

# Number of PET cases: the uncertainty of the parameter values and cost were accounted by
inputting estimates as triangular distribution with maxima and minima set as +/-10% of each
parameter values and then run 10000 iterations.
# theta is the mode or the most likely number of patients that were given PET from 2001 to
2008
# theta is also the most likely cost for each parameter
# Multiplication by 0.9 or 1.1 is the +/-10%
# TCV means tissue culture vaccine given
# total number of PET cases from 2001 to 2008 was 10223
##########################################################

TCV<-rtriangle(n=n_iterations, theta=10223, lower=10223*0.9, upper=10223*1.1)
hist(TCV); summary(TCV)

#####################
# annual number of PET cases =10222/8 years=1278

TCV_annual<-rtriangle(n=n_iterations, theta=1278, lower=1278*0.9, upper=1278*1.1)
hist(TCV_annual); summary(TCV_annual)

###############################################

#common costs for PEP
Tissue culture vaccine cost; material cost per vaccine injection; material cost for wound
dressing; doctor consultation time cost per patient; overhead/delivery cost for vaccine inj.
and wound dressing per patient per visit
# number of visit to health centre for one course = 5 times and no of inj. per patient = 5
times
# theta is the most likely cost
####################################

number_visits_TCV<-5
vaccine_cost_per_dose<-rtriangle(n=n_iterations, theta=305, lower=305*0.9, upper=305*1.1)
summary(vaccine_cost_per_dose)

#95 % confidence intervals
q_vaccine_cost_per_dose<-round(quantile(vaccine_cost_per_dose, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_vaccine_cost_per_dose

############################

material_cost_per_injection_TCV<-rtriangle(n=n_iterations, theta=1.31, lower=1.31*0.9,
upper=1.31*1.1) # only for swabs and spirit)
summary (material_cost_per_injection_TCV)

#95 % confidence intervals

q_material_cost_per_injection_TCV<round(quantile(material_cost_per_injection_TCV, probs =
c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T,type=6), 3);
q_material_cost_per_injection_TCV
 ############################
material_cost_dressing<-rtriangle(n=n_iterations, theta=10,lower=10*0.9, upper=10*1.1)  #
bandages and antiseptics, one time)
summary(material_cost_dressing)
```

```
#95 % confidence intervals

q_material_cost_dressing<-round(quantile(material_cost_dressing, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.8, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_material_cost_dressing

##############################

delivery_cost_TCV<-rtriangle(n=n_iterations, theta=9, lower=9*0.9, upper=9*1.1) # per
injection cost-nurse salary)
summary(delivery_cost_TCV)

#95 % confidence intervals

q_delivery_cost_TCV<-round(quantile(delivery_cost_TCV, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_delivery_cost_TCV

##############################

delivery_cost_dressing<-rtriangle(n=n_iterations, theta=17, lower=17*0.9, upper=17*1.1) #
wound dressing cost, one time nurse salary)
summary(delivery_cost_dressing)

#95 % confidence intervals

q_delivery_cost_dressing<-round(quantile(delivery_cost_dressing, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_delivery_cost_dressing

##############################

consultation_cost_doctor<-rtriangle(n=n_iterations, theta=14, lower=14*0.9, upper=14*1.1)
summary(consultation_cost_doctor)

#95 % confidence intervals

q_consultation_cost_doctor<-round(quantile(consultation_cost_doctor, probs = c(0, 0.025,
0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_consultation_cost_doctor

##############################
```

 # Total direct costs per patient per course (5 times visits): which is the sum of above costs, multiplied by the number of visits. Here the doctor consultation cost, and the wound dressing materials and dressing cost is calculated once only, assuming each patient consult only one time and also require only one time wound dressing).

