Understanding and mitigating the risk of pathogen transmission from wild animals to domestic pigs in Australia.

Hayley E. Pearson

B. Animal and Veterinary Bioscience (Hons) (University of Sydney)

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The University of Sydney

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Declaration

I hereby declare that this thesis is my own original work and that it contains no material previously published or written by another person, except where due acknowledgment is made. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

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Contributions to Chapters

The ideas, development, data analysis and writing of all chapters were my principal responsibility, working independently under the supervision of Jenny-Ann Toribio (principal supervisor), Marta Hernandez-Jover (associate supervisor) and Steven Lapidge (associate supervisor). Comment on drafts was provided by all three supervisors for Chapters 1, 2, 4, 5, 6, and 8. Comment on drafts of Chapter 3 and 7 were provided by Jenny-Ann Toribio and Marta Hernandez-Jover.

Chapter 4

Partial funding for this study was provided by the Wildlife Health Network. Starling trapping and sample collection was performed by myself in conjunction with licensed personnel; Tina Bentz in 2008 and Thomas Bradley in 2009. Culture of samples for *Escherichia coli*, *Salmonella spp*. and *Campylobacter spp*. and serotyping for *Campylobacter spp*. was conducted by Gribbles Laboratory, Adelaide, South Australia. Serotyping of *Escherichia coli* was conducted by the Bendigo *Escherichia coli* Reference Laboratory, Bendigo, Victoria. Serotyping of *Salmonella spp*. was conducted by the Australian Salmonella Reference Centre (Institute of Medical and Veterinary Science, Adelaide, South Australia).

I performed the calculations to determine the sample size. These results were subject to calculations performed by myself to determine the probability of pathogen presence and true prevalence of pathogens in the starling populations on each piggery. I have drawn conclusions based on my findings and written the chapter in this thesis.

Chapter 5

This chapter contains my own work as well as some results from a co-authored paper:

Collins, A.M., Fell, S., Pearson, H., Toribio, J-A (2011). Colonisation and shedding of *Lawsonia intracellularis* in experimentally inoculated rodents and in wild rodents on pig farms. Veterinary Microbiology, 150, p 384-388.

The concept for the study reported in this chapter arose from discussions between myself, Jenny-Ann Toribio and Steven Lapidge, as well as Ross Cutler and Alison Collins. The study proposal funded by the Victorian Swine Compensation Fund was prepared by Jenny-Ann Toribio, Steven Lapidge and I. Piggeries that employed one of the three desired

control techniques were identified through recommendations by Ross Cutler, Tony Fahy and Patrick Daniel. I performed the calculations to determine the sample size. I collected samples by dissection from all 299 rats, performed DNA extraction on all samples, and ran all Multiplex Polymerase Chain Reactions (PCR) and gel electrophoresis after initial training provided by staff at the Bendigo Pig Health Laboratory.

The DNA extracted from the rats was later subjected to an additional quantitative real-time PCR conducted free of charge by Alison Collins at the Elizabeth Macarthur Agricultural Institute. The results of the real-time qPCR were included in the above co-authored paper. Parts of the results for this test have been included in the results section of this chapter under the following headings: 5.3.1.3. Quantitative real-time PCR *Lawsonia intracellularis*; 5.3.2.2. Quantitative real-time PCR *Lawsonia intracellularis*; 5.3.2.3. A comparison of *Lawsonia intracellularis* true prevalence determined by enteric multiplex PCR and quantitative real-time PCR. These results were subject to calculations performed by myself to determine the true prevalence of this pathogen in the rat populations on each piggery. I have drawn conclusions based on my findings and written the chapter in this thesis.

Chapter 6

I performed the calculations to determine the sample size. Feral pig trapping and shooting was performed as part of an ecological damage study by the Queensland Murray Darling Committee (QMDC). The majority of sample collection and serum extraction was performed by me with the remainder obtained from feral pig shooters or Darren Marshall and Renee Stephens of the QMDC. Analysis of samples was conducted by various Australian laboratories: for *Brucella suis*, by the Elizabeth MacArthur Agricultural Institute, Menangle, NSW; for *Leptospira spp*., by Queensland Health Forensic and Scientific Services Laboratory, Brisbane, Queensland; for *Lawsonia intracellularis,* by Ace Laboratory Services, White Hills, Victoria; for *Mycoplasma hyopneumoniae,* ELISA by the Elizabeth MacArthur Agricultural Institute, Menangle, NSW; and for *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*, PCR by the Department of Primary Industries Pig Health Laboratory, Bendigo, Victoria.

Figure 6.3 was produced by Joel Pettiford from QMDC, with the remainder of GPS collar movement data analysed by Tony Buckmaster.

These results were subject to calculations performed by myself to determine the probability of pathogen presence and true prevalence of pathogens in the feral pig populations around each piggery. I have drawn conclusions based on my findings and written the chapter in this thesis.

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Abstract

The pork industry in Australia accounts for 2.1% of all agricultural production with a gross value of AU $865 million. It is comprised of approximately 1,350 pork producers and 2.3 million pigs. Pigs in Australia, similar to most other developed countries, are raised in three different production systems: traditional intensive production, ecoshelters and free-range. Each of these production systems provide opportunities for pathogen introduction and spread by wild animals. Wild animal species, both introduced and native to Australia, pose a disease threat to domestic animals and humans in relation to the introduction, maintenance and spread of emerging, exotic and endemic pathogens. Australia has had few outbreaks of emerging infectious diseases and is well protected from transboundary animal diseases due to stringent quarantine procedures, which protect Australia's livestock industries. As such, the main threat to domestic pig health in Australia posed by wildlife is transmission of infectious pathogens currently endemic in wild animal populations.

Eighty five percent of the pig producing members of Australian Pork Limited that responded to a postal survey experienced wild animal incursions on their piggery. The wild animals presenting the greatest risk to commercial piggeries, determined by the number of pig producers reporting an observation of the species and the frequency of observations, included the European starling (*Sturnus vulgarus*), rodents (black rat *Rattus rattus*, brown rat *Rattus norvegicus*, and the house mouse *Mus musculus*) and feral cats (*Felis catus*). The species-specific pathogen transmission potential from feral pigs (*Sus scrofa*) also presented a high risk.

The role of the cat in the transmission of *Toxoplasma gondii* to domestic pigs in piggeries has been thoroughly studied and consequently has not been examined further in this study. Pig pathogens were detected in European starlings, rats and feral pigs. These pathogens could be transmitted to pigs through direct and indirect pathways, via contaminated food, water and air.

The current study detected *Escherichia coli*, *Salmonella spp*. and *Campylobacter spp*. in European starling populations around four large-scale commercial piggeries in South Australia. *Escherichia coli* was detected in starlings on all four piggeries, while *Salmonella spp*. and *Campylobacter spp*. were only detected on one piggery. *Salmonella spp*. and

Lawsonia intracellularis were detected in rats on three large-scale commercial piggeries, two of which were located in Victoria, and one in South Australia. *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* were not detected in rats in the current study. *Lawsonia intracellularis*, *Brucella suis*, *Leptospira spp*. and *Mycoplasma hyopneumoniae* were all detected in feral pigs within 10 km of two large-scale commercial piggeries in southern Queensland. Results from six collared feral pigs showed that for 5 pigs the majority of their movement was within 5 km of the piggeries. One individual, a large male boar, moved to within 100 m of a free-range piggery.

Based on the results of an exposure assessment, rats presented the highest probability of exposure of pathogens to domestic pigs, and *Lawsonia intracellularis* (median 0.13, 5% and 95% CI 0.05–0.23) and *Brachyspira hyodysenteriae* (median 0.10, 5% and 95% CI 0.05–0.19; based on the prevalence in rats obtained from literature) were the most likely pathogens to be transmitted. The probability of exposure of domestic pigs to pathogens from European starlings was estimated to be lower than the exposure from rats. However, pathogenic *Escherichia coli* had a 0.03 (5% and 95% CI 0.02–0.04) median probability of exposure, which was the highest probability among the three pathogens studied in starlings. The probability of pathogen exposure from feral to domestic pigs was found to be lower than rats and starlings for some pathogens. The highest probability of domestic pig exposure to feral pig pathogens was found to be for *Mycoplasma hyopneumoniae* (median 0.01, 5% and 95% CI 0.004–0.02) and *Lawsonia intracellularis* (median 0.01, 5% and 95% CI 0.005–0.03) for pigs in free-range piggeries in a region with a high number of feral pigs. The models developed in this thesis identified the presence and number of wild animals around piggeries, their access to piggeries and pig food and water, and their proximity to piggeries, as critical points to mitigate the risk of pathogen exposure.

Findings from this thesis indicate that the estimated probability of exposure of domestic pigs to pathogens from wild animals is not negligible. As such, the implementation of mitigation strategies should be further investigated, considering also the magnitude of the impacts of this exposure, the costs involved with the mitigation measures and the practical implications. This would support decision-making to determine the need for and benefits of these mitigation strategies.

List of abbreviations

APIQ✓™	Australian pork industry quality assurance program
APL	Australian Pork Limited
AU	Australian
bp	base pairs
CFT	complement fixation test
CI	confidence interval
cm	centimetre
CSV	comma-separated values
DNA	deoxyribonucleic acid
DPI	Department of Primary Industries
DRC-1339	3-chloro-4-methylbanzenamine hydrochloride
ELISA	enzyme-linked immunosorbent assay
Eq	equation
FMD	foot-and-mouth disease
g	gram
G	gravitational force
GCS	geographic coordinate system
GIS	geographic information system
GPS	global positioning system
h	hour
HDOP	horizontal dilution of precision
kg	kilogram
km	kilometre
L	litre
m	metre
MAT	microscopic agglutination test
mg	milligram
min	minute
ml	millilitre
Nm	nanometre
nox	NADH (Nicotinamide adenine dinucleotide) oxidase
NSW	New South Wales

OIE	World Organisation for Animal Health
PCR	polymerase chain reaction
QMDC	Queensland Murray Darling Committee
qPCR	quantitative real-time polymerase chain reaction
RBT	Rose Bengal test
se	sensitivity
sec	second
sp	specificity
SQL	structured query language
Taq	Taq polymerase
Tp	true prevalence
UHF	ultra high frequency
US	United States
WGS	world geodetic system
XLD	xylose lysine desoxycholate
µl	microlitre
µM	micromolar
ºC	degrees Celsius
>	greater than
<	less than
1080	compound 1080 (Sodium fluoracetate)

1 General Introduction

Domestic pig production in Australia is a relatively small but important industry in agricultural terms. The pig industry comprises 2.1% of total domestic agricultural production in Australia, with a gross value of AU $865 million (APL 2010), while Australian pork production comprises 0.4% of world pork production (APL 2010). Due to the very small size of the Australian pork industry, an outbreak of the exotic virus foot-and-mouth disease in the naïve pig population would have a significant effect on the industry. Not only pigs, but all cloven hoofed animals are susceptible to this devastating disease. The Productivity Commission (2002) estimated that a foot-and-mouth disease outbreak in Australia lasting 3 months would cost $5.5 billion to livestock industries and a loss of $8 billion in gross domestic product. Animal welfare is also a major concern in any infectious disease outbreak. An epidemic of the emerging disease Nipah virus in Malaysia during 1999 led to a cull of over one million pigs (Chua et al. 2000).

At present, Australia has substantial advantages in livestock production and trade due to being free of 71% of World Organisation for Animal Health (OIE) listed terrestrial animal diseases, 32% of which infect pigs (WAHID 2010). Australia's continuing disease free status is attributable to its remote location and stringent quarantine procedures which protect its livestock industry. However, livestock health is threatened by infectious pathogens currently emerging and endemic in wild animal populations in this country. The role that wild animal populations could play in the event of exotic disease introduction also requires investigation to underpin approaches to farm biosecurity practice and more broadly to disease preparedness and response planning. This thesis investigates two principle modes for pathogen introduction onto livestock farms from wild native and non-native animals: direct transmission through contact between animals; and indirect transmission through infected air, food and water (Frölich et al. 2002; Coetzer and Tustin 2004; Kyriazakis and Whittemore 2006). The transmission of infectious pathogens from wild animals to domestic pigs is the focus of this thesis.

In order to determine which wild animals are the most important sources for infectious pathogen transmission to pigs in Australian piggeries, a questionnaire was distributed

amongst members of Australian Pork Limited who worked on commercial piggeries. The procedure, rationale, and outcomes of this survey of commercial pig producers are presented in detail in Chapter 2. The animals identified were: European starlings (*Sturnus vulgarus*), rodents (rats: *Rattus rattus* and *Rattus norvegicus*; house mouse: *Mus musculus*) and feral pigs (*Sus scrofa*). Once identified, these wild animal species were subjected to further investigation in order to detect the presence of specific pig pathogens in naturally occurring populations of each species in close proximity to commercial piggeries.

European starlings are associated with more than 20 pathogens of animals and humans (Weber 1979). Consequently, starlings have been deemed a possible source of pathogens with economic and animal welfare impact to livestock industries (Kirk et al. 2002; Gaukler et al. 2009; Carlson et al. 2011a). In the present study, the starling was one of the most frequently reported birds on piggeries in Australia (Chapter 2). The three bacterial enteropathogens *Salmonella spp*., *Escherichia coli* and *Campylobacter spp*. have been previously isolated from starlings (Morishita et al. 1999; Craven et al. 2000; Tizard 2004). Salmonella and Campylobacter alone are responsible for 90% of bacterial enteritis infections in humans derived from food sources world-wide (Thorns 2000). Despite this evidence, the extent of the contribution of starlings to the transmission of pathogens to livestock remains unclear.

Rodents have been shown to be reservoirs of a large number of pathogens, including bacteria, viruses and parasites (Le Moine et al. 1987; Amass and Clark 1999; Henderson 2009). Some diseases that involve transmission from rodents, such as the Bubonic Plague and Leptospirosis, are zoonotic in nature, being transmissible to humans, and can also be transmitted to other animals (Twigg 1975; Alderton 1996; Singleton et al. 1999). Consequently, rodents have been identified as a likely vehicle for transmission of pathogens to and between domestic animals (Le Moine et al. 1987; Hampson et al. 1991; Drummond 2001). The carrier status of rodents for many pathogens that can infect pigs has been well documented. Arguably the two most important endemic pathogens in Australian piggeries in terms of cost of production and animal welfare are *Brachyspira hyodysenteriae* and *Lawsonia intracellularis* (Hampson 1997; Straw et al. 2006; Holyoake et al. 2010a). These pathogens have been isolated from rodents captured in piggeries (Hampson et al. 1991; Friedman et al. 2008; Collins et al. 2011). However, transmission of these pathogens from rodents to livestock has not been investigated in detail.

Feral pigs have been considered responsible for a number of significant disease outbreaks in livestock. Classical swine fever, African swine fever and pseudorabies are some international examples where feral pig populations that harbour disease have contributed to outbreaks in livestock. Among 93 primary outbreaks of classical swine fever in domestic pigs in Germany during 1993-1998, 59% were proven to be caused by transmission of the virus from feral pigs (Fritzemeier et al. 2000). Feral pigs in Australia are known reservoirs of 21 different pathogenic species of bacteria, many of which are transmissible to other animals and humans, and all of which are transmissible to domestic pigs (Bensink et al. 1991; Pavlov et al. 1992; Godfroid 2002; Phillips et al. 2009). Similar to starlings and rodents, limited information is available on the role played by feral pigs in the transmission of endemic pathogens to domestic pigs and other livestock.

The cat (*Felis catus*) was also identified as an important wild animal source for infectious pathogen transmission to pigs in the survey described in Chapter 2. Perhaps the most significant zoonotic pathogen associated with cats is the protozoan parasite *Toxoplasma gondii.* Cats are the definitive host of *Toxoplasma gondii* and are the only animal to excrete *Toxoplasma gondii* oocysts (Elmore et al. 2010). *Toxoplasma gondii* can infect the majority of birds and mammals, including humans. Consumption of undercooked pork meat is considered to be one of the main sources of *Toxoplasma gondii* infection in humans (Dubey et al. 1986; Lehmann et al. 2003). Infection in pigs is considered to come predominantly from a feline source, through infection with excreted oocysts (Lehmann et al. 2003). The role of the cat in the transmission of this protozoan to domestic pigs in piggeries has been thoroughly studied by the above authors, and as such was not examined further in this study.

The aim of this thesis is to characterise the role of European starlings, rats and feral pigs in the transmission of specific pathogens to domestic pigs on commercial piggeries in Australia. While other wild animal species may also pose a risk for pathogen introduction, this study is limited to the characterisation of these three wild animal species. Chapter 3 of this thesis presents a review of the literature associated with each of these species and the transmission characteristics for each species is presented in separate chapters (Chapters 4, 5 and 6).

