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Anthropogenic and environmental risk factors for rabies occurrence in Bhutan

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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

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.

2. Materials and methods

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.

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).

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™ 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).

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 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 1).

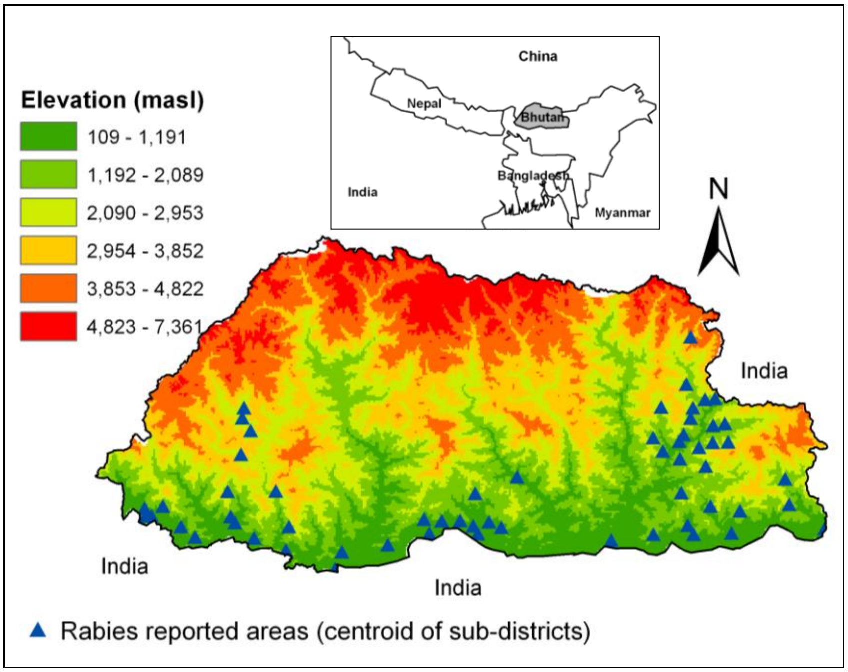

Figure 1: Map of Bhutan showing the elevation and the distribution of rabies occurrence in domestic animals, 1996–2009. The (▲) indicates the centroid location of sub-districts that reported rabies in domestic animals during the period 1996–2009.

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 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™ 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 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 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; $P=0.831$).

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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