```
total_direct_cost_TCV  <-(vaccine_cost_per_dose + material_cost_per_injection_TCV +
delivery_cost_TCV)  * number_visits_TCV + (delivery_cost_dressing +consultation_cost_doctor+
material_cost_dressing)

total_direct_costs<- total_direct_cost_TCV
hist(total_direct_costs); summary(total_direct_costs)

#95 % confidence intervals

q_total_direct_costs<-round(quantile(total_direct_costs, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_direct_costs

##############################################
```

 # total direct cost for the period from 2001 to 2008 for 10223 patients) including cost of vaccine and administration cost. Rabies immunoglobulin was not given to the patients

```
total_direct_costs_8_years<- total_direct_cost_TCV * TCV
hist(total_direct_costs_8_years); summary(total_direct_costs_8_years)
total_direct_costs_8_years<- total_direct_cost_TCV * TCV
hist(total_direct_costs_8_years); summary(total_direct_costs_8_years)


#95 % confidence intervals

q_total_direct_costs_8_years<-round(quantile(total_direct_costs_8_years, probs = c(0, 0.025,
0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_direct_costs_8_years
```

```
################################################

# Annual cost from 2001 to 2008 (8 years): total cost divided by 8 years

total_direct_costs_annual<-(total_direct_cost_TCV * TCV)/8
hist(total_direct_costs_annual); summary(total_direct_costs_annual)

#95 % confidence intervals
q_total_direct_costs_annual<-round(quantile(total_direct_costs_annual, probs = c(0, 0.025,
0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_direct_costs_annual

##########################################

# RABIES IMMUNOGLOBULIN COST
# Equine rabies immunoglobulin (ERIG) cost per dose
# 5 ml vial contain 1500IU and cost Nu.432
# 50 kg body weight patient may require 2 vials @ dose rate of 40IU per kg body weight
# also included cost of delivery and material cost for injection

ERIG_cost_per_vial<-rtriangle(n=n_iterations, theta=432, lower=432*0.9, upper=432*1.1)
ERIG_cost_per_dose<-rtriangle(n=n_iterations, theta=864, lower=864*0.9, upper=864*1.1) # dose
rate of 40IU per kg,
summary(ERIG_cost_per_dose)
q_ERIG_cost_per_dose<-round(quantile(ERIG_cost_per_dose, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_ERIG_cost_per_dose

#####
delivery_cost_ERIG<-rtriangle(n=n_iterations, theta=17, lower=17*0.9, upper=17*1.1) # per
injection cost-nurse salary)

#####
material_cost_ERIG<<-rtriangle(n=n_iterations, theta=1.31, lower=1.31*0.9, upper=1.31*1.1)

#####
total_ERIG_costs_dose<-ERIG_cost_per_dose + delivery_cost_ERIG+material_cost_ERIG
summary(total_ERIG_costs_dose)

#95 % confidence intervals
q_total_ERIG_costs_dose<-round(quantile(total_ERIG_costs_dose, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_ERIG_costs_dose

################################################
# Human rabies immunoglobulin (HRIG) cost per dose

# 5 ml vial contain 152 IU per ml (~760IU) and cost about Nu.6000
# 50 kg patient may require 3 vials and cost ~Nu.6000*3 = Nu.18000
# also included cost of delivery and material cost for injection

HRIG_cost_per_vial<-rtriangle(n=n_iterations, theta=6000, lower=6000*0.9, upper=6000*1.1)
HRIG_cost_per_dose<-rtriangle(n=n_iterations, theta=18000, lower=18000*0.9, upper=18000*1.1)
# dose rate of 20IU per kg,
summary(HRIG_cost_per_dose)

#95 % confidence intervals
q_HRIG_cost_per_dose<-round(quantile(HRIG_cost_per_dose, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_HRIG_cost_per_dose

######
delivery_cost_HRIG<-rtriangle(n=n_iterations, theta=17, lower=17*0.9, upper=17*1.1) # per
injection cost-nurse salary)

######
material_cost_HRIG<<-rtriangle(n=n_iterations, theta=1.31, lower=1.31*0.9, upper=1.31*1.1)

######
total_HRIG_costs_dose<-HRIG_cost_per_dose + delivery_cost_HRIG +  material_cost_HRIG
summary(total_HRIG_costs_dose)

#95 % confidence intervals
```

```
q_total_HRIG_costs_dose<-round(quantile(total_HRIG_costs_dose, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_HRIG_costs_dose

########################################################################

# Overall direct medical costs (includes vaccine plus ERIG administration)
overall_direct_medical_cost_ERIG<-total_direct_costs+ total_ERIG_costs_dose
summary(overall_direct_medical_cost_ERIG)

#95 % confidence intervals
q_overall_direct_medical_cost_ERIG<-round(quantile(overall_direct_medical_cost_ERIG, probs =
c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_overall_direct_medical_cost_ERIG