The outcomes of the studies characterising the role of the three wild animal species are used to determine the probability of exposure of domestic pigs to specific pathogens carried by each species. Following a risk analysis approach, an exposure assessment is conducted to estimate this probability. The assessment incorporates prevalence data on the specific pathogens as well as the biosecurity practices of the piggeries included in the studies presented in Chapters 4, 5 and 6. This measure of the risk of pathogen transmission to domestic pigs will enable the provision of informed recommendations to the Australian pork industry that aim to minimise this biosecurity hazard to domestic pigs in Australia.

2 Producer reported wildlife incursions on commercial piggeries

2.1 Introduction

Progress in minimising the potential for cross-species transmission of disease is impeded by lack of knowledge on which wild animal species are likely to exchange pathogens, and indeed, which species are routinely coming into contact with animals in our agricultural industries. While there is good evidence of interactions between wildlife and domestic pigs (Amass and Clark 1999; Bengis et al. 2002; Lapidge et al. 2006), and well established cases of pathogen transmission between them (Fritzemeier et al. 2000; Lehmann et al. 2003; Breed et al. 2006), there are currently few well documented studies that have identified the range of wildlife species, their distributions and numbers on and around piggeries.

The objectives of this study were to obtain baseline data on the following: first, the proportion and geographical distribution of commercial piggeries experiencing wildlife incursions/presence in Australia; secondly, the control techniques in use on piggeries for wildlife intruders and the producer reported effectiveness of those techniques; and, thirdly, the proportion of piggery producers concerned about the occurrence of specific diseases that are carried by wildlife hosts.

2.2 Materials and Methods

2.2.1 Overview

A nation wide survey of commercial pig producers who were members of Australian Pork Limited (APL; n=444) was conducted. APL is a producer owned registered company that supports and promotes the Australian pork industry. Commercial piggeries included in the current study were identified through the APL confidential members list. A self-administered questionnaire was distributed through APL to protect the privacy of the organisations members. Two subsequent mailings were sent to non-respondents. Procedures involving contact with and collection of information from pig producers were

approved by the Human Research Ethics Committee of the University of Sydney, Australia (Reference number: 08-2007/10271).

2.2.2 Questionnaire design, distribution and processing

The one-page questionnaire (Appendix 1) was designed for return postage with a postage-paid code and return address on the reverse side. A cover letter and a participant information statement were included in the envelope describing the purpose of the survey, instructions on survey completion and a statement to reassure recipients of the confidentiality of the results.

The questionnaire contained open questions regarding piggery location and sow herd size. Closed-ended questions established the piggery housing type and whether the piggeries experienced any wild animal incursions. Remaining questions pertaining to the wild animal types observed, the frequency of these observations, the methods for controlling wild animals, the wildlife related diseases of concern to pork producers, their sources of information about these diseases and areas for future research related to wildlife were open-ended with space for further comments. The questionnaire was piloted on three respondents who had been piggery managers during their career and questions then modified to improve clarity and ease of administration.

The first mailing of the questionnaire was distributed to all commercial piggery owners and managers who were members of APL via the 'Pork It Up' newsletter distributed by APL on the 18th of September, 2007. This was followed up with two subsequent repeat mail-outs to non-respondents administered by APL at six and nine weeks following a modified version of the Dillman protocol for postal surveys (Dillman 2000).

Data from returned questionnaires was entered into Microsoft Excel (PC/Windows XP, 2003) and checked for data entry errors. Each respondent reported on one piggery; each piggery was assigned a piggery identification number and the postal area code listed by the respondent was the only identifying information from each questionnaire maintained in the database. Some responses were coded to create dichotomous variables (Yes=1, No=0). Other questions, such as sow herd size and the number of animals per wildlife species observed each week provided numerical data. Responses for the rest of the questions (piggery type, wildlife control method, information regarding pig producers' diseases of

concern, sources of information and future research needs) were categorical. Not all survey participants responded to every question, this is indicated in the results.

2.2.3 Analysis

The overall response rate and proportions were calculated using all responses. Insufficiently completed questionnaires with more than 50% of the information missing were excluded from further analysis. The geographic distribution of all respondents was mapped using ArcGIS (Professional 9.2. MapInfo Corporation, USA) based on postal codes.

Some animal species were grouped into the following categories for analytical convenience:

- Marsupials: kangaroos, wallabies, possums, wombats, koalas, sugar gliders
- Passeriformes: crows, magpies, butcher birds, swallows, martins
- Psittaciformes: cockatoos, galahs, parrots
- Anseriformes: ducks, geese
- Reptiles and Amphibians: reptiles (snakes and lizards), amphibians (toads)
- Lagomorpha: rabbits, hares
- Rodents: rats and mice
- Wild Canids: foxes, dingoes, wild dogs.
- Non-specific wild birds: used when specific bird species were not named

Some producers did not specify species of wild bird observed; therefore, all of these birds were grouped under the category of Non-specific wild birds. Other piggeries specified native bird species that were observed, and these were categorised by bird group.

Descriptive analysis of the questionnaire data variables was performed using Microsoft Excel (PC/Windows XP, 2003), and data was then exported to Genstat 10th edition (release 10.2, VSN International Ltd., United Kingdom) for further statistical analysis. Differences in the proportion of piggery producers in each state that responded to the survey compared to APL members were assessed by a Fisher's exact test. Significant differences between the proportion of animal group observations on piggeries, as well as the proportion of control techniques used for each individual animal or animal group were assessed using a z-test. Statistically significant differences were considered when P-value ≤ 0.05.

Association between the outcome variable, presence or absence (1/0) of wildlife or of specified individual animal or animal group observed on a piggery, and the explanatory variables was investigated in separate binomial multivariate logistic regression models for each outcome variable. The explanatory variables included in the models as fixed effects were the state where the piggery was located (based on postal code), sow herd size (no sows - grower herd, 1–150 sows, 151–1000 sows and >1000 sows) and piggery type (intensive, ecoshelter, free-range, intensive and ecoshelter, or other mixed types). Other mixed piggery types for a piggery reported by respondents in this study included intensive and free-range production; ecoshelter and free-range production; and all three forms of production. Significant variables with a P-value ≤0.05 in the multivariable model were retained in the final model.

2.3 Results

2.3.1 Description of study population

Of the 444 APL members invited to complete the questionnaire, 171 (38.5%) responded. The first mailing resulted in 50 responses, representing a response rate of 11.3%, the second mailing gained a further 71 responses, with a mailing response rate of 18.0%, and the third mailing gained a final 50 responses with a mailing response rate of 15.5%. Data from one respondent was omitted from the analysis due to a recent piggery closure. In addition, another respondent had 2 piggeries, but answered the questionnaire only for the largest piggery. There was no statistically significant difference in geographic distribution by state between survey respondents and the 444 APL members who received the questionnaire. The geographic distribution of respondents within states of Australia is illustrated in Figure 2.1.

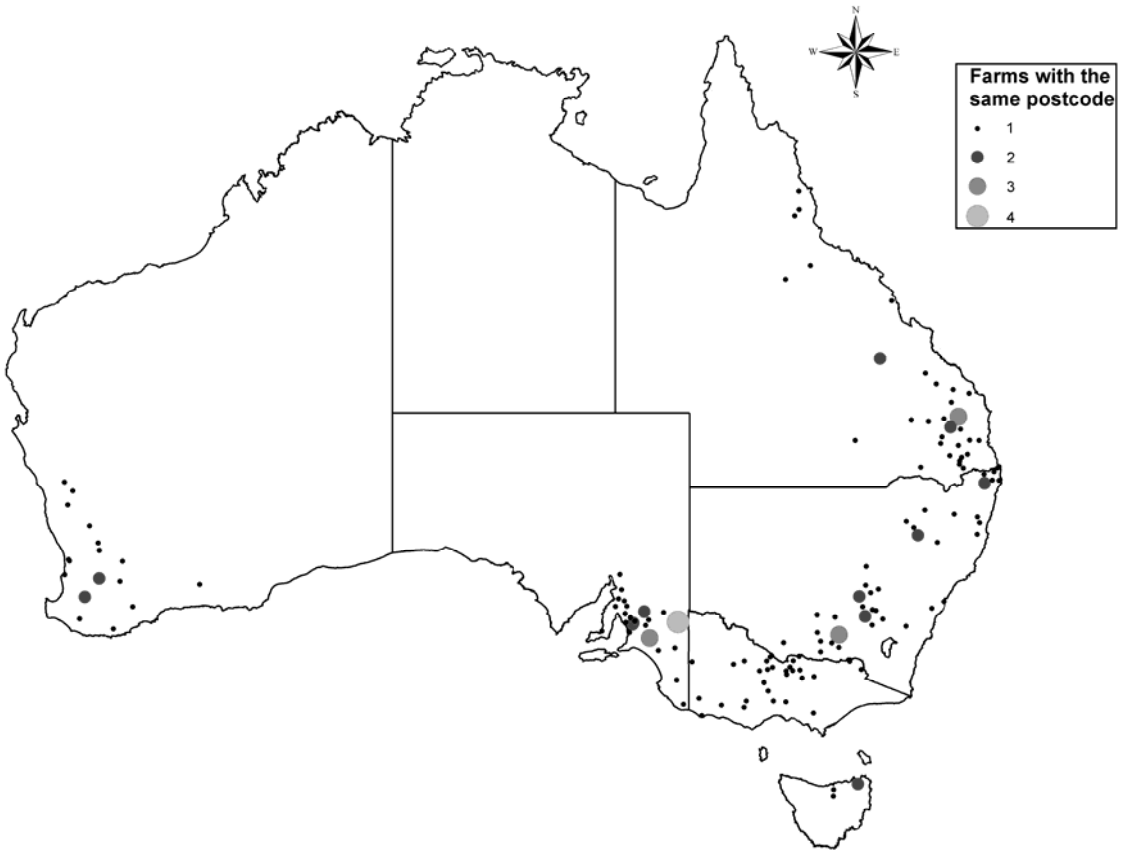

Figure 2.1. Map of the geographic distribution of 171 respondents to a postal survey on domestic pig-wildlife interactions on commercial piggeries in Australia conducted in 2007. Size of the points indicates the number of commercial piggeries in a particular postal code.

Of the 170 piggeries, nine were grower herds with no sows and the remainder ranged in size from 5 to 6,000 breeding sows (mean 418, median 180). Intensive production was reported for 87 (51.2%) of the 170 piggeries and was the most common single piggery type, followed by free-range production for 19 (11.2%) piggeries and ecoshelter production for 6 (3.5%) piggeries. However, 58 piggeries were engaged in more than one production type, with intensive and ecoshelter production reported for 46 piggeries (27.0%), intensive and free-range production reported for 7 piggeries (4.1%), ecoshelter and free-range production for 3 piggeries (2.0%), and all three forms of production for 2 piggeries (1.0%).

2.3.2 Wildlife incursions

Some level of wild animal incursion was reported by 145 of the 170 respondents (85.3%), either into buildings used to house pigs or within 5 m of pig housing facilities.

Birds were statistically the most common animals observed intruding on piggeries with 114 (78.6%) pork producers reporting bird observations (Figure 2.2). The most common individual species were the House sparrow, reported by 29 (*Passer domesticus*, 25.4%) of the pork producers and the European starling by 24 (21.1%) (Figure 2.3). Feral cats were the most common single species observed intruding on piggeries, being reported by 88 (60.7%) of the 145 responders.

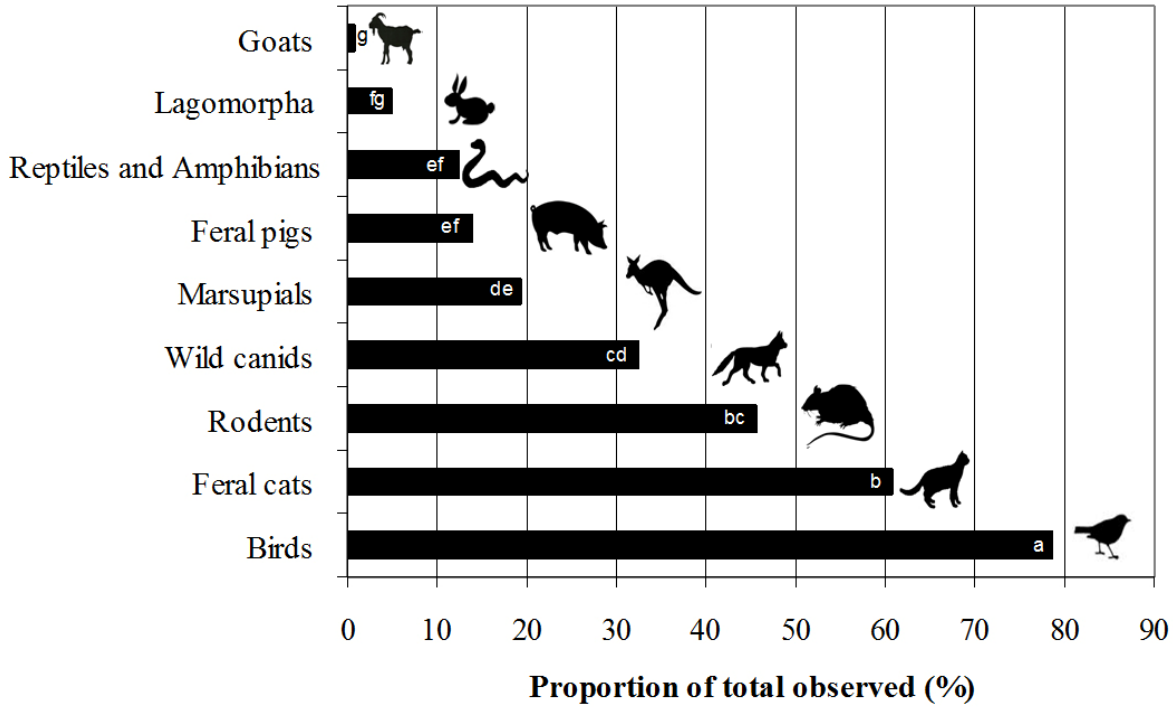

Figure 2.2. Proportion of all wild animals and animal groups observed on commercial piggeries by 145 pig producers in Australia during 2007. Different letters represent significant differences ($P<0.05$) between animal group observations. For example, a is significantly different to b, but ab is not significantly different to either a or b.

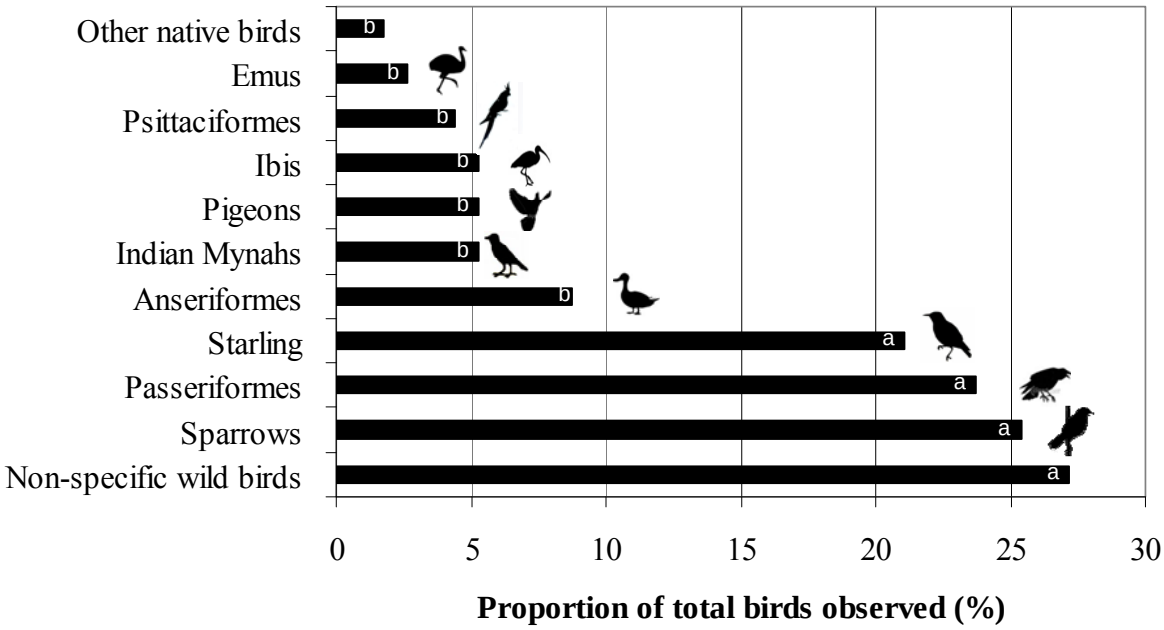

Figure 2.3. Proportion of all bird groups observed on commercial piggeries by 114 pig producers in Australia during 2007. Different letters represent significant differences ($P<0.05$) between bird group observations.

Despite feral cats being the most commonly reported species, the average count of these animals seen in a piggery varied from 1 per year to 30 per week. Feral pigs (n = 20 producers, 13.8%) were observed in the lowest numbers, ranging from an average of 1 per year to 5 per week, with European starlings observed in the greatest numbers, ranging from 30 to 10,000 animals per week.

Results from the logistic regression analysis suggest that the presence or absence of wildlife on commercial piggeries in Australia did not differ according to location (state) of the piggery, piggery type or piggery size. However, significant differences were observed for the presence of some individual wildlife species (Table 2.1). The presence of feral cats and wild canids on commercial piggeries in Australia was significantly different ($P<0.05$) according to piggery type and piggery size; however, no differences were observed according to the location (state) of the piggery. For feral cats, the highest number of observations was recorded by piggeries using mixed production systems (83.3%), which did not differ from intensive/ecoshelter piggeries (65.2%). More feral cats were observed on piggeries with >1000 sows (68.8%) and with 151 to 1000 sows (63.8%). Wild canids were observed in more piggeries using mixed (66.7%) production systems that any other

production system, except for ecoshelters. A higher proportion of piggeries with >1000 sows (56.3%) recorded wild canid incursions than other piggery types, except for grower herds.

Observations of marsupials on piggeries differed according to piggery location and piggery size ($P<0.05$). Tasmania was the only state which differed from the other states on the reported marsupial observations. The presence of marsupials in large piggeries (>1000 sows) was higher (50.0%) than in piggeries with 1 to 150 sows (12.3%), 151 to 1000 sows (14.5%) and grower herds (11.1%), which were not different to each other. The proportion of piggeries observing feral pigs on or around the piggery differed between locations ($P < 0.001$), being greater in Queensland (37.1%) compared to the other states, with the exception of Western Australia. No feral pigs were observed in Tasmania or South Australia.

Table 2.1. Observations of wild mammals on 145 commercial piggeries in Australia during 2007, according to location by state, piggery type and piggery size.

Parameter	Number of piggeries	Number (percentage) of piggeries with species reported				
		Feral Cats	Rodents	Wild Canids	Marsupials	Feral Pigs
State		NS	NS	NS	*	**
NSW	46	22 (47.8)	20 (42.5)	12 (26.1)	5 (10.9)[b]	3 (6.5)[b]
Queensland	35	18 (51.4)	13 (37.1)	8 (22.9)	6 (17.1)[b]	13 (37.1)[a]
Victoria	28	12 (42.9)	13 (46.6)	8 (28.6)	6 (21.4)[b]	1 (3.6)[b]
South Australia	33	20 (60.6)	13 (39.4)	10 (30.3)	4 (12.1)[b]	0
Tasmania	4	1 (25.0)	0	0	4 (100)[a]	0
Western Australia	20	14 (70.0)	6 (30.0)	8 (40.0)	3 (15.0)[b]	3 (15.0)[ab]
Piggery Type		*	NS	*	NS	NS
Intensive	87	40 (46.0)[b]	41 (47.1)	19 (21.8)[b]	14 (16.1)	10 (11.5)
Ecoshelter	6	2 (33.3)[b]	1 (16.7)	3 (50.0)[ab]	1 (16.7)	0
Free-range	19	6 (31.6)[b]	3 (15.8)	5 (26.3)[b]	4 (21.1)	4 (21.1)
Intensive / Ecoshelter	46	30 (65.2)[ab]	17 (37.0)	12 (26.1)[b]	8 (17.4)	4 (8.7)
Mix	12	10 (83.3)[a]	4 (33.3)	8 (66.7)[a]	1 (8.3)	2 (16.7)
Sow Herd Size		*	NS	*	*	NS
No Sows - Grower herd	9	2 (22.2)[a]	2 (22.2)	4 (44.4)[ab]	1 (11.1)[b]	0
1–150 Sows	73	30 (41.1)[a]	24 (32.9)	15 (20.5)[b]	9 (12.3)[b]	10 (13.7)
151–1000 Sows	69	44 (63.8)[b]	33 (47.8)	19 (27.5)[b]	10 (14.5)[b]	6 (8.7)
>1000 Sows	16	11 (68.8)[b]	6 (37.5)	9 (56.3)[a]	8 (50.0)[a]	4 (25.0)

*P<0.05; ** P<0.001; NS= not significant

[a,b,c] Different superscripts within a column and parameter (location, piggery type and piggery size) (P<0.05)

Data from four respondents was excluded from the State parameter due to the absence of piggery location information on their completed questionnaire. Data from three respondents was excluded from the Sow Herd Size parameter due to the absence of piggery size information on their completed questionnaire.

Table 2.2 shows the number of observations of different species of wild birds according to piggery location, piggery type and sow herd size. Non-specific wild bird observations differed (P<0.05) according to piggery type. Ecoshelter (66.7%) and mixed (58.3%) piggeries reported more incursions than intensive (27.6%) and free-range (26.3%) piggeries. Intensive/ecoshelter farm systems (34.8%) did not differ from the other piggery types. Proportion of farms where sparrows were observed differed according to farm location (P<0.001), with fewer observations in Queensland than in other states. No observations were reported in Western Australia. Ecoshelter and free-range piggeries did not report any sparrow observations. Observations of starlings were significantly different

(P<0.05) according to piggery location, with higher observations in New South Wales (23.9%) and South Australia (21.2%) than in Queensland (2.9%).

Table 2.2. Observations of wild birds on 145 commercial piggeries in Australia during 2007, according to location by state, piggery type and piggery size.

Parameter	Number of piggeries	Number (percentage) of piggeries with species reported				
		Non-specific wild birds	Sparrows	Passeriformes	Starlings	Anseriformes
State		NS	**	NS	*	NS
NSW	46	15 (32.6)	9 (19.6)[a]	7 (15.2)	11 (23.9)[a]	3 (6.5)
Queensland	35	14 (40.0)	1 (2.9)[b]	7 (20.0)	1 (2.9)[b]	3 (8.6)
Victoria	28	10 (35.7)	6 (21.4)[a]	3 (10.7)	4 (14.3)[ab]	1 (3.6)
South Australia	33	12 (36.4)	10 (30.3)[a]	3 (9.1)	7 (21.2)[a]	2 (6.1)
Tasmania	4	0	2 (50.0)[a]	0	0	0
Western Australia	20	5 (25.0)	0	7 (35.0)	0	1 (5.0)
Piggery Type		*	NS	NS	NS	NS
Intensive	87	24 (27.6)[b]	16 (18.4)	13 (14.9)	12 (13.8)	7 (8.0)
Ecoshelter	6	4 (66.7)[a]	0	0	2 (33.3)	0
Free-range	19	5 (26.3)[b]	0	3 (15.8)	2 (10.5)	1 (5.3)
Intensive / Ecoshelter	46	16 (34.8)[ab]	12 (26.1)	6 (13.0)	8 (17.4)	2 (4.3)
Mix	12	7 (58.3)[a]	1 (8.3)	5 (41.7)	0	0
Sow Herd Size		NS	NS	NS	NS	NS
No Sows - Grower herd	9	3 (33.3)	1 (11.1)	1 (11.1)	3 (33.3)	1 (11.1)
1–150 Sows	73	21 (28.8)	14 (19.2)	15 (20.5)	5 (6.8)	5 (6.8)
151–1000 Sows	69	24 (34.8)	12 (17.4)	9 (13.0)	13 (18.8)	2 (2.9)
>1000 Sows	16	7 (43.8)	2 (12.5)	2 (12.5)	3 (18.8)	2 (12.5)

*P<0.05; ** P<0.001; NS= not significant

[a,b,c] Different superscripts within a column and parameter (location, piggery type and piggery size) (P<0.05)

Data from four respondents was excluded from the State parameter due to the absence of piggery location information on their completed questionnaire. Data from three respondents was excluded from the Sow Herd Size parameter due to the absence of piggery size information on their completed questionnaire.

2.3.3 Control measures for wildlife

A total of 26 producers (17.9%) did not implement any control measures for the wild animals visiting their piggery, with the main lack of control measure implementation for birds and native species (including marsupials, reptiles and amphibians) (Figure 2.4).

When a control measure was applied, the most common techniques included shooting, baiting and trapping. Baiting was used predominantly for rodents, being reported alone by 57 (86.4%) of 61 respondents who implemented rodent control, and in combination with trapping or shooting by the remainder. Of the control techniques used for bird control, shooting was the most common (Figure 2.4). There was no significant difference (P>0.05) between trapping, shooting, other techniques and no control use for feral cats. This was similar to wild canids except baiting was used in place of trapping. Other control techniques for feral pigs were the use of fences and a single producer who used a combination of baiting, shooting and trapping.

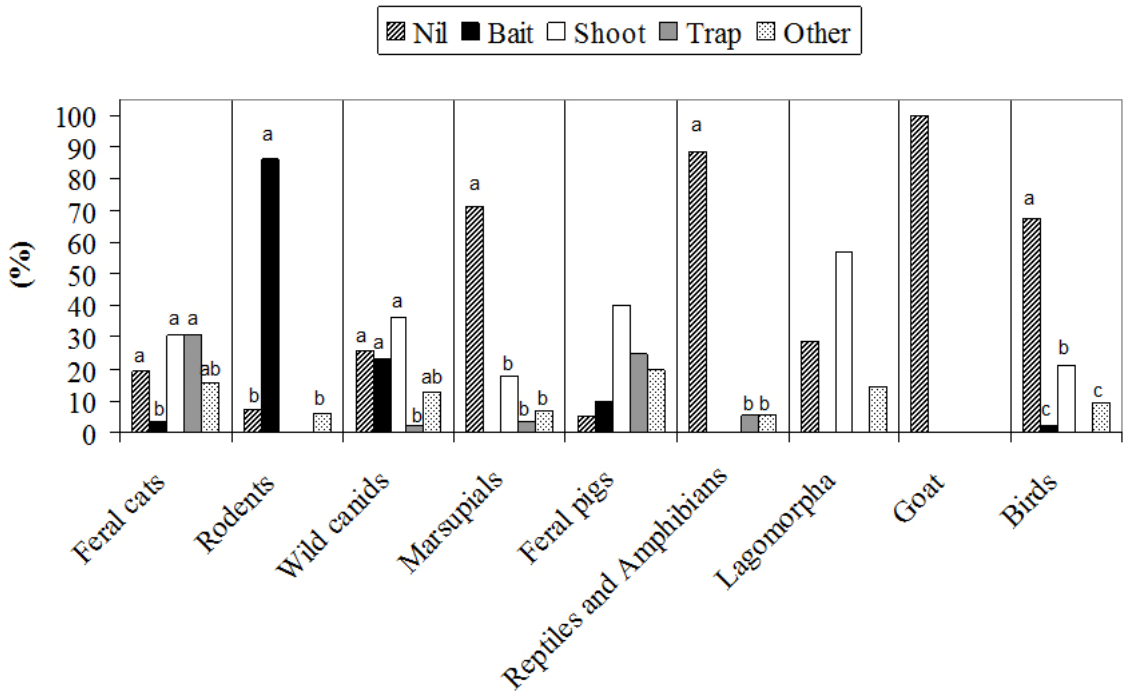

Figure 2.4. Proportion of the main control techniques used for the control of wild animals intruding on 145 commercial piggeries in Australia during 2007. Control techniques included in the 'Other' heading were combinations of 'baiting, trapping and shooting', fencing, and environmental alterations. Different letters represent significant differences (P<0.05) between control techniques used for each individual animal or animal group. Absence of letters indicates no significant difference.

Respondents using control measures to combat wildlife incursions were also asked to evaluate the effectiveness of these measures. Of those pork producers, 77% found their control measures effective for feral cats and 88% for rodents. The less effective control measures were those applied for European starlings, with only one of the 10 pork

producers who implemented control for these birds reporting their control techniques to be effective.

2.3.4 Wildlife diseases

Of the 87 respondents that reported the sources used when seeking information regarding diseases carried by wildlife, 60 consulted veterinarians (69%) followed by 30 consulting industry (34% – people and publications from the Department of Primary Industries and Australian Pork Limited), 13 from literature (15%) and 8 consulted friends (9%). In the opinion of 86 pig producers, the most important focus for future research was the European starling (39.5%), followed by the Indian mynah (29.0%) and the House sparrow (29.0%). Other topics of interest mentioned by producers were other wildlife species, diseases and wildlife control measures.

In terms of diseases, salmonella (24%), toxoplasmosis (21%) and leptospirosis (20%) were the 3 diseases most commonly of concern to pork producers (Table 2.3). Salmonella was considered by pork producers to be associated with birds (57%; House sparrows, Passeriformes, non-specific wild birds, European starlings, Ibis and pigeon) with House sparrows being the most commonly listed. Leptospirosis was reported to be mainly associated with rodents (66%) and feral pigs (20%), and toxoplasmosis was associated with cats (86%) (Table 2.3). Avian influenza was only reported in birds, with Passeriformes and pigeons being equally of concern, followed by Indian mynahs and European starlings. *Escherichia coli* was reportedly associated with birds (40%; Passeriformes, pigeons, Indian mynahs and European starlings) followed by rodents (30%). Rodents (40%) were also the main animal of concern associated with swine dysentery.

Table 2.3. Opinions of survey respondents regarding diseases associated with wild animals intruding on commercial piggeries based on a survey of 170 pig producers in Australia during 2007.

Disease	**Total number of respondents that listed disease**	**Number of respondents that associated disease with species (%)**					
	n (%)	Feral cats	Rodents	Wild canids	Feral pigs	Marsupials	Birds
Salmonella	42 (24%)	6 (14)	9 (21)	1 (2)	1 (2)	1 (2)	24 (57)
Toxoplasmosis	36 (21%)	31 (86)	2 (6)	1 (3)			2 (6)
Leptospirosis	35 (20%)	1 (3)	23 (66)	2 (6)	7 (20)		2 (6)
Escherichia Coli	10 (6%)	2 (20)	3 (30)	1 (10)			4 (40)
Swine Dysentery	10 (6%)	1 (10)	4 (40)		3 (30)		2 (20)
Mange	9 (5%)	1 (11)	1 (11)	5 (56)	1 (11)	1 (11)	
Erysipelas	7 (4%)		3 (43)		1 (14)		3 (43)
Avian influenza	6 (3%)						6 (100)
Lice	5 (3%)				2 (40)		3 (60)
Worms	4 (2%)	1 (25)			2 (50)		1 (25)
Coccidiosis	3 (2%)				1 (33)		2 (67)
Parvovirus	3 (2%)	1 (33)			1 (33)		1 (33)
Enzootic pneumonia	3 (2%)				1 (33)		2 (67)
Encephalomyocarditis	3 (2%)		3 (100)				

2.4 Discussion

The aims of this work were to identify the wildlife species that intrude on commercial piggeries in Australia, and the extent of their intrusion. It also aimed to identify control techniques for these wildlife intruders and the associated diseases of concern to pork producers. Eighty-five percent of producers that responded to the questionnaire experienced wild animal incursion, illustrating that there are multiple pathways involving wild animals by which domestic pigs may be exposed to potential pathogen hazards. In total, 37 wild animal species were reported entering piggery buildings or coming within 5 m of areas where domestic pigs were housed. It is uncertain whether the 61% of non-responders did not experience wildlife incursions, or did not respond to this questionnaire due to other concerns. Direct follow-up was not possible for confidentiality reasons.

Birds were the most reported wildlife on Australian commercial piggeries. Birds are known to be common on commercial piggeries in Australia and will enter piggery buildings and sheds. Farming practices such as open windows for cooling purposes and air circulation facilitate bird access to piggeries. As pig feed and water sources are usually accessible and exposed to wild birds, contamination with faecal matter can easily occur. A postal survey conducted in 2005 in Australia reported that almost three quarters of commercial pork producers experience bird impacts to their enterprise (Lapidge et al. 2006). These perceived impacts included damage to buildings, crops, vegetation and infrastructure; fouling of exposed sources of food and water being fed to pigs with droppings; direct feed loss; and increased pathogen spread (Lapidge et al. 2006). Despite the evidence of producer concern about bird presence, this study found that birds are still present in particularly large numbers on piggeries. Ten pork producers reported observations of starling flock sizes of one thousand birds or more; one pork producer noted starling flock sizes approximating 10,000 birds.

Registered baiting techniques to assist with control are not available for birds in Australia. Most pork producers did not control birds, and of those who did, using methods such as exclusion techniques and shooting, only 10% were satisfied with their effectiveness. In comparison, control strategies used for rodents, which focused on specifically developed baits, had a reported effectiveness of 88%. This could be one reason why European starlings were stated by respondents as the preferred area for future research.