########

# Overall direct medical costs (includes vaccine plus HRIG administration)
overall_direct_medical_cost_HRIG<-total_direct_costs+ total_HRIG_costs_dose
summary(overall_direct_medical_cost_HRIG)

#95 % confidence intervals

q_overall_direct_medical_cost_HRIG<-round(quantile(overall_direct_medical_cost_HRIG, probs =
c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_overall_direct_medical_cost_HRIG

########################################################################

INDIRECT PATIENT COSTS ESTIMATION (income loss and transport cost)

# number of PET visit from 2001 to 2008 was 10223,
# it was assumed that 4% of 10223 would be accompanied by adults, especially accompanying
children to visit hospital
# therefore 4089 would be an accompanying adults and the total visit to the hospital from
2001 to 2008 is calculated to be 14311 patients (10223+4089)
# accompanying adults would add in additional transport cost.

# Accompanying adults
num_PET_visits <-TCV; summary(num_PET_visits)
prop_with__working_adult <- rtriangle(n=n_iterations, theta=0.4, lower=0.4*0.9,
upper=0.4*1.1)
visits_with_working_adult <- num_PET_visits * prop_with__working_adult;
summary(visits_with_working_adult)
total_PET_visits <- num_PET_visits + visits_with_working_adult; summary(total_PET_visits)
total_PET_visits_1 <- num_PET_visits; summary(total_PET_visits_1)

####################################

# Income loss calculation
# Children would have no income but the accompanying adults would results in income loss. #
Here the 4089 accompanying adults would incur income loss but their children 4089 children
will not have any income. Here income loss is calculated for only 10233 patients
# daily income was calculated based on daily Gross National Income (GNI) and the hours lost
per visit (assumed to lost 3 hours per visit @daily GNI of Nu.109)

hours_per_visit <-rtriangle(n=n_iterations, theta=3, lower=3*0.9, upper=3*1.1)
equivalent_days_lost_per_visit <-  hours_per_visit/8; summary(equivalent_days_lost_per_visit)
daily_GNI <-109     # Gross National Income for 2006 was Nu.39639
income_loss_per_visit <-  equivalent_days_lost_per_visit * daily_GNI ;
summary(income_loss_per_visit)

#95 % confidence intervals
q_income_loss_per_visit<-round(quantile(income_loss_per_visit, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_income_loss_per_visit

############################

# income loss for one course (5 times visits)

income_loss_per_5_visit <-income_loss_per_visit * 5; summary(income_loss_per_5_visit)
```

```
#95 % confidence intervals
q_income_loss_per_5_visit<-round(quantile(income_loss_per_5_visit, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_income_loss_per_5_visit

############

# income loss for all patients visit (10222) from (2001 to 2008)

total_PET_visits_1 = 10222
income_loss_total <-income_loss_per_5_visit * total_PET_visits_1 ;summary(income_loss_total)
# children data substracted

#95 % confidence intervals
q_income_loss_total<-round(quantile(income_loss_total, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_income_loss_total

############
# income loss annual (2001 to 2008) : divide by 8 years

income_loss_annual <-income_loss_per_5_visit * total_PET_visits_1/8
;summary(income_loss_annual)

#95 % confidence intervals
q_income_loss_annual<-round(quantile(income_loss_annual, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_income_loss_annual

########################

# Transport costs (for each PET patients and also the accompanying adults)
# transport cost per person=Nu.40

transport_costs_per_person <-rtriangle(n=n_iterations, theta=40, lower=40*0.9, upper=40*1.1)
transport_costs_per_person_per_visit <- transport_costs_per_person;
summary(transport_costs_per_person_per_visit)

#95 % confidence intervals
transport_costs_per_person_per_visit<-round(quantile(transport_costs_per_person_per_visit,
probs = c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
transport_costs_per_person_per_visit

# transport cost for one course (5 times visits)
transport_costs_per_5_visit <-transport_costs_per_person_per_visit * 5;
summary(transport_costs_per_5_visit)

#95 % confidence intervals

q_transport_costs_per_5_visit<-round(quantile(transport_costs_per_5_visit, probs = c(0,
0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_transport_costs_per_5_visit

########## ###################

# Total transport cost for 10222 patients plus 4089 accompanying adults from 2001 to 2008

transport_costs_total <-transport_costs_per_5_visit *
total_PET_visits;summary(transport_costs_total)

#95 % confidence intervals

q_transport_costs_total<-round(quantile(transport_costs_total, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_transport_costs_total

# Transport cost annual from 2001 to 2008: total PET visits divided by 8 years

transport_costs_annual <-(transport_costs_per_5_visit *
total_PET_visits)/8;summary(transport_costs_annual)

#95 % confidence intervals
q_transport_costs_annual<-round(quantile(transport_costs_annual, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_transport_costs_annual
```

```
###################################

#Total indirect costs per visit (income loss plus transport cost)

total_indirect_costs_human_per_visit <-(income_loss_per_visit +
transport_costs_per_person_per_visit); summary(total_indirect_costs_human_per_visit)

#95 % confidence intervals

q_total_indirect_costs_human_per_visit<-
round(quantile(total_indirect_costs_human_per_visit, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_indirect_costs_human_per_visit


#total indirect costs for 5  visit

total_indirect_costs_human_per_5_visit <- (income_loss_per_visit +
transport_costs_per_person_per_visit)*5 ; summary(total_indirect_costs_human_per_5_visit)
#95 % confidence intervals
q_total_indirect_costs_human_per_5_visit<-
round(quantile(total_indirect_costs_human_per_5_visit, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_indirect_costs_human_per_5_visit

############################


#Overall total indirect costs for the period 2001 to 2008

total_indirect_costs_human <-income_loss_total + transport_costs_total ;
summary(total_indirect_costs_human)

#95 % confidence intervals

q_total_indirect_costs_human<-round(quantile(total_indirect_costs_human, probs = c(0, 0.025,
0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_indirect_costs_human

# Annual indirect costs for the period 2001 to 2008

total_indirect_costs_human_annual <- (income_loss_total + transport_costs_total)/8 ;
summary(total_indirect_costs_human_annual)

#95 % confidence intervals

q_total_indirect_costs_human_annual<-round(quantile(total_indirect_costs_human_annual, probs
= c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_total_indirect_costs_human_annual

#################################################################
SOCIETAL COST  (Direct medical cost plus indirect patient cost)

# societal cost for 5 times PET  visits for vaccine administration only without ERIG
administration

societal_cost_ARV<- total_direct_costs+ total_indirect_costs_human_per_5_visit
summary (societal_cost_ARV)

#95 % confidence intervals

q_societal_cost_ARV<-round(quantile(societal_cost_ARV, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_societal_cost_ARV

# Total societal cost from 2001 to 2008 with vaccine administration only

societal_cost_total_ARV<- total_direct_costs_8_years + total_indirect_costs_human
summary (societal_cost_total_ARV)

#95 % confidence intervals
q_societal_cost_total_ARV<-round(quantile(societal_cost_total_ARV, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_societal_cost_total_ARV

#################################
```

 # Societal cost for 5 times PET visits (includes vaccine and ERIG administration and indirect patient cost)

```
societal_cost_ARV_ERIG_indirect<-(total_direct_costs + total_ERIG_costs_dose +
total_indirect_costs_human_per_5_visit)
summary (societal_cost_ARV_ERIG_indirect)

#95 % confidence intervals
q_societal_cost_ARV_ERIG_indirect<-round(quantile(societal_cost_ARV_ERIG_indirect, probs =
c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_societal_cost_ARV_ERIG_indirect

###################
```

 # Societal cost for 5 times PET visits (includes vaccine and HRIG administration and indirect patient cost)

```
societal_cost_ARV_HRIG_indirect<-(total_direct_costs + total_HRIG_costs_dose +
total_indirect_costs_human_per_5_visit)
summary (societal_cost_ARV_HRIG_indirect)


#95 % confidence intervals

q_societal_cost_ARV_HRIG_indirect<-round(quantile(societal_cost_ARV_HRIG_indirect, probs =
c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_societal_cost_ARV_HRIG_indirect