Cats were the species most observed by survey respondents (61%). They are most likely found in the vicinity of piggeries due to a number of interrelated factors, including relative proximity to urban centres, readily available water and food sources (such as rodents, birds, and young piglets), and sufficient shelter for den sites. Cats were observed in numbers up to 30 per week on two piggeries. These numbers of cats are of concern due to the status of feral cats as the definitive host species for the *Toxoplasma gondii* parasite (Dubey et al. 1995 Dubey 2006; Dubey 2008). Infected cats shed oocyst in their faeces, which are resistant to decomposition in the environment (Elmore et al. 2010; Hutchison 1965). Feed and water contaminated by feline faecal material may be consumed by pigs or other animals, such as mice, opening up a wide variety of potential transmission modes (Dubey 2006). This parasite causes mortality in pigs, particularly neonatal piglets (Dubey 2009). Reducing the contact between feral cats and domestic pigs either via restricting direct entry to cats and the intermediate hosts, rats and mice, or through prevention of feed contamination, significantly reduces risk of infection (Weigel et al. 1995).

Rodents are also an important source of pig pathogens on piggeries. Their size and high reproductive capacity makes them a difficult animal to eradicate and exclude from farms and farming areas that house pigs. Contact and proximity of rodents with pigs may result in the increased likelihood of co-transmission of infections (Le Moine et al. 1987). Rats and mice have been shown to be carriers of a number of significant pig and human related pathogens, including *Leptospira spp*., *Salmonella spp*. and *Escherichia coli*, while others including *Brachyspira hyodysenteriae* and *Lawsonia intracellularis* not only cause illness in pigs, but also pose a significant economic burden to the pig industry in prevention and control (Smythe et al. 2000).

Reported control methods for rodents on piggeries were not focused on exclusion, but more on population management; the most popular was the use of rat baits in piggeries. The efficiency of baits to control rat populations can be highly variable. In some cases, where alternative food sources are constantly available, there may be no impact at all on rat populations (Leung and Clark 2005).

Feral pigs were only observed by a relatively small number of respondents (13.8%) when compared to birds, cats and rodents. A greater number of these observational reports were

from piggeries in Queensland; the population density of wild pigs is known to be larger in Queensland than in other states (West 2008). Seventy-nine percent of those pork producers reporting feral pig observations used a control technique and were satisfied with its effectiveness. However, there were several reports in the 'Other Comments' section of the questionnaire which suggested that feral pigs were not deterred by physical barriers to piggeries, with one case mentioning feral pigs digging under fences to gain access to commercial herds.

Bats and flying foxes have been identified as the reservoir for several emerging pathogens affecting humans and livestock including Menangle virus, which was transferred from flying foxes to commercial pigs in Australia on one occasion in 1997 (Halpin et al. 1999). It is therefore of interest that no producers in this survey reported observations of bats or flying foxes in or around their piggeries. If bats are present on piggeries surveyed they must be present in low numbers, or only visit piggeries seasonally or at night in periods of human absence.

Pork producers expressed particular concern about three main pathogens: *Salmonella spp*., *Toxoplasma gondii* and *Leptospira spp*. in wildlife on piggeries. As previously mentioned these pathogens can be carried by a multitude of wildlife hosts (birds, rodents, cats, feral pigs), and many of these hosts are wild animals that were reported entering the piggeries participating in this study on regular occasions and in large numbers. Thus, exposure of pigs to these pathogens is a justified concern of pig producers, both due to losses in pig production and the zoonotic potential of the pathogens.

Birds, rodents, feral cats and feral pigs emerge as animals presenting a greater risk of pathogen transmission to commercial pigs. For birds, rodents and feral cats this risk arises predominantly from their more regular occurrence on piggeries, their high numbers, and in turn their greater rate of contact. In the case of feral pigs, the species-specific pathogen transmission potential heightens risk although piggeries reporting feral pigs were more geographically limited. The reported effectiveness of control methods used by pork producers was not 100%, suggesting that control measures need to be improved to satisfy the biosecurity requirements necessary to mitigate the occurrence of disease.

Observations of some animals were more frequent in particular locations, such as feral pigs in Queensland, starlings in New South Wales and South Australia compared to Queensland, and sparrows were observed less frequently in Queensland. This was most likely due to climate and habitat preferences of the animals. Animals were also observed more often on particular piggery types, such as feral cats and wild canids on mixed piggeries. Similarly, non-specific wild birds were also observed more frequently on mixed piggeries and ecoshelters. Some possible reasons may have to do with easier access to food, or greater sources of food, as well as possible den sites or places of concealment. Greater food sources and accessibility may also be the reason behind more observations of some animals on larger piggeries, as was the case with feral cats, wild canids and marsupials. All of these results can be used to direct managerial approaches to minimise the biosecurity risk posed by these species.

The study design presented a potential bias in the response to some questions. The opinions on diseases of importance may have been influenced by the introductory letter distributed with the questionnaire, where previous cases of *Toxoplasma gondii* in cats and *Leptospira spp*. in rats were stated. This could have also influenced respondents to consider the presence of these particular animals on their piggeries, as well as encouraged producers with these animals to respond to the survey. However, bats were also mentioned in the introductory letter and no reports of this animal were observed among respondents. A comparison of the geographic distribution by state of survey respondents and the 444 APL members who received the questionnaire indicated that the survey captured a representative population of registered pig producers in Australia. However, the questionnaire distribution was limited to APL members, who represent only a proportion (n = 444) of the total number of piggeries in Australia (n = 1,350; APL 2010). This estimation of total piggeries is based on the Australian Bureau of Statistics and may be an under estimate (Personal communication, Holyoake 2012). Respondents were not specific to a single group of producers, evidenced by the piggery types covering all available methods (intensive, ecoshelter and free-range) and all sow herd sizes (range from 0 to 6,000 breeding sows, including nine grower herds).

Literature provides numerous examples of pathogens of importance to humans and livestock that are carried by wild animals. However, little is known about the mechanisms and necessary conditions for introduction and reintroduction of pathogens to commercial

livestock. This study provides information on the range of wild animal species, their distributions and numbers that are routinely coming into contact with domestic pigs in the Australian domestic pig producing industry. This information will provide the foundation to further examine the risk of pathogen transmission from the most common wildlife intruders to commercial pigs in Australia. Animals to be targeted for future pathogen risk assessments on piggeries include the European starling, rodents and feral pigs. Cats will not be a focus for this thesis due to the existence of comprehensive research into the involvement of the cat in transmission of *Toxoplasma gondii* to domestic pigs, as well as other animals and humans.

3 Literature review

3.1 The Australian Pork Industry

The pork industry in Australia accounts for 2.1% of all agricultural production, with a gross value of AU $865 million (APL 2010). On an international scale, Australian pork production accounts for 0.4% of world pork production (APL 2010). The Australian pork industry is thus relatively small when compared to the top two producing countries, China and the United States (with 51% and 9%, respectively, of the total world pork production; APL 2010).

The Australian pork industry is comprised of approximately 1,350 pork producers and 2.3 million pigs (APL 2010), with the majority of pork being produced along the East, South and South Western coasts of Australia in the grain growing regions of each state (Figure 3.1) (ABARE and MAF 2006; APL 2010). New South Wales has the largest proportion of pig herds, followed by Victoria, Queensland, South Australia, Western Australia and Tasmania (Figure 3.2) (APL 2010). The Northern Territory and Australian Capital Territory do not have any commercial pig herds.

Piggeries in Australia can be separated in to small and large-scale production units. The Australian pork industry quality assurance program (APIQ✓™), defines small-scale pig producers as those with 20 or fewer sows who sell or send to slaughter 400 or less pigs per year (APIQ 2010). Approximately 40% of pig producers have less than 50 sows (APL 2010). All remaining producers with greater than 20 sows can be defined as large-scale.

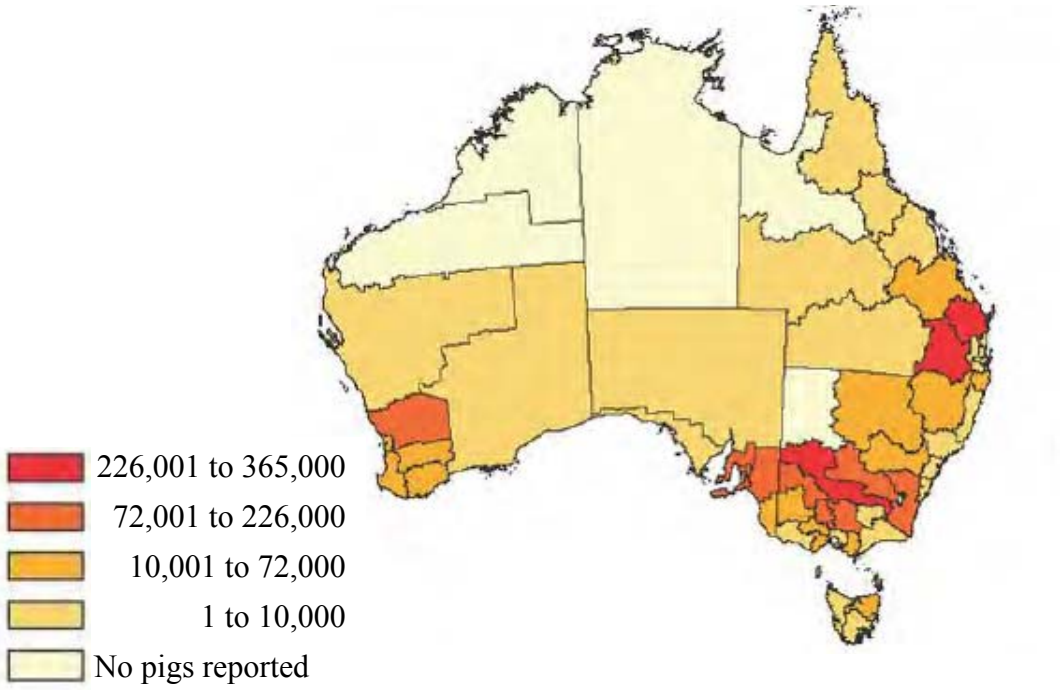

Figure 3.1. Distribution of domestic pigs throughout Australia in 2009 (APL 2010).

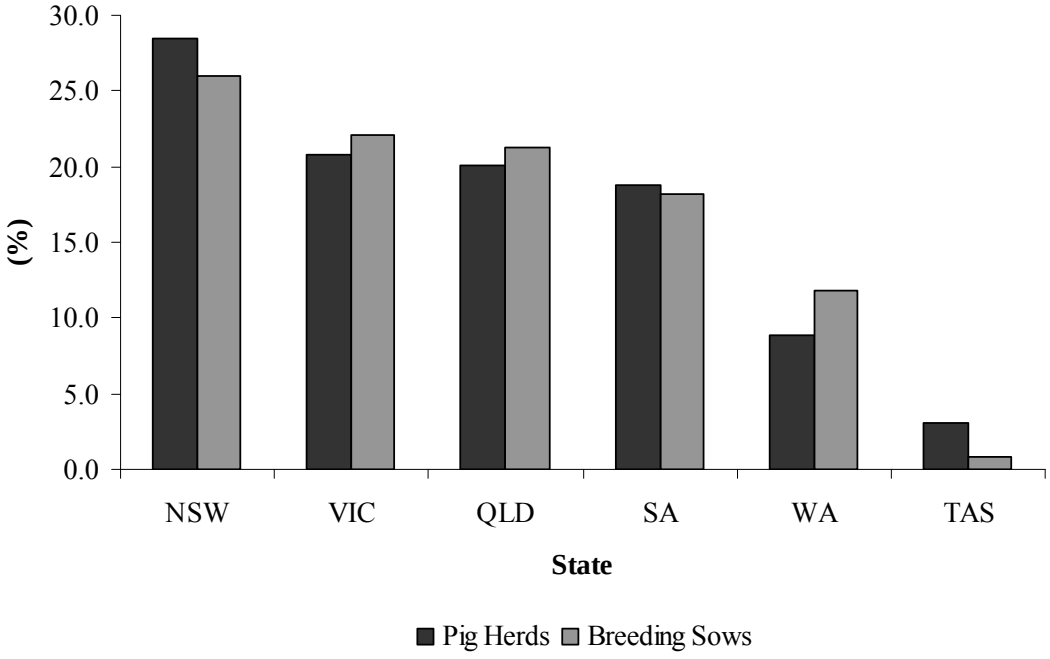

Figure 3.2. Distribution of pig herds and breeding sows by state throughout Australia in 2009 (adapted from APL 2010).

Pigs in Australia are mainly raised in three different production systems: traditional intensive production, ecoshelters and free-range (Primary Industries Standing Committee 2008). The majority of commercial pigs in Australia are produced in intensive piggeries (Chapter 2). Intensive piggeries house pigs in enclosed buildings with adjustable windows for ventilation and temperature control. Pigs are kept in pens and farrowing crates are used for sows during late pregnancy and lactation. This type of housing enables high animal density and an all-in all-out method or a continuous animal throughput process. An all-in all-out method is also implemented in ecoshelters, which are stand alone sheds with pigs of the same age group moving unconfined within a shed. Free-range piggeries house pigs almost entirely outdoors in paddocks, with the provision of limited housing, such as arcs or huts, for protection from climatic elements (Primary Industries Standing Committee 2008). Free-range piggeries can only be located in temperate areas with no severe cold season (FAO 2010). Each of these production systems provides opportunities for pathogen introduction and spread.

Although pork production in Australia is small on an international scale, it has a competitive advantage in terms of the health status of the nation's pig herd. Australia is free of 71% of World Organisation for Animal Health (OIE) listed terrestrial animal pathogens, and 32% of the listed pathogens that infect pigs (WAHID 2010). This list includes the more devastating pig diseases that have impacted most other pork producing countries over the past 5 to 10 years, such as post-weaning multi-systemic wasting syndrome (PMWS) and porcine reproductive and respiratory syndrome (PRRS), both of which result in high mortality and reduced productivity (APL 2009). Australia is also free of foot-and-mouth disease and classical swine fever, which have a devastating effect on livestock industries in regard to mortality, production losses, animal slaughter for disease control and international meat export restrictions (APL 2009).

3.2 Biosecurity in Australian piggeries

Biosecurity is: "*The implementation of measures that reduce the risk of the introduction and spread of disease agents*" (FAO 2010, p 3). There are standard biosecurity practices utilised and recommended in the livestock industry to minimise pathogen introduction and spread. These practices are based on reducing the chance of pathogen introduction to farms while considering the modes of pathogen transmission. Pathogens can be transmitted

through either direct contact with an infected individual or through indirect contact by exposure to contaminated air, water, food, fomites, vectors and biological products (Frölich et al. 2002; Coetzer and Tustin 2004). APIQ✓™ outlines the recommended standards and procedures to follow to attain a high standard of management, food safety, traceability, animal welfare and biosecurity (APIQ 2010). The four protocols are:

1. On-farm systems are in place to minimise the risk of contamination or disease spread
2. The risk of disease introduction from new stock and semen is controlled
3. Staff are trained in emergency animal disease awareness and follow biosecurity procedures
4. Pest control procedures are in place.

Pig producer adherence to the APIQ✓™ guidelines is used by the Australian Quarantine Inspection Service to verify that pork products meet international market requirements. Similarly, a quality assurance program is required by the State Food Authorities for pigs destined to the domestic market (Hernandez-Jover et al. 2009). Without this quality assurance, pig producers are not permitted to sell their stock to these markets. Many small-scale pig producers tend to have a reduced level of quality assurance implementation on their piggeries and as such a lower biosecurity standard than large-scale commercial pig producers (Hernandez-Jover et al. 2011).

The main source of pathogen entry into Australian piggeries is through new or replacement stock, or semen for artificial insemination (Kyriazakis and Whittemore 2006). As such, it is recommended that new animals or semen are obtained from reliable sources such as breeding companies, which can demonstrate freedom from diseases (APIQ 2010). Any new stock that arrives on a piggery should, if possible, be quarantined away from the main herd for a recommended period of three weeks (Kyriazakis and Whittemore 2006; APIQ 2010).

Other modes of pathogen entry include vehicles, infected individuals, equipment, feed, water and wild animals (APIQ 2010; OIE 2010). Visitors to piggeries may be carrying pathogens on their vehicles, clothing or equipment. As such, to minimise the risk of contamination or pathogen spread on farm, clothing and footwear is generally provided to visitors, with showers before and after entry also recommended (APIQ 2010). Most

piggeries also require visitors to have a three day period of no contact with other pigs before entering their piggery. Equipment, food, water and ecoshelter bedding materials, such as straw, can all be contaminated with pathogens. The source of each product used must be identified and the cleanliness of vehicle and equipment maintained when transporting products to many different livestock enterprises (APIQ 2010). Piggery location in relation to other piggeries and farms is another factor to be considered due to the possibility of aerosol transmission.

Further pathogen entry can occur through wild animals coming into the vicinity of piggeries. The structure of pig housing facilities used in each of the three piggery types, previously explained, allows many different species of animals to enter buildings and come into contact with pigs and their feed or water sources. The biosecurity measures that must be implemented to meet the APIQ✓™ standards include prevention of feral pig access to domestic pigs, as well as pest control programs to prevent rodents and other pests accessing pigs, feed stores and bedding (APIQ 2010). Entry of pathogens via a feed and water source can be minimised through the use of fully enclosed feed silos to prevent contamination by wild animals, as well as perimeter fencing to restrict entry of larger animals. However these measures are not effective in preventing piggery access for many pest and native animals, which is demonstrated by the vast array of wild animals observed in and around piggery facilities by pig producers in Australia (Chapter 2).

3.3 Wildlife and pathogen transmission to humans and domestic animals

Wild animals have been associated with outbreaks of novel and transboundary pathogens around the world. Around 60% of the emerging infectious disease events recorded in literature between 1940 and 2004 in humans were caused by zoonotic pathogens, which are transmissible between animals and humans. Approximately 72% of these zoonotic emerging infectious pathogens were from a wild animal source (Jones et al. 2008).

Recent emerging infectious disease outbreaks in Australia affecting domestic animals include Hendra virus and Menangle virus. Hendra virus is transmitted from fruit bats (*Pteropus spp*.) to horses and on to humans and is a continuing problem (Breed et al. 2006; Playford et al. 2010). Menangle virus was transmitted from fruit bats to pigs at one piggery in 1997 (Philbey et al. 2008). Impacts of these viruses are described in Section 3.8.2. Based

on these disease outbreaks and others internationally, wild animal species, both introduced and native to Australia, pose a disease threat to domestic animals and humans in relation to introduction, maintenance and spread of emerging, exotic and endemic pathogens.

Australia has had few outbreaks of emerging infectious diseases and is well protected from transboundary animal diseases due to stringent quarantine procedures, which protect Australia's livestock industry. As such the main threat to human and domestic animal health in Australia posed by wildlife is transmission of infectious pathogens currently endemic in wild animal populations. Wild animals are reservoirs, hosts and definitive hosts of many endemic infections in Australia. The cat (*Felis catus*) is the definitive host of *Toxoplasma gondii*, and is essential to the lifecycle of the parasite (Elmore et al. 2010). Dogs, including *Canis lupus familiaris* and *Canis lupus dingo*, are definitive hosts for *Neospora caninum*, a parasite causing significant reproductive disease and abortion in cattle (King et al. 2010). Wild canids are also definitive hosts for *Echinococcus granulosus*, a tape worm causing hydatidosis in animals and humans in Australia and around the world (Jenkins 2006).

Pathogens can be transmitted in a number of different ways, including direct and indirect transmission methods. Biosecurity practices on piggeries are developed to minimise transmission and spread of pathogens via these routes. Chapter 2 of this thesis identified the wild animal species that are frequently observed on piggeries in Australia and, as such, may pose a risk of pathogen transmission to domestic pigs. Those wild animal species providing the greatest risk, due to the number of pig producers reporting an observation of the species and the frequency of observations, included the European starling (*Sturnus vulgarus*), rodents (black rat *Rattus rattus*; brown rat *Rattus norvegicus*; and the house mouse *Mus musculus*) and feral cats. The species-specific pathogen transmission potential from feral pigs (*Sus scrofa*) also presents a high risk.

3.4 Birds and pathogen transmission

Some wild birds have readily adapted to human urbanised settings, which has resulted in severe epidemiologic and economic consequences, and their subsequent classification as pests (Pimentel et al. 2001). The three greatest bird pests include the European starling, the house sparrow (*Passer domesticus*) and the common pigeon (*Columba livia*) (Pimentel et

al. 2001). Some of the most obvious problems associated with close interactions of these birds and urban environments are damage to infrastructure and losses to agricultural operations, particularly crops, orchards and intensive livestock production facilities (Bomford and Sinclair 2002; Lapidge et al. 2006). In addition to these detrimental impacts is the possibility of interaction between wild birds and domestic animals (as identified in Chapter 2).

Weber (1979) reported that many pathogens can be carried by birds, with a list of 40 pathogens being associated with starlings, sparrows and pigeons prior to 1979. This list of pathogens has since been expanded. Wild and pet birds have been associated with outbreaks of common and exotic diseases in humans, domestic animals and wildlife (Mackenzie 1988; Daszak et al. 2000; Gaukler et al. 2009). Thus, it is important to determine what pathogens may be carried by birds and have the potential to be transmitted to domestic animals. There are two well known and significant pathogens associated with wild birds that have had large health and economic consequences in birds, mammals and humans on an international scale. Highly pathogenic avian influenza, specifically H5N1, and West Nile virus, for which birds are the primary hosts, have been associated with epidemics in livestock and humans during the last decade.

Highly pathogenic avian influenza, H5N1, has had significant economic and health consequences associated with infections in poultry and zoonotic transmission to humans (Peiris et al. 2007), with a case fatality rate of 59% for human cases (WHO 2012a). Wild birds are considered reservoirs of low pathogenic avian influenza and can be infected with highly pathogenic avian influenza. The predominant wild bird reservoirs are water and shore birds with a migratory capacity (Rappole and Hubálek 2006). As such, the potential involvement of wild birds in the spread of this virus to humans and other animals has been identified. However, the respective contributions of wild birds and of legal and illegal movements and trade of domestic poultry and birds to the spread of avian influenza are still being investigated (Kilpatrick et al 2006; van den Berg 2009). Infection of pigs with avian influenza based on antibody detection has been previously confirmed in Asia (Choi et al. 2005). Experimental exposure of pigs has resulted in infection, but pig-to-pig transmission has not been detected (Choi et al. 2005). The main concern about infection of pigs with avian influenza viruses is the potential for re-assortment of the virus in a pig host, which could result in a pandemic disease (Peiris et al. 2007).

Avian influenza H5N1 has not become a worldwide disease in humans despite the infection of migratory birds. However, the consequences of the disease have been considerable, particularly in domestic poultry and humans. In Hong Kong in 1997 there were 18 human cases, 6 resulting in fatalities, and 1.5 million poultry were culled (Chen et al. 2004). Human cases of avian influenza H5N1 reported to the World Health Organisation (2012a) total 583 with 344 fatalities, from 2003 to 2012.

West Nile virus is also an important public health concern as evidenced by its introduction to the United States in 1999. The virus was introduced in New York, where the first bird, human and horse cases were detected, and it subsequently spread to other parts of the United States and to Canada. The outbreak has resulted in the death of thousands of domestic horses and more than 12,000 human cases, causing over 1,100 human fatalities from 1999 to 2010 (Murray et al. 2010; Hofmeister 2011). West Nile virus is transmitted to humans, birds and other mammals by mosquitoes, with birds being the main introductory and amplifying hosts (Rappole et al. 2000).

Large surveillance programs for West Nile virus, among other vector-borne pathogens, have incorporated testing of wild birds. In the New York district alone, 430 birds were caught at the beginning of the outbreak and 33% of birds were infected with West Nile virus (Komar et al. 2001). Large mortalities of wild birds occurred concurrently with human infections. The pathogen is now established endemically in the United States and will continue to require rising financial inputs to control the disease in humans and horses (vaccination for horses only) and for ongoing surveillance (Murray et al. 2010).

A more common endemic pathogen with zoonotic potential and a worldwide distribution associated with wild birds is *Salmonella spp*. The presence of *Salmonella spp*. in wild birds has been recognised for over a hundred years. However, its prevalence has been increasing over the past 40 years, with the most significant upsurge of *Salmonella spp*. infections in the Passerines (Tizard 2004). Not only has this pathogen rapidly spread among birds, but it has been transmitted from birds to other animals and humans. Instances of human infection have occurred from direct handling of birds, and contact with domesticated animals infected by birds (Cizek et al. 1994; Tauni and Österlund 2000; Alley et al. 2002).

3.5 European starlings

3.5.1 Origin and spread of starlings

The starling is a bird from the Sturnidae family in the order Passeriform. It has a natural origin which includes Europe, Asia and North Africa (Feare and Craig 1999). However, the starling population and distribution expanded through the introduction of this bird into other countries including Iceland, the United States, South Africa, New Zealand, Australia, New Habrides, Fikian Group and Tonga (Long 1981). Some countries introduced starlings as an insect pest control measure, while others did so for historical reasons. An example was in New York, where starlings were introduced by an Acclimatisation Society in order to have all birds mentioned in Shakespeare's plays present in the city (Feare 1984).

Three separate introductions of European starlings into Australia occurred in 1856, 1857 and 1858 for private collections (Long 1981). It is unknown whether the wild population was established at this point in time. Regardless, local Acclimatisation Societies were responsible for introductions into the wild of a large number of starlings in Victoria, Queensland, South Australia, New South Wales, and Phillip Island in the mid-19th century (Long 1981). Due to the highly adaptable nature of the starling, it was able to rapidly colonise and spread throughout the settled areas of those states (Feare 1984; Feare and Craig 1999). The starling was not introduced into Western Australia, and despite regular incursions from South Australia into this state, the population has not been able to successfully establish due to continuing eradication programs (Rollins et al. 2009).

3.5.2 Starlings as a pest to humans and agriculture

Starlings live in high density populations and communal roosts, with some roosts containing tens of thousands of individuals, or even over a million birds (Campbell and Lack 1985). They not only roost in dense populations but also tend to feed as large aggregations, sometimes with other bird species. This proves to be a significant burden to their chosen feeding locations, as well as providing the opportunity for pathogen transmission between the birds (Morishita et al. 1999). Starlings are selective, omnivorous eaters and tend to feed on the more valuable agricultural products, such as grains from poultry, cattle and pig feed, as well as cherries, apples, wheat, barley and grapes (Feare 1984; Tracey and Saunders 2003).

Large wintering populations of starlings are supported by feed at livestock operations in the United States (Weber 1979). Consumption of feed at these operations is substantial; one starling has been estimated to consume around 28.3 grams of animal feed per day (Besser et al. 1968). As well as feed consumption, feed contamination can result in additional negative impacts. A study in Scotland reported 25 bird droppings per metre squared of stored feed on four different cattle farms (Daniels et al. 2003). There are a significant number of organisms, some of which could be pathogenic for livestock that can be transferred from the bird excrement into the animal feed and water (Weber 1979).

Initial figures of the bird pest problem in relation to feedlots and piggeries in Australia were documented by Lapidge et al. (2006). Almost three quarters of respondents of a survey among 310 piggery producing members of Australian Pork Limited and 545 cattle feedlot producers in Australia reported negative impacts of birds on their enterprises. These perceived impacts included damage to infrastructure, crops and vegetation, fouling of feed and water, feed loss and increased pathogen spread.

3.5.3 Starlings and the biosecurity threat to piggeries

The very few overseas studies looking at pathogens in wild birds on piggeries, most of which did not consider starlings, have shown low prevalence levels for the pathogens included in the current study. In the United States, *Mycobacterium avium* was isolated from seven of 127 starlings on an infected piggery (Bickford et al. 1966). However, recent evidence has shown that *Mycobacterium avium* does not infect pigs via infected birds (Norwegian School of Veterinary Science 2010). Transmissible gastroenteritis virus was shown to be transmitted from starlings to pigs in experimental conditions (Pilchard 1965). More recently, *Salmonella spp*. were identified in wild bird faeces on a piggery in Illinois (Barber et al. 2002). In Europe, *Escherichia coli* was identified in a single starling on a piggery in Denmark (Nielsen et al. 2004), and in the same country *Salmonella spp*. were found in wild birds, including one starling (Skov et al. 2008).

Starlings have been identified as carriers of numerous pathogens on other livestock enterprises, such as cattle feedlots, dairy and poultry farms. *Salmonella spp*., *Escherichia coli* and *Mycobacterium avium* sub species *paratuberculosis* have been isolated from starlings on cattle feedlots and dairy farms in the United States (Kirk et al 2002; Corn et al.

2005; LeJeune et al. 2008; Gaulker et al. 2009; Carlson et al. 2011), and *Campylobacter jejuni* was identified in starlings on poultry farms in England (Colles et al. 2003).

As starlings are a particularly big problem on piggeries in Australia, as evidenced in Chapter 2 and the survey by Lapidge et al. (2006), and are known to carry zoonotic pathogens from studies documented above, they potentially pose a high risk for pathogen transmission to pigs. Of those pathogens that have been identified in wild birds and starlings, *Escherichia coli*, *Campylobacter spp*. and *Salmonella spp*. are the main bacterial pathogens isolated (Morishita et al. 1999; Benskin et al. 2009). These pathogens, particularly *Escherichia coli*, are considered to be endemic on piggeries, and in their pathogenic forms are responsible for causing illness, loss of condition and occasional mortality in pigs, with animal welfare and economic implications for piggeries.

As birds are potential vectors of pathogens to pigs in commercial piggeries, further knowledge about the pathogens carried by starlings present on piggeries would provide an informed assessment of the biosecurity risk posed by these birds. Such knowledge would benefit the pig industry and government agencies responsible for surveillance of animal and human health. It is essential that the pig industry has a solid understanding of the ability of wild birds to transfer pathogens of production-limiting diseases to better inform disease control and eradication procedures.

3.5.3.1 Escherichia coli

Escherichia coli are common bacteria of the intestinal flora. However, there are pathogenic strains which can cause illness when pigs are exposed. Pigs can be exposed via direct invasion of the respiratory tract or the anterior of the small intestine via the faecal-oral route to cause septicaemia in non-immune piglets (Taylor 2006).

Escherichia coli can cause diarrhoea in young pigs, including neonatal and post weaning pigs, as well as infections such as septicaemia in young pigs, and cystitis and mastitis in adult sows (Straw et al. 2006). Diarrhoea is one of the most common diseases of suckling and post-weaned pigs (Alexander 1994). In a study of preweaning disease in pig herds in the United States, diarrhoea resulted in the highest morbidity in piglets and accounted for 10.8% of preweaning mortality (Tubbs et al. 1993). *Escherichia coli* has been reported as

responsible for over 50% of all pig enteritis (intestinal inflammation often resulting in diarrhoea) in the United Kingdom (Taylor 2006).

Escherichia coli can also produce illness in young pigs, via attachment of the bacteria to mucous overlying the epithelium, or to brush borders of the mucosal epithelium due to the bacteria's specific adhesive antigens (Taylor 2006). *Escherichia coli* can also multiply in the small and large intestine, destroying epithelial cells and causing diarrhoea. Some *Escherichia coli* strains (Enterotoxigenic *Escherichia coli*) produce enterotoxins which affect cell membrane receptors, instigating the secretion of chloride ions in the lumen which is passively followed by water and sodium ions (Taylor 2006). This leads to diarrhoea and results in metabolic acidosis and dehydration and eventual circulatory collapse and death (Taylor 2006).

Genetic factors as well as physiological factors (such as stomach acidity, infection with other agents and gut flora changes) within piglets and adults can affect the multiplication of *Escherichia coli* bacteria and immune response effectiveness in individuals (Taylor 2006). Symptoms of neonatal septicaemia due to *Escherichia coli* include standing alone, drooping tails, unconsciousness, and are generally exhibited within 12 hours of birth, with mortality occurring within 48 hours (Straw et al. 2006). Antimicrobial treatments can be provided to individual pigs to treat the condition with varied success. Depopulation of piggeries and repopulation with new stock is the only way to eradicate large-scale herd infections.

3.5.3.2 Campylobacter jejuni

Campylobacter jejuni is another bacterial pathogen that mainly causes diarrhoea in piglets. Infection of piglets occurs via the faecal-oral route from infected faeces or contaminated water usually from 3 days to 3 weeks of age (Taylor 2006). In addition, *Campylobacter jejuni* is a public health concern as it is the main food-borne pathogen causing human bacterial enteritis in Australia (Blumer et al. 2003).

Symptoms in piglets usually include a high temperature lasting a few days, as well as watery or creamy diarrhoea that may contain mucous and streaks of blood (Taylor 2006). Loss of condition usually occurs but mortality is rare. While the prevalence of the serotype *Campylobacter coli* is similar to that of *Escherichia coli*, in that it may be present in 100%

of pigs (Taylor 2006), the level of the significant illness-causing serotype, *Campylobacter jejuni*, is much lower. The reported incidence of this bacterium in pig production facilities was 4.6% in Japan and 5% in Denmark (Taylor 2006).

3.5.3.3 Salmonella spp.

Salmonella spp. bacteria cause septicaemia and diarrhoea in pigs. *Salmonella spp*. are also a food-borne pathogen, being the second most likely food-borne pathogen causing enteritis in humans after *Campylobacter jejuni* (Todd 1995; Blumer et al. 2003). Pork meat is an important source of infection of both of these pathogens (Beran 1995).

Infection in pigs usually occurs via ingestion of the organism, but can also occur via the respiratory route (Straw et al. 2006). Three forms of *Salmonella spp*. infection have been described in pigs: septicaemia, acute enteric (enteritis with some local invasion) and chronic enteric (colonisation of intestinal mucosa) (Taylor 2006). The septicaemic form usually results in death within 24 to 48 hours of infection with animals appearing dull, weak and depressed prior to death (Taylor 2006). Animals with the acute enteric form have watery, yellowish diarrhoea and a fever (Taylor 2006). Pigs with the chronic form have persistent diarrhoea, intermittent fever and become emaciated (Taylor 2006).