######################
```

DOG VACCINATION COST

 # STRAY DOG VACCINATION COST

```
# have to pay incentives to people who bring dogs for vaccination or employ dog catchers
# theta is the number of dog vaccinated and the cost per dog vaccination
# between 2001 to 2008, 64074 stray dogs were vaccinated @ Nu 75 per dog

num_dogs_vacc_stray <-  rtriangle(n=n_iterations, theta=64074, lower=64074*0.9,
upper=64074*1.1)
   summary ( num_dogs_vacc_stray)

cost_per_dog_vacc_stray <- rtriangle(n=n_iterations, theta=75, lower=75*0.9, upper=75*1.1)
   summary (cost_per_dog_vacc_stray)

total_dog_vacc_cost_stray <- num_dogs_vacc_stray * cost_per_dog_vacc_stray;
summary(total_dog_vacc_cost_stray)

#95 % confidence intervals
q_total_dog_vacc_cost_stray<-round(quantile(total_dog_vacc_cost_stray, probs = c(0, 0.025,
0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_dog_vacc_cost_stray

################################################
```

 # PET DOG VACCINATION

```
# Have to consider income loss for the owners to bring dogs to the centre
# assumed about 2 hours would be lost for the dog owners to bring dog for vaccination
# Transport cost would be negligible since the vaccination point will be located near the
town or at the centre
# do not have to pay dog catching incentives
# the following formula is to calculate pet dog vaccination cost without including income
loss
# between 2001 and 2008, 42716 pet dogs was vaccinated @Nu. 45 per dog (this is theta)

num_dogs_vacc_pet <-  rtriangle(n=n_iterations, theta=42716, lower=42716*0.9,
upper=42716*1.1)
summary (num_dogs_vacc_pet)
```

```
cost_per_dog_vacc_pet <- rtriangle(n=n_iterations, theta=45, lower=45*0.9, upper=45*1.1)
summary (cost_per_dog_vacc_pet)

total_dog_vacc_cost_pet <- num_dogs_vacc_pet * cost_per_dog_vacc_pet;
summary(total_dog_vacc_cost_pet)

#95 % confidence intervals
q_total_dog_vacc_cost_pet<-round(quantile(total_dog_vacc_cost_pet, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_dog_vacc_cost_pet


# Total dogs vaccination cost (stray and pet) from 2001 to 2008

total_dog_vacc_cost <- total_dog_vacc_cost_stray + total_dog_vacc_cost_pet ;
summary(total_dog_vacc_cost)

#95 % confidence intervals
q_total_dog_vacc_cost<-round(quantile(total_dog_vacc_cost, probs = c(0, 0.025, 0.05, 0.25,
0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_dog_vacc_cost

##############################################

DOG STERILIZATION COST

# theta is the number of dog sterilized and cost per dog sterilized

# Male dog castration cost:

number_male_dogs_castrated<- rtriangle(n=n_iterations, theta=39502, lower=39502*0.9,
upper=39502*1.1)
summary(number_male_dogs_castrated)
cost_per_male_dog_castrated<- rtriangle(n=n_iterations, theta=281, lower=281*0.9,
upper=281*1.1)
cost_per_male_dog_castrated <- number_male_dogs_castrated * cost_per_male_dog_castrated;
summary(cost_per_male_dog_castrated)

#95 % confidence intervals
q_cost_per_male_dog_castrated<-round(quantile(cost_per_male_dog_castrated, probs = c(0,
0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_cost_per_male_dog_castrated


# Female dog spaying cost_pet
number_female_dogs_spayed<- rtriangle(n=n_iterations, theta=29321, lower=29321*0.9,
upper=29321*1.1)
summary(number_female_dogs_spayed)
cost_per_female_dog_spayed<- rtriangle(n=n_iterations, theta=296, lower=296*0.9,
upper=296*1.1)
cost_per_female_dog_spayed <- number_female_dogs_spayed * cost_per_female_dog_spayed;
summary(cost_per_female_dog_spayed)

#95 % confidence intervals
q_cost_per_female_dog_spayed<-round(quantile(cost_per_female_dog_spayed, probs = c(0, 0.025,
0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_cost_per_female_dog_spayed

# total cost for male and female dog sterilization
total_cost_dog_sterlization<- (cost_per_male_dog_castrated +  cost_per_female_dog_spayed)
summary (total_cost_dog_sterlization)

#95 % confidence intervals
q_total_cost_dog_sterlization<-round(quantile(total_cost_dog_sterlization, probs = c(0,
0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_total_cost_dog_sterlization