3.5.4 Starling control

There are a number of methods available for the control of starling populations. The availability of these methods depends on the regulations and laws in the region in which the starling population is to be controlled. The available methods include: exclusion techniques; scaring; population reduction through shooting, trapping and poisoning; repellents and toxicants (Johnson and Glahn 1994; Bomford and Sinclair 2002).

Exclusion techniques include closing off all openings to food and buildings, and erecting netting to prevent roosting. All openings greater than 2.5cm need to be closed to increase effectiveness (Johnson and Glahn 1994). Additionally, for birds that are nesting or roosting at pig facilities, removal of nests and placement of wire mesh barriers or spikes on roosting surfaces may reduce their presence on piggeries. Frightening techniques such as loud noises, bright lights and recorded alarm calls have been reported to have little to no effect on birds (Bomford and Sinclair 2002).

Shooting has also been found to be largely ineffective as a means to control bird populations (Bomford and Sinclair 2002). Fleming (1990) found the use of shooting to control bird damage to be the "*most universally practiced and most ineffective bird control technique used in Australia*". Trapping can be equally ineffective against a large population, and is also not species specific, requiring frequent checking and maintenance to minimise non-target bird losses and animal cruelty. Additionally, the fate of translocated birds is unknown and hence the humane nature of this technique as an alternative to direct killing is unknown (Bomford and Sinclair 2002). Repellents do not cause pain and suffering to the birds; however, they do require constant maintenance and replacement. Repellents used in the United States are polybutenes which are soft and sticky and are applied to ledges (Johnson and Glahn 1994).

Toxicants for starlings are also available, with the avicide DRC-1339 (3-chloro-4-methylbenzenamine hydrochloride) poison being the most common toxicant used (Carlson et al. 2011b). The avicide DRC-1339 was effectively used in a cattle feedlot in Texas (US), to minimise the starling population (Carlson et al. 2011b). The reduction in the starling population reportedly had a corresponding reduction in the presence of *Salmonella enterica* in cattle feed and water (Carlson et al. 2011b). This toxicant is not registered for use in Australia.

Most of these methods could be used on livestock enterprises, such as piggeries, to minimise starling population size and corresponding impacts in Australia. However, a combination of techniques may need to be used to achieve a sufficient level of effectiveness for population control.

3.6 Rodents

Wild rat species and the house mouse are an economic and health concern in locations where food is readily accessible. Intensive farming practices, such as those of piggeries, provide an ideal environment for rodents to live and reproduce. Wherever rodents are found there are associated damages, such as destruction of infrastructure, financial losses through food consumption and contamination, and increased disease incidence, which results in livestock production losses due to reduced growth rates, reduced feed conversion efficiency and increased treatment costs.

It has been estimated that rats eat 10% of their body weight per day and produce a daily average of 50 droppings as well as millilitres of urine (Drummond 2001). In a study of rodent faecal contamination in stored animal feed in Scotland, there was an average of 80 rodent droppings per metre squared of feed (Daniels et al. 2003). This contamination of food and the environment is of concern due to the presence of disease-causing organisms in rodent excrement (Twigg 1975).

3.6.1 Origin and spread of rodents

The rodent species that have become worldwide pests of human environments are just three of approximately 1,500 different known rodent species (Alderton 1996): the house mouse (*Mus musculus)*, the black rat (*Rattus rattus*) and the brown rat (*Rattus norvegicus*). All three of these rodent species originated from Asia: the house mouse originating in the north of the continent in the dry steppelands of Russia, and the two rats originating in south eastern Asia, in the vicinity of China (Twigg 1975; Alderton 1996). Human activities, such as improvements to agriculture and transportation, have enabled the ranges of these rodents to expand to all continents of the world, even including Antarctica for the house mouse (Alderton 1996). The house mouse started its expansion outside of Asia many thousands of years before the rats, reaching the United Kingdom by 1200 B.C. (Alderton 1996). The black rat reached the United Kingdom in the late 12th century A.D., followed by the brown rat around 1720 A.D. (Twigg 1975). The spread of the black rat has been linked to bubonic plague outbreaks throughout history around the world.

The house mouse and the black rat are believed to have arrived in Australia with infested settlement ships in 1788 (Caughley et al. 1998). The brown rat is also present in Australia, although its time of arrival is not as clear. It could have arrived with the house mouse and black rat with the first settlers, or on subsequent settlement or trading ships (Twigg 1975).

3.6.2 Rodents as a pest to humans and agriculture

"The rate of propagation of field mice in country places, and the destruction they cause, is beyond all telling." Aristotle (384–322 BC).

Since their spread, these three rodents have been a significant pest to humans, and the increase in their populations with expansion of agricultural production has added to their

impact. There is a significant economic toll on human populations due to damage to crops and stored food, buildings, insulation, electrical wiring, water plumbing, irrigation lines and undermining foundations. The costs associated with control and disease transmission are also significant (Singleton et al. 1999).

In recent times, a large mouse plague in Australia during 1993 cost an estimated AU $100 million in production losses and infrastructural damage in South Australia alone (CSIRO 1997). This plague had an impact on all agricultural industries. In plague-affected areas feed costs for piggeries increased by approximately 50% and production indicators such as litter size, conception rates and growth rates all decreased, in some of the worst cases by up to 50%, reportedly due to stress from constant harassment and physical injury (Caughley et al. 1998). These impacts were also accompanied by the possibility of pathogen transmission (CSIRO 1997).

3.6.3 Rodents and pathogen transmission

Rats and mice have been shown to be reservoirs of a large number of pathogens and parasites (Le Moine et al. 1987; Amass 1999; Henderson 2009). The most well known disease carried by the black rat is the bubonic plague. The black rat enabled and perpetuated the spread of fleas and transmission of this disease (causative bacteria *Yersinia pestis*) from fleas to millions of people throughout the world, causing, for example, approximately 120 million deaths worldwide between 1900 and 1903 A.D. (Alderton 1996). A proposed additional vector of the plague was body lice, allowing human to human transmission (McLean and Fall 2010). Cases of the plague in humans involving fatalities are still occurring at the present time, with recent occurrences in Peru in 2010, China in 2009 and the Democratic Republic of the Congo in 2005 and 2006 (WHO 2012b).

Rodent species are a potential vehicle for transmission of pathogens to and between domestic animals and humans. Some of the most important zoonotic pathogens that have been found in rodents include bacteria, such as *Leptospira spp*., *Yersinia spp*., *Listeria spp*., *Pasturella spp*. and *Salmonella spp*.; viruses, such as Hantavirus; and protozoa, such as *Cryptosporidium parvum* and *Toxoplasma gondii* (Twigg 1975; Alderton 1996; Singleton et al. 1999).

3.6.4 Rodents and the biosecurity threat to piggeries

The biosecurity threat to Australian piggeries posed by rodents for the introduction, maintenance, and reintroduction of a number of pathogens has not been confirmed. However, the carrier status of rodents for many pathogens that can infect pigs has been well documented. A review of biosecurity considerations for pork producing units by Amass and Clark (1999) documents some of the pathogens that have been isolated from rodents captured on piggeries. These pathogens include the following: *Bordetella bronchiseptica*, *Salmonella spp*., *Escherichia coli*, *Brachyspira hyodysenteriae*, *Toxoplasma gondii*, *Leptospira spp*., rotavirus, and encephalomyocarditis virus. Two other pathogens which were not included in the review by Amass and Clark (1999), but have been isolated from rodents, include *Trichinella spiralis* (Leiby et al. 1990) and *Lawsonia intracellularis* (Friedman et al. 2008).

Rats are often passive carriers of pathogenic bacteria. They are able to harbour bacteria, such as *Leptospira spp*., and excrete it into the environment without having any clinical signs (Twigg 1975). Rats also seem to experience less severe clinical disease when infected with some gastrointestinal pathogens (such as *Lawsonia intracellularis*) compared to pigs. Collins et al. (2011) challenged rats with *Lawsonia intracellularis*, resulting in infection and faecal shedding, but the infected rats did not develop diarrhoea. Only one rat developed lesions in the intestine typical of proliferative enteropathy. The same strain and dose of this bacteria resulted in clinical signs such as diarrhoea and extensive proliferative enteropathy lesions in the intestines of pigs. Pathogens of importance to the pig industry in Australia due to their extensive negative health and economic impacts that have been isolated from rats include *Brachyspira hyodysenteriae*, *Lawsonia intracellularis* and *Salmonella spp*. *Brachyspira pilosicoli* has not been previously isolated from rodents. However, given this pathogen is related to *Brachyspira hyodysenteriae* and has similar, though less severe implications, its presence in rat populations on piggeries is also important to ascertain.

3.6.4.1 Brachyspira hyodysenteriae

Swine dysentery is an acute or chronic diarrhoea disease caused by the bacteria *Brachyspira hyodysenteriae* (Straw et al. 2006). Swine dysentery was present on 33% of 106 Western Australian piggeries sampled in a serological survey between 1988 and 1990, with a herd prevalence ranging from 2.5 to 47.5% (Mhoma et al. 1992). Swine dysentery

can have a severely detrimental effect on enterprise profitability due to costs associated with pig deaths, reduced growth rate and diminished feed conversion efficiency. In addition, the costs of disease control through in-feed medication, or eradication by disinfection and total depopulation or partial Swiss-depopulation of pig facilities, are also significant (Hampson 1997; Straw et al. 2006).

Swine dysentery significantly reduces the profit margin on finishing pigs. While there have been no recent evaluations of the cost, Polson et al. (1992) showed the profit margin for 100 kg live weight was close to US $7.44 for pigs on a swine dysentery-free piggery, compared to US $1.67 on a swine dysentery-infected piggery. In Australia, swine dysentery is still generally considered to be one of the most costly endemic diseases of pig production.

Infection with this bacterium in pigs occurs via the oral route, through ingestion of faeces from affected animals (Jackson and Cockcroft 2007). Invasion of the mucosa and crypts of the large intestine occurs within two hours of exposure. Multiplication of the bacteria ensues, followed by invasion of the goblet and epithelial cells, resulting in damage or disruption to these cells (Taylor 2006). The incubation period of the disease is between 7 and 60 days, and faecal shedding continues for a period of around 90 days after clinical recovery (Taylor 2006).

Research has shown that experimentally inoculated mice shed *Brachyspira hyodysenteriae* in their faeces for over 180 days after initial infection (Joens 1980). This indicates that mice on infected piggeries could be a reservoir for swine dysentery, and as such impede control and eradication of this disease in the pig population. Rats should also be considered as a possible source of re-infection of destocked facilities due to their wider movement range and their potential carrier status (Joens et al. 1982; Hampson et al. 1991). The involvement of rodents in the transmission of this pathogen in pigs needs to be better understood to gauge their possible role in infection and re-infection of piggeries.

3.6.4.2 Lawsonia intracellularis

Lawsonia intracellularis causes intestinal haemorrhage and the wasting disease proliferative enteropathy in pigs (Lawson and Gebhart 2000). Holyoake et al. (2010b) reported that 100% of a sample of 63 finisher piggeries in Australia had antibodies against

Lawsonia intracellularis, with a within-herd prevalence ranging from 31.3 to 100%. The cost of a subclinical infection of *Lawsonia intracellularis* on net revenue was estimated to be AU $8.33 per pig, while a clinical infection reduced net revenue by AU $13.00 per pig (Holyoake et al. 2010a).

Infection with *Lawsonia intracellularis* also occurs via the faecal-oral route (Jackson and Cockcroft 2007). The organism invades the epithelial cells of the crypts of the small intestine and causes increased cell division, resulting in abnormal proliferation of cells and reduced absorption of feed (Taylor 2006). The incubation period of the disease is between 3 to 6 weeks, and clinical disease lasts for about 6 weeks (Taylor 2006). Disease is most common in recently weaned pigs, leading to weight loss or failure to gain weight, vomiting, and distinctive faeces which may be black or similar to the colour and consistency of wet cement (Straw et al. 2006). *Lawsonia intracellularis* has been found in mice and rats trapped on commercial piggeries (Friedman et al. 2008). Experimentally inoculated laboratory rats and mice shed the bacteria in their faeces for two weeks (Collins et al. 2011).

3.6.4.3 Salmonella spp.

Salmonella spp. has previously been discussed in the context of European starlings in Section 3.5.3.3. Previous studies reported that rodents on different livestock enterprises were infected with *Salmonella spp*. Skov et al. (2008) found infected rodents on *Salmonella spp*. infected cattle and pig farms in Denmark. Similarly, Letellier et al. (1999) and Meerburg et al. (2006) reported infected rodents on a piggery in Québec and on an organic piggery in the Netherlands, respectively. One of the most commonly isolated *Salmonella spp*. serotypes in the world from humans, domestic animals and wild animals is *Salmonella typhimurium* (Davis 1948; Jones and Twigg 1976; Le Moine et al. 1987). This serotype alone was associated with 36.2 food-borne disease outbreaks per 100,000 people in 2001 in Australia (Page 18; Blumer et al. 2003). *Salmonella spp*. in general were responsible for between 33.2 and 90.7 salmonellosis disease outbreaks per 100,000 people in 2011 in Australia (DHA 2012c).

3.6.4.4 Brachyspira pilosicoli

Infection of pigs with *Brachyspira pilosicoli* can result in diarrhoea, intermittent dysentery and reduced weight gain (Taylor 2006). The prevalence of this pathogen on two Western

Australian piggeries was estimated to be 2.4% on the first piggery and 12.2% on the second (Oxberry and Hampson 2003). Infection of the host occurs via the oral route, as for *Brachyspira hyodysenteriae* (Taylor 2006). Organisms densely colonise the colonic mucosa in the large intestine, resulting in reduction and failure of absorption (Taylor 2006). The organism infects a number of different hosts apart from the pig, including humans, dogs and birds (Oxberry et al. 1998). *Brachyspira pilosicoli* has not been isolated from rodents.

3.6.5 Rodent control

Methods available to control rodent pests are similar to those used for birds and have varying effectiveness. The two most common methods for rodent control on piggeries include poisoning and trapping (Chapter 2). Other methods include shooting and habitat modification.

Poisoning through the use of anticoagulant rodenticides is the control method recommended for use in piggeries (CSIRO 1997). Pre-feeding should be used prior to baiting to encourage rodents to use a particular feeding site. Leung and Clark (2005) recommend the placement of baits within 17 metres of each other to account for the minimum home range of rats in the studied population, and to place baits inside the piggery building to reduce non-target species bait consumption.

Live traps, which capture rats alive and without injury, and mortality-causing traps are available (CSIRO 1997). A pre-feeding phase is recommended for traps as for baiting, to habituate rodents to the traps' presence, increasing effectiveness. Shooting is not as effective on a large-scale as poisoning and trapping. Habitat modification, such as reducing ground cover vegetation around sheds or paddock, rodent-proofing feed and water sources and removal of spilt grain and standing water, should be implemented along with any other techniques for active rodent population reduction (Cowan et al. 2002).

The best techniques for rodent control involve integration of a number of the previously mentioned control methods (Singleton et al. 2002). Continual maintenance and evaluation of effectiveness are necessary, through the monitoring of rodent numbers, bait uptake and trapping numbers. Reinvasion of rodents from surrounding areas can occur, so rodent management must be integrated with neighbouring agricultural enterprises (CSIRO 1997).

3.7 Feral pigs

Feral pigs (*Sus scrofa*) are the wild ancestors of many different breeds of domesticated pigs (Auty 2003). They adapt to and live in many different climatic conditions, with the result that they are one of the most widely distributed mammals in the world (Massei and Genov 2004). Being a large omnivorous mammal, feral pigs cause damage to natural ecosystems and extensive damage to agricultural cropping and grazing lands, with associated damage to infrastructure (Choquenot et al.1996; DEH 2005).

Additional to these impacts, the pathogens that feral pigs may harbour and transmit are also a cause for concern. Feral pigs and their relatives are reservoirs for significant pig diseases, such as classical swine fever and African swine fever; diseases of cloven-hoof animals, such as foot-and-mouth disease; as well as reservoirs of zoonotic diseases, such as brucellosis and leptospirosis. In Australia, feral pigs are known reservoirs of *Brucella spp*. and *Leptospira spp*., transmissible to livestock and humans. To date, feral pigs have also been identified as reservoirs of 21 bacterial species and six viral pathogens in Australia (Henderson 2009).

3.7.1 Origin and spread of feral pigs

Feral pigs have been introduced to many continents of the world through human exploration and colonisation. In Australia, the time period when pigs established in sufficient numbers to produce a self-maintained population of feral animals is not known, though it is assumed that feral pig numbers would have begun growing after the arrival of the first fleet when pigs were introduced as a source of food (Rolls 1969). The feral pig population was estimated to be 13.5 million (95% confidence interval 3.5 to 23.5 million) in the late 1980s (Hone 1990), with a present inhabitation of approximately 45% of Australia, including Queensland, the Northern Territory, New South Wales, and the Australian Capital Territory (West 2008). Isolated populations occur in Victoria, Kangaroo Island in South Australia, Western Australia, and Tasmania (Wilson 1992; West 2008). The feral pig population in regions of abundant water and food can reach levels as high as 20 pigs per square kilometre (Dexter 1990; Choquenot et al. 1996).

3.7.2 Feral pigs as a pest to humans and agriculture

The sayings "*eats like a pig*" or "*as greedy as a pig*" are due to the eating and foraging behaviour of pigs (Seward et al. 2004). They are omnivorous generalists that are constantly foraging for food due to their stomachs' relative inefficiency at digestion compared to ruminants (Seward et al. 2004). Their habitat often overlaps with agricultural enterprises, and as a result, crops and livestock are included in their diet (Massei and Genov 2004).

Feral pigs damage crops, including grain, sugar cane and tropical fruits, and reduce their yields; predate livestock, mainly lambs; damage fences and destroy dams (Benson 1980; Caley 1993a; Choquenot et al. 1996). Their impact on agriculture alone in New South Wales and Queensland has been estimated to be around AU $100 million annually (DEH 2005). Their agricultural impacts are not the only source of damage. Feral pigs are also considered an important threat to biodiversity in Australia due to adverse impacts on the survival of at least 18 nationally listed threatened species, including mammals, frogs, birds, fish, turtles and plants (Braysher and Moore 2003; DEH 2005). Feral pigs are also a source of disease in many parts of the world, including Australia.

3.7.3 Feral pigs and pathogen transmission

Pathogens carried by pigs have been a source of infection for humans and livestock for centuries. There is historical evidence from 1918 of an influenza-like illness in pigs at the same time as the Spanish flu pandemic in humans (Easterday 2003; Weingartl et al. 2009), and pigs were considered a possible source of this infection. Feral pigs specifically have been deemed responsible for pathogen infection in humans in Australia. The nationally notifiable disease Brucellosis has been isolated from humans in all states and territories of Australia, with the greatest frequency occurring in Queensland (DHA 2012a). The number of human cases per year ranged from 16 to 54 between 1991 and 2011 (DHA 2012a). Of the 32 human cases of Brucellosis retrospectively studied by Eales et al. (2010) and reported between 1996 and 2009 in Townsville, 30 had been feral pig hunting. Feral pig hunting is the greatest risk for Brucellosis infection in Queensland (Eales et al. 2010).

Some highly contagious and financially damaging disease outbreaks in livestock have been spread by feral pigs. Some examples are classical swine fever, African swine fever and pseudorabies virus. Feral pigs were determined to be responsible for 59% of the primary domestic pig classical swine fever outbreaks in Germany between 1993 and 1998

(Fritzemeier et al. 2000). The consequences of classical swine fever epidemics on the pork industry are extreme, with an outbreak in the Netherlands in 1997/1998 costing an estimated US $2.3 billion (Meuwissen et al. 1999). Costs included in this figure related to the depopulation and slaughter of pigs; lost opportunity costs due to idle time prior to piggery repopulation; and related industry financial losses, such as slaughter houses, feed suppliers and replacement pig breeders. The disease results in haemorrhagic fever with a mortality rate up to 90% when infected with high virulence strains (Artois et al. 2002; Penrith et al. 2011).

African swine fever was identified as a novel disease, distinct from classical swine fever, in Kenya in 1928 (Penrith and Vosloo 2009). It is highly contagious in pigs and results in mortality up to 100% in naïve domestic pigs (Penrith 2009). The disease is also very costly, with an outbreak in Lagos Nigeria in 1998 costing farmers US $8.4 million in just six months (Babalobi et al. 2007). Contact between domestic pigs, warthogs (*Phacochoerus spp*.) and bush pigs has been identified as the key factor in infection and spread of the disease in southern and eastern Africa (Penrith and Vosloo 2009). The sylvatic cycle of the virus between warthogs and argasid ticks (O*rnithodoros moubata* complex) allows for the transmission of the virus to domestic pigs from time-to-time. An outbreak in Brazil between 1978 and 1984 cost an estimated US $14.5 million, including direct and indirect costs of the disease and eradication, as well as compensation to farmers for the cull of 67,000 domestic pigs (Lyra 2006).

Pseudorabies virus causes almost 100% total mortality in infected newborn piglets, 40 to 60% mortality in four week old pigs and up to 15% mortality in one to five month old pigs (Taylor 2006). Infection in adult pigs is often exhibited through abortions, stillbirths and decreased growth and production (Taylor 2006). These major impacts have led to eradication programs for this virus in domestic pigs in many countries, including the United Kingdom and the United States. Eradication was successful in both of these countries; however, isolated outbreaks in the United States have identified feral pigs as a source of residual infection and transmission to domestic pigs (Hahn et al. 2010). In various regions of the United States, pseudorabies has been detected in feral pigs at prevalence levels of 8 to 38% (Pirtle et al. 1989; Corn et al. 2004; Wyckoff et al. 2009). Infection of feral and domestic pigs has been determined to occur mainly by direct

oronasal and venereal contact (Romero et al. 2001; Hahn et al. 2010), and less commonly via cannibalism of infected carcasses (Hahn et al. 1997).

While Australia remains free of these devastating livestock diseases, there is ongoing substantial investment in biosecurity and emergency animal disease surveillance and response at pre-border, border and post-border points, due to the severe consequences of incursions for our livestock industries. An outbreak of foot-and-mouth disease in Australia lasting just 3 months has been estimated to cost AU $5.5 billion for the livestock industries and a loss of AU $8 billion in gross domestic product (Productivity Commission 2002). These figures primarily consist of the cost of control and eradication for the government and livestock industries, as well as a loss of revenue due to a reduction in domestic and export sales. The greatest revenue loss would be for the beef industry, followed by the pig industry. In the event of a foot-and-mouth disease incursion in Australia, feral pigs would likely become one of the main reservoir hosts and constitute the greatest wild animal threat to foot-and-mouth disease control and eradication (Productivity Commission 2002).

Henderson (2009) summarised the list of pathogens isolated from feral pigs in Australia, which includes 21 different pathogenic species of bacteria (Bensink et al. 1991; Pavlov et al. 1992; Godfroid 2002; Phillips et al. 2009), six viruses (Pavlov et al. 1992; Choquenot et al. 1996; Johansen et al. 2005), three parasites, nine worms and three protozoa.

3.7.4 Feral pigs and the biosecurity threat to piggeries

Due to the species-specific nature of several infectious diseases affecting pigs, domestic pigs are at greater risk of contracting diseases from feral pigs than other animal species. Outdoor pig production systems are at particularly high risk of pathogen transmission from feral pigs due to the opportunity for direct contact to occur (FAO 2010). Prevention of contact requires fencing with a double fence, a practice not routinely implemented and not always possible due to cost or geographic factors (FAO 2010). Additional to the risk of direct contact, is the possibility of domestic pigs contacting surface waters or streams that are not secured from access by wild animals (FAO 2010). The biosecurity standards defined by the Australian pork industry quality assurance program require piggeries to have a system to prevent feral pigs contacting domestic pigs, using well-fenced paddocks. However, the standards do not mention the need to restrict domestic pig access to a surface water runoff source from outside their paddocks (APIQ 2010). Further, there is no

description of the most effective fence or fencing systems. Fences that physically prevent feral pig movement have been proven to be ineffective over the long term due to damage and poor maintenance (Choquenot 1996). Additionally, electric wires on fences are not 100% pig-proof (Reidy et al. 2008). Free-range and indoor piggeries are also both susceptible to aerosol transmission of pathogens.

Surveillance testing in feral pigs for pathogens in Australia in the past focused on zoonotic pathogens. The exception to this is a recent study by Phillips et al. (2009), which determined the presence of the endemic production-limiting pathogens *Brachyspira hyodysenteriae*, *Lawsonia intracellularis* and *Brachyspira pilosicoli* in feral pigs in Western Australia. Although these pathogens can infect some other mammalian species, the health and financial consequences of diseases caused are most felt by the domestic pig industry.

The two most common bacteria looked at and isolated from feral pigs in Australia are *Brucella spp*. and *Leptospira spp*. (Choquenot et al. 1996). The zoonotic implications and the major consequences for livestock of these pathogens might explain why most of the studies in feral pigs have focused on these pathogens. In addition, these pathogens are subject to control strategies in livestock, such as quarantine and for *Leptospira spp*., vaccination programs. As the focus of pathogen surveys of feral pigs in Australia has been on detection of pathogens transmissible and harmful to multiple species, some of the most important pathogens that pose a risk of transmission to domestic pigs, and have the most significant impacts on the pig industry, have been neglected.

The two pneumonia-causing bacteria, *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*, are capable of being transmitted via the air. They have not been investigated in feral pigs in Australia. These pathogens have been isolated from wild pigs in Slovenia (Vengust et al. 2006), while *Mycoplasma hyopneumoniae* has also been isolated from wild pigs in the United States (Baker et al. 2011). However, neither study specifically selected feral pigs in close proximity to piggeries.

3.7.4.1 Mycoplasma hyopneumoniae

Enzootic pneumonia is an economically important respiratory disease caused by infection with the bacteria *Mycoplasma hyopneumoniae*, resulting in high morbidity and an adverse

impact on food conversion efficiency and weight gain (Jackson and Cockcroft 2007). Enzootic pneumonia was present in 86.5% of Australian piggeries prior to 2001 and was determined to be a disease eradicable from pig herds (Cutler 2001). Chronic infection, the most common form of the disease, causes fever, dry barking cough and diarrhoea in all age groups, lasting for about 50 days (Taylor 2006). Growth rate and feed conversion ratio can be reduced by as much as 15.9% and 13.8%, respectively, in 5 to 85 kilogram pigs (Taylor 2006). In the less common acute form, clinical symptoms include anorexia, fever and coughing, and may result in up to 50% of piglet deaths and reduced reproductive performance in breeding stock (Taylor 2006).

Infection with *Mycoplasma hyopneumoniae* occurs through the inhalation of infected aerosols or direct contact with infected pigs, and attachment of the pathogen to the ciliated cells of the trachea, bronchi and bronchioles (Jackson and Cockcroft 2007). The bacteria compromises epithelial function by causing cilia to clump together, which are subsequently shed, resulting in impaired clearance of normal secretory products from the respiratory tract (Taylor 2006).

As previously mentioned, feral pig infection with *Mycoplasma hyopneumoniae* has not been studied in Australia, with only some studies conducted overseas. The prevalence of this pathogen in the feral pig population in Slovenia (Vengust et al. 2006) and the United States (Baker et al. 2011) was estimated to be 21% and 32%, respectively. An accurate determination of the presence of this pathogen in feral pigs in the vicinity of piggeries in Australia is essential to determine the risk of transmission to domestic pigs. This pathogen may be transmitted in aerosols over a distance of 5 km (Cutler 2001; Dee et al. 2009); thus, infection of isolated piggeries may be the result of transmission from feral pigs. Determining this risk will identify the need for management procedures to reduce the likelihood of infected aerosols entering the piggery building. Some measures that would reduce this likelihood would be enclosed buildings with temperature control and ventilation through purification systems, though this may not be economically feasible, as well as feral pig population control in proximity to piggeries.

3.7.4.2 Actinobacillus pleuropneumoniae

Pleuropneumonia is caused by the bacteria *Actinobacillus pleuropneumoniae*. The clinical disease may be peracute, acute or chronic in pigs (Straw et al. 2006). Death in the peracute

form of the disease normally occurs within 24 to 36 hours after the onset of the symptoms. The common symptoms in this form are fever, apathy, anorexia, diarrhoea, vomiting and bluish tinge of the extremities such as the ears, nose and feet (Straw et al. 2006). The acute form also results in fever, depression, apathy and skin reddening, as well as respiratory symptoms such as coughing. Chronic disease develops in animals surviving the acute form, and is exhibited as coughing, reduced appetite, exercise intolerance and hairy appearance (Taylor 2006; Straw et al. 2006).

Prior to 2001, pleuropneumonia was estimated to be present at a similar herd prevalence in Australian piggeries (approximately 85%) to that of enzootic pneumonia (Cutler 2001). When *Actinobacillus pleuropneumoniae* serovar 1 is present in a piggery, the subsequent problems with growth performance and mortality cost were estimated to be in excess of AU $100/sow/year (Cutler and Gardner 1988). Mortality rates as a result of acute disease are variable but may reach up to 50% of affected pigs (Taylor 2006).

Infection occurs via the same routes as *Mycoplasma hyopneumoniae*, though the pathogen colonises tonsillar epithelium and adheres to alveolar epithelium (Straw et al. 2006). The *Actinobacillus pleuropneumoniae* bacteria produce toxins to reduce the host immunological response against the pathogen; as a consequence, these toxins exert cytotoxic effects on different cell types, resulting in many of the symptoms of disease (Straw et al. 2006). Alveolar walls also become damaged due to capillary congestion, and bacteria can enter the blood and colonise other sites in the body, such as joints (Taylor 2006).

Actinobacillus pleuropneumoniae has only been investigated in feral pigs in a single study in Slovenia, where a prevalence of 52% was reported for the 178 wild pigs shot and tested throughout the country (Vengust et al. 2006). Further investigation in feral pigs is crucial to determine the presence of this pathogen among feral pigs and estimate the level or infection in feral pig populations in proximity to piggeries. As *Actinobacillus pleuropneumoniae* can also be transmitted through aerosols over a distance anecdotally estimated at approximately 500 m (Cutler 2001), infected feral pigs pose a potential risk of transmission of the pathogen to piggeries. This has great implications on feral pig control in the vicinity of piggeries.

3.7.4.3. Brucella suis

Brucella suis infection in the majority of pigs does not present with recognisable symptoms; the main indicator of infection is infertility, abortions and stillbirths (Straw et al. 2006). Any symptoms, if exhibited, include lameness due to swollen tendons, joints and bursa and paralysis (Nielsen and Duncan 1990). Infected animals can remain infected and infectious for up to 64 months and boars can be infected for life (Taylor 2006). *Brucella suis* is considered rare in domestic pigs and has been maintained at very low levels in domestic pig herds in Australia by depopulating entire herds when infection has been identified (Nielsen and Duncan 1990). Past surveys among commercial pigs reported 3 positive results from 4,814 domestic pigs in Queensland in the early 1980s (QDPI 1983), and 15 positive results from 20,000 pigs sampled in Queensland between 1991 and 1996, 14 of which were negative when re-tested (AHSQ 1997). At the present time, there have been no cases of *Brucella suis* in domestic pigs reported in any state of Australia with the exception of Queensland, where a free-range piggery was found to have positive cases of the pathogen towards the end of 2010 (AHA 2010).

Infection with *Brucella suis* occurs by direct contact via the oral or venereal route, resulting in direct infection of the lymphatic system (Taylor 2006). The bacteria reproduce in lymph nodes for 1 to 7 weeks, and remain in lymph nodes, bone marrow, joints and the genital tract (Taylor 2006).

Brucella suis has been detected in feral pigs in international studies as well as in Australia. Studies in Queensland detected the pathogen in feral pigs, with a prevalence of 8.8% in Cape York (Pavlov 1988), and from 1.9% to 4.1% in Central Queensland (Pavlov 1991; Mason and Fleming 1999). *Brucella suis* has also been detected in the Northern Territory (Pavlov 1992). In addition, feral pigs have been deemed responsible for infection of humans with *Brucella suis* in Australia: refer to Section 3.7.3.

3.7.4.4. Leptospira spp.

Leptospira spp. can cause leptospirosis in acute or chronic forms in pigs of all ages. Acute leptospirosis exhibits as listlessness, loss of appetite, irritability, fever, red eyes, convulsions, and sometimes diarrhoea and jaundice (Faine 1994). Abortions, still birth and neonatal disease are also common symptoms of *Leptospira spp*. infection (Straw et al. 2006). Infected animals can continue to harbour, grow and excrete leptospires in urine and

genital fluids from days to years after infection (Faine 1994). In Queensland from 1972 to 1976, the proportion of pig herds infected with *Leptospira pomona* was 29.8%, with 18.5% of all pigs tested found to be positive (Elder and Ward 1978). A vaccine has since been developed against *Leptospira pomona* and is now incorporated into general practice on piggeries. The national pig herd prevalence was last estimated (in the late 1990s) to be less than 6% (Cutler 2001).

Infection with *Leptospira spp*. occurs via abrasions or breaches of the skin, the mucous membrane of the eye, nose and mouth, the vaginal route and inhalation of aerosols from urine. Vertical transmission is also possible (Faine 1994; Straw et al. 2006). Direct infection of the blood stream or lymphatics occurs via these routes of entry. The incubation period for horizontal infection is between 3 and 7 days (Faine 1994).