############################

LIVESTOCK LOSSES COST


# cattle loss cost
```

```
# average cost per head of death cattle = Nu.11132 (calculated based on age, breed and sex of
death cattle). The actual cost of a cow would be much higher than this while the cost would
be lower for male and calves)
# there were 287 deaths of cattle between 2001 to 2008 (See Chapter 12, Table 12.4 for
details)

cattle_deaths <-rtriangle(n=n_iterations, theta=287, lower=287*0.9, upper=287*1.1)
cost_per_cattle_death <-rtriangle(n=n_iterations, theta=11132, lower=11132*0.9,
upper=11132*1.1)
cattle_loss_costs <-cattle_deaths * cost_per_cattle_death;
summary(cattle_loss_costs)

#95 % confidence intervals
q_cattle_loss_costs<-round(quantile(cattle_loss_costs, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_cattle_loss_costs


#Horse death cost # there were 14 deaths between 2001 and 2008, average cost per
horse=Nu.25000

horse_deaths <-rtriangle(n=n_iterations, theta=14, lower=14*0.9, upper=14*1.1)
cost_per_horse_death <-rtriangle(n=n_iterations, theta=25000, lower=25000*0.9,
upper=25000*1.1)
horse_loss_costs <-horse_deaths * cost_per_horse_death;   summary(horse_loss_costs)

#95 % confidence intervals
q_horse_loss_costs<-round(quantile(horse_loss_costs, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_horse_loss_costs


# pig death cost # there were 9 deaths and the average cost per pig was Nu.10000
pig_deaths <- rtriangle(n=n_iterations, theta=9, lower=9*0.9, upper=9*1.1)
cost_per_pig_death <- rtriangle(n=n_iterations, theta=10000, lower=10000*0.9,
upper=10000*1.1)
pig_loss_costs <- pig_deaths * cost_per_pig_death;   summary(pig_loss_costs)

#95 % confidence intervals
q_pig_loss_costs<-round(quantile(pig_loss_costs, probs = c(0, 0.025, 0.05, 0.25, 0.5, 0.75,
0.95, 0.975, 1), na.rm = T, type=6), 3); q_pig_loss_costs


# goat death cost # there were 5 deaths of goat and the average cost was Nu.2000
goat_deaths <- rtriangle(n=n_iterations, theta=5, lower=5*0.9, upper=5*1.1)
cost_per_goat_death <-rtriangle(n=n_iterations, theta=2000, lower=2000*0.9, upper=2000*1.1)
goat_loss_costs <-goat_deaths * cost_per_goat_death;   summary(goat_loss_costs)

#95 % confidence intervals
q_goat_loss_costs<-round(quantile(goat_loss_costs, probs = c(0, 0.025, 0.05, 0.25, 0.5, 0.75,
0.95, 0.975, 1), na.rm = T, type=6), 3); q_goat_loss_costs

# Total livestock losses cost
total_livestock_losses<-(cattle_loss_costs+ horse_loss_costs + pig_loss_costs+
goat_loss_costs); summary(total_livestock_losses)

#95 % confidence intervals
q_total_livestock_losses<-round(quantile(total_livestock_losses, probs = c(0, 0.025, 0.05,
0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_livestock_losses

######################

# rabies sample tests cost  # a totaol of 234 samples were tested, average cost per test was
Nu. 1246
number_rabies_tests <-rtriangle(n=n_iterations, theta=234, lower=234*0.9, upper=234*1.1)
cost_per_test <-rtriangle(n=n_iterations, theta=1246, lower=1246*0.9, upper=1246*1.1)
lab_test_costs <-number_rabies_tests * cost_per_test; summary(lab_test_costs)

#95 % confidence intervals
q_lab_test_costs<-round(quantile( lab_test_costs, probs = c(0, 0.025, 0.05, 0.25, 0.5, 0.75,
0.95, 0.975, 1), na.rm = T, type=6), 3); q_lab_test_costs
```

```
##############################################
# Grand total Direct cost (ONLY DIRECT COST) from 2001 to 2008

# sum of rabies vaccine administration to humans, dog vaccination cost, dog sterilization
cost, livestocl losses cost and laboratory test cost)
# does not include rabies immunoglobulin cost
# does not include indirect patient cost and indirect dog owners income loss cost

grand_total_cost <- (total_direct_costs_8_years+ total_dog_vacc_cost +
total_cost_dog_sterlization + total_livestock_losses+ lab_test_costs)
summary (grand_total_cost)

#95 % confidence intervals
q_grand_total_cost<-round(quantile( grand_total_cost, probs = c(0, 0.025, 0.05, 0.25, 0.5,
0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_grand_total_cost

#########################################################

From 2009 to 2011, there were 10320 PET cases in Bhutan

# total of 51600 doses of human rabies vaccine were procured and distributed between 2009 and
2011 (2009= 15800 doses, 2010=17000 doses; 2011=18800 doses)

# Assuming each patient received 5 doses; about 10320 patients (51600/5) were given PEP
during 3 years period
# the direct cost for the period 2001 to 2008 and between 2009 and 2011 were almost same
# An annual average of 3440 patients would receive PEP in future (10320/3 years) assuming if
dog bite cases remain constant and each patient are given PEP
# the following code calculate the total cost for 3 years
# also calculate the expected annual future cost of PEP in humans
# assumption: 10% of the 3440 patients would receive rabies immunoglobulin
# to calculate the following parameters, have to run the above code as well, because they are
linked

##################

Total for 3 years (2009 to 2011)
ARV<-rtriangle(n=n_iterations, theta=10320, lower=10320*0.9, upper=10320*1.1)
  hist(ARV); summary(ARV)

# Annual PET cases 2009 to 2011
ARV_annual<-rtriangle(n=n_iterations, theta=3440, lower=3440*0.9, upper=3440*1.1)
hist(ARV_annual); summary(ARV_annual)

# total direct cost for the period from 2009 to 2011 was calculated as:

total_direct_costs_3_years<- total_direct_cost_TCV * ARV
hist(total_direct_costs_3_years); summary(total_direct_costs_3_years)

#95 % confidence intervals

q_total_direct_costs_3_years<-round(quantile(total_direct_costs_3_years, probs = c(0, 0.025,
0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3); q_total_direct_costs_3_years


# Annual cost with VACCINE ONLY (2009 to 2011)

total_direct_costs_3_years_annual<-total_direct_cost_TCV * ARV_annual
hist(total_direct_costs_3_years_annual); summary(total_direct_costs_3_years_annual)

#95 % confidence intervals

q_total_direct_costs_3_years_annual<-round(quantile(total_direct_costs_3_years_annual, probs
= c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_total_direct_costs_3_years_annual

# Future expected annual direct medical cost for ERIG if 10% of the 3440 patients (i.e 344
people) are given ERIG

expected_annual_future_cost_ERIG<-total_ERIG_costs_dose*344
summary(expected_annual_future_cost_ERIG)
```

```
#95 % confidence intervals
expected_annual_future_cost_ERIG<-round(quantile(expected_annual_future_cost_ERIG, probs =
c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
expected_annual_future_cost_ERIG
```

 # Future expected annual direct medical cost (vaccine administration plus if 10% of patients are given ERIG)

```
expected_annual_future_cost_PET<-(total_direct_costs *3440)+ (total_ERIG_costs_dose*344)
summary(expected_annual_future_cost_PET)

#95 % confidence intervals
q_expected_annual_future_cost_PET<-round(quantile(expected_annual_future_cost_PET, probs =
c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_expected_annual_future_cost_PET

########################
```

 # Future expected annual direct medical cost for HRIG if 10% of the 3440 patients (i.e 344 people) are given HRIG

```
expected_annual_future_cost_HRIG<-total_HRIG_costs_dose*344
summary(expected_annual_future_cost_HRIG)

#95 % confidence intervals
expected_annual_future_cost_HRIG<-round(quantile(expected_annual_future_cost_HRIG, probs =
c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
expected_annual_future_cost_HRIG
```

 # Future expected annual direct medical cost (vaccine administration plus if 10% of patients are given HRIG)

```
expected_annual_future_cost_PET_HRIG <-(total_direct_costs *3440)+(total_HRIG_costs_dose*344)
summary(expected_annual_future_cost_PET_HRIG)

#95 % confidence intervals
q_expected_annual_future_cost_PET_HRIG<-round(quantile(expected_annual_future_cost_PET_HRIG,
probs = c(0, 0.025, 0.05, 0.25, 0.5, 0.75, 0.95, 0.975, 1), na.rm = T, type=6), 3);
q_expected_annual_future_cost_PET_HRIG
```