Leptospira spp. has been detected in feral pigs in Australia and around the world. It is the most common bacterial pathogen isolated from feral pigs in Australia (Choquenot et al. 1996). Previous studies from the 1970s to the present time have reported a wide range in the prevalence of this pathogen among the feral pig populations in different states of Australia. *Leptospira pomona* and *tarassovi* has been isolated in feral pigs in NSW with a prevalence reaching as high as 51% (Giles 1980; Mason et al. 1998). In the 1970s, *Leptospira* serovars pomona, tarassovi and hardjo were isolated from feral pigs in Western Australia with a prevalence of 2 to 9% (Masters 1979). In the 1980s, *Leptospira spp*. were also isolated from feral pigs in Northern Queensland and Cape York at a prevalence of 4 to 22% (Pavlov 1988; Pavlov et al. 1992). More recently, of two feral pig populations culled in South Australia in 2003, while one was negative, in the other population 70% of individuals were positive for *Leptospira pomona* (AHA 2003). Recent seropositive results were obtained from feral pigs in the Kimberly region in Western Australia, at a prevalence of 5.7% (95% confidence interval: 2.6 to 11.9%) (Personal communication, Cowled 2012).

Elder and Ward (1978) compiled the serological test results on *Leptospira spp*. from serum obtained from cattle and pigs in Queensland from 1972 to 1976. During this study, feral pigs captured on 43 of the 359 pig enterprises were also tested, and 31.2% of the animals were positive. Pig production has undergone significant changes since the time of this survey, when pig herds (averaging 12.4 sows per herd) were commonly a sideline for dairy enterprises and pigs were mainly kept in free-range style housing (Richardson and

O'Connor 1978). By 2007, the average herd size was 180 sows per herd (Australian Pork Limited 2008), with most herds housed indoors, reducing the likelihood of feral pig access. The introduction of the vaccination for domestic pigs against *Leptospira pomona* may have also resulted in a corresponding change in the prevalence in wild animals. An updated assessment of the presence and prevalence of *Leptospira spp*. in feral pigs near piggeries is necessary to estimate the risk of transmission of this pathogen to domestic pigs.

3.7.4.5 Lawsonia intracellularis

A description of this pathogen can be found in Section 3.6.4.2 on rodents. *Lawsonia intracellularis* has been isolated from free ranging wild pigs in the Czech Republic, Sweden, Germany and Australia (Tomanová et al. 2002; Jacobson et al. 2005; Phillips et al. 2009). However, all of these studies obtained samples from hunter-harvested pigs, without targeting locations in proximity to piggeries.

3.7.5 Feral pig control

There are a number of common techniques available to landholders for control of feral pigs. They involve both lethal and non-lethal practices, with the most common methods being shooting, poisoning, fencing and trapping (followed by euthanasia) (Choquenot et al. 1996). These control measures are used equally by pig producers to manage feral pigs around their piggeries (Chapter 2).

Shooting of feral pigs on a large-scale is conducted from the air, and is mainly cost effective in open habitats with high pig density over a number of connecting properties (Choquenot et al. 1996). Shooting from the ground is largely ineffective if implemented as a means of damage control unless it is intensively conducted on a small population. For all shooting control methods, time and cost increases when the number of individuals to be managed decreases.

Poisoning with sodium monofluoroacetate (compound 1080) can be effective in a range of feral pig densities, and is particularly effective in remote areas when alternative food options are scarce (Choquenot and Lukins 1996). As such, 1080 poisoning in these areas is considered more cost effective than shooting or trapping (Dall 2010). However, non-target poisoning of animals with 1080 could occur. To reduce the non-target poisoning, poison delivery systems targeted to pigs, including the PIGOUT® bait (Animal Control

Technologies, Australia; Cowled et al. 2006), the Hog-Hopper™ (Animal Control Technologies, Australia; Dall 2010) and the Boar Operated System (BOS™; Massei et al. 2010) delivery containers have been developed. These targeted delivery systems could also be used for contraceptives or vaccines after further research and development (Massei et al. 2010; Ballesteros et al. 2011).

Trapping, although not as effective as poisoning, can also be used to control feral pig populations. Records of population capture up to 80% have been reported (Saunders et al. 1993). Benefits of trapping include euthanasia being entirely target-specific and maintenance of the traps can be incorporated into daily farm procedures (Choquenot 1996).

Fencing is typically used to protect small valuable land areas, and is a recommended biosecurity practice for all piggeries (Choquenot 1996; APIQ 2010). One fence design has been identified as being the most effective at preventing feral pig movement (Hone and Atkinson 1983). This fence is approximately 1 metre tall, with steel posts, hinge joints and an electric outrigged wire (Hone and Atkinson 1983). However, fencing to prevent feral pig access has been described as ineffective over the long term, regardless of the fence used, due to physical damage, poor maintenance, human error and electrical failure (Choquenot 1996; Seward et al. 2004; Reidy et al. 2008). These attributes, as well as the expense of installation and maintenance, can lower the adoption rate of this biosecurity practice on piggeries. An alternative, relatively inexpensive fence design has been described by Lavelle et al. (2011), in Texas, for temporary containment of feral pigs followed by eradication. The 0.86 m tall, hog-panel fence, had a containment effectiveness ranging between 83 to 100% when feral pigs were motivated to escape.

3.8 Other animals

3.8.1 Introduced species

The postal survey distributed to pig producers identified a number of other species of animals that were present on piggeries (Chapter 2). Feral cats were observed by many of the survey respondents. A survey of pathogens in wild cats in Australia reported the presence of bacterial, viral, helminth and protozoal pathogens. Among these, 50 pathogenic species were not host-specific to cats (Moodie 1995: unpublished report referenced in Dickman 1996). As mentioned in Chapters 1 and 2, one of the most

important pathogens for human and domestic animal health associated with cats is *Toxoplasma gondii*. Prevalence of *Toxoplasma gondii* in wild cats in Australia ranged from 4.9% in Western Australia to 89% on Kangaroo Island (Milstein and Goldsmid 1997; Adams 2003; O'Callaghan et al. 2005). *Toxoplasma gondii* can infect many mammals, including pigs and humans. Infection in pigs is considered to originate predominantly from a feline source, resulting generally in a marked decline in reproduction and loss of condition (Lehmann et al. 2003; Taylor 2006). Cats were not included in this thesis as their role in the transmission of *Toxoplasma gondii* has been previously well studied.

Wild canids, including wild dogs (*Canis lupus familiaris* and *Canis lupus dingo*) and foxes (*Vulpes vulpes*), have been associated with a number of different pathogens. Henderson (2009) summarised a list of pathogens in wild canids, which included bacterial, viral, helminth, protozoal and ectoparasitic pathogens. While the number of pathogen species isolated from the wild dog is far greater than from the fox, a pathogen common in both canids in Australia and worldwide is the helminth *Echinococcus granulosus* tapeworm, which causes hydatidosis. Wild dogs are the most important definitive host of *Echinococcus granulosus* in Australia, while marsupials, feral pigs and sheep are the most common intermediate host, with cattle and humans acting as less significant intermediate hosts (Jenkins and Macpherson 2003). The prevalence of *Echinococcus granulosus* in wild dogs has ranged from 25 to 100% in Victoria, New South Wales and Queensland (Baldock et al. 1985; Durie and Riek 1995; Jenkins and Morris 2003).

Wild dogs are also definitive hosts of the protozoa *Neospora caninum* in Australia as previously mentioned, and play a role in the sylvatic and domestic lifecycles of the protozoan (King et al. 2010; King et al. 2011). Dogs and dingoes are capable of indirectly transmitting *Neospora caninum* to cattle by contaminating food and water with faeces containing oocysts (King et al. 2011). The significant consequence of infection in cattle is reproductive disease, such as abortions, resulting in an estimated cost to beef and dairy industries of AU$110 million annually (Reichel and Ellis 2009). This protozoan has not been recorded as leading to disease in pigs.

The three bacterial pathogens *Salmonella spp*., *Campylobacter spp*. and *Leptospira spp*., discussed in Sections 3.5, 3.6 and 3.7 of this literature review, have also been isolated from wild dogs (Henderson 2009). Dogs sampled in South Australia were infected at varying

prevalence rates for different *Campylobacter spp*. serotypes: 34% of dogs carried *Campylobacter upsaliensis*, 7% *Campylobacter jejuni* and 2% *Campylobacter coli* (Baker 1999). *Leptospira spp*. were detected in 1.9% of domestic dogs sampled at animal shelters in Australia (Zwijnenberg et al. 2008).

Cane toads (*Bufo marinus*), rabbits (*Oryctolagus cuniculus*) and goats (*Capra hirsus*) also carry a variety of different pathogens. The number and range of the pathogenic species isolated in Australia is small in comparison to those isolated from feral cats and wild canids (Henderson 2009), but some are of significance to livestock industries. Eleven serotypes of *Salmonella spp*. were isolated from the cane toad in a survey in Queensland between 1978 and 1998 (Thomas et al. 2001). Culture of a small number of rabbit faecal samples collected from the environment around Sydney water sources in 2002 isolated *Cryptosporidium* and faecal coliforms (Cox et al 2005). *Cryptosporidium* has been shown to cause diarrhoea in experimentally infected pigs (Tzipori et al. 1981).

3.8.2 Native species

Marsupials were observed by the survey respondents in and around piggery facilities (Chapter 2). Numerous different pathogens have been identified in Australian marsupials. Possum populations in the Sydney area had a *Toxoplasmosis gondii* prevalence of 6.3% (Eymann et al. 2006), and the prevalence of *Leptospira interrogans* serovar Hardjo was 9.6% (Eymann 2007). *Leptospira spp*. have also been isolated from Eastern grey kangaroos, with Roberts et al. (2010) identifying a high prevalence of 47% among free-ranging kangaroos sampled in the Warragamba Catchment Area, Sydney. All seropositive animals were found to have the pathogen *Leptospira weilii* serovar Topaz (Roberts et al. 2010), which was first identified in 2008 on a North Queensland dairy farm (Corney et al. 2008). Despite this significant prevalence level, the ability of the Eastern grey kangaroo to act as a carrier of this pathogen, transmit and infect other animals is still poorly understood (Roberts et al. 2010). These findings are concerning, as piggeries do not have measures in place to prevent marsupial access, considering the ability of possums to climb and kangaroos to jump fences. Furthermore, the health consequences of infection with the new *Leptospira weilii* serovar Topaz have not been determined in animals or humans.

Reptiles were observed by a number of pig producers who responded to the survey. Many of the pathogens carried by reptiles in Australia are not zoonotic. The exception to this is

the isolation of *Salmonella spp*. from eight snakes, four lizards and two turtles between 1978 and 1998 in Queensland in a study by Thomas et al. (2001).

Fruit bats were not observed by pig producers surveyed in 2007 (Chapter 2). This is an important observation, as fruit bats are a source of a number of infectious pathogens to livestock and humans. Two well known examples of these diseases are the Hendra virus infection in horses and humans in Australia, and the Nipah virus infection in pigs and humans in Malaysia (Breed et al. 2006). Both of these viruses are *Henipavirus*, a genus of the family *Paramyxoviridae*.

The first outbreak of Hendra virus was described in Australia in 1994. The disease resulted in the death of 14 horses and one human (Murray et al. 1995). Outbreaks in horses and subsequent human infection have continued to occur up until the present. A recent study by Li et al (2010) has also identified that pigs may be experimentally infected with this virus, exhibiting fever, depression and respiratory symptoms. A major epidemic of Nipah virus in pigs and humans in Malaysia during 1999 led to 105 human fatalities and a cull of over one million pigs (Chua et al. 2000). Fruit bats were also found to be the source of Menangle virus infection in pigs, which caused a significant increase in stillborn piglets and a significant decrease in the pregnancy rate on one piggery in Australia in 1997 (Philbey et al. 1998). Fruit bats are also carriers of pathogenic *Leptospira spp*. and shed leptospires into the environment (Cox et al. 2005). Of the fruit bat urine samples analysed for pathogenic Leptospirosis by Cox et al. (2005) in Australia, 39% tested positive.

3.9 Evaluating pathogen presence and prevalence

Studies involving pathogen surveillance within a defined population must focus on either substantiating freedom from a pathogen, or measuring prevalence of a pathogen (Cameron and Baldock 1998). These two different survey aims require different sample sizes. The freedom from pathogen studies generally require a smaller sample size and a predetermined minimum expected prevalence for inclusion in the calculations of sample sizes (Dohoo et al. 2003). The prevalence of the pathogen can also be determined from freedom of pathogen surveys, though the confidence intervals around this prevalence estimate will be wider than if the sample size required for a survey to measure prevalence were used (Cameron and Baldock 1998).

Often studies that present a prevalence value for pathogens within a population report the apparent prevalence; that is, the proportion of individuals determined to be positive in a survey (Gardner 2004; Reiczigel et al. 2010). However, the presentation of apparent prevalence as the prevalence estimate for a population is based on the assumption that the diagnostic procedure used to determine the pathogen positive/negative status of a sample is a perfect test, providing conclusive and error-free results. There is a flaw in this assumption when the diagnostic techniques performed are not perfect, with sensitivity and/or specificity values lower than 100% (Gardner 2004). The accuracy of a diagnostic technique, the sensitivity and specificity, is determined by comparison of the technique against a gold standard method (Gardner 2004). The sensitivity (ability of a technique to identify a diseased individual as positive) is determined by investigating how well the test identifies known positives; the specificity (ability of a technique to identify a non-diseased individual as negative) is determined by how well the test determines non-infected samples to be negative, and reflects ability of a test to differentiate between the specific pathogen of interest and other pathogens. Therefore, false negative results will arise when a test has low sensitivity and false positive results when there is low specificity. Transformation of this apparent prevalence to account for the sensitivity and specificity values of the diagnostic procedure used provides an estimate of the true prevalence of the pathogen. Calculation of the true prevalence makes the results more accurate and allows comparisons to other pathogen surveillance studies using different diagnostic techniques (Gardner 2004).

3.10 Evaluating risk

Definitive identification of the source of infection for an animal population and proof of the pathogen transmission process is often difficult to obtain, particularly in relation to endemic diseases. For an outbreak of a new pathogen, as is the case with emerging infectious diseases; or an outbreak of a known pathogen in a new region; it is usually possible to identify an index case or source. However, for an endemic disease, where there may be multiple potential sources of the pathogen, determining the source of infection for a previously free population requires consideration of all the potential transmission pathways and sources. In this context, an estimation of the probability of transmission occurring, considering different event determinants, could provide useful information regarding the most likely source of infection.

Using a risk analysis approach enables the likelihood and consequences of pathogen transmission to be estimated and measured, and procedures to reduce the risk to be evaluated. Estimation and evaluation of the risk posed by pathogens (and other types of hazards) is an internationally accepted standard underpinning international trade, called import risk analysis. There are three different frameworks for analysing risk endorsed for use by the World Trade Organisation (WTO): the Codex Alimentarius Commission (CAC) of the Food and Agriculture Organisation of the United Nations (FAO)/World Health Organisation (WHO) framework for microbial food safety risk assessments; the International Plant Protection Convention framework relating to plant health; and the World Organisation for Animal Health (OIE 2010) framework for animal disease.

The OIE risk analysis framework consists of four inter-related components: hazard identification, risk assessment, risk management and risk communication (OIE 2010) (Figure 3.3). The risk analysis process following the OIE framework is summarised below.

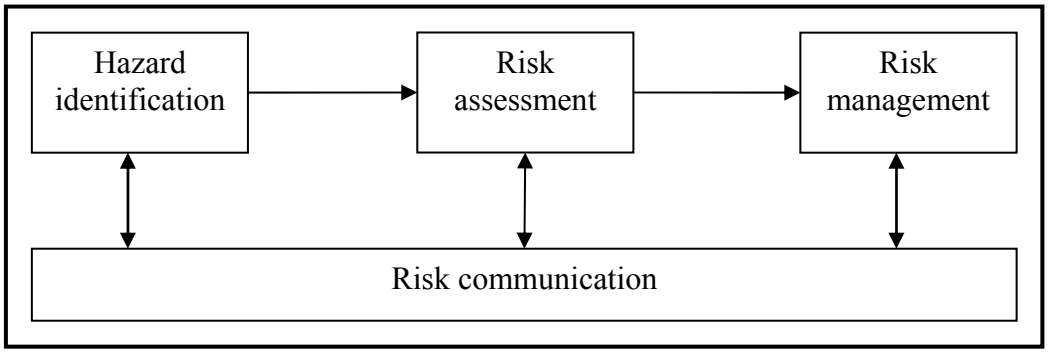

Figure 3.3. Four components of a risk analysis (OIE 2010).

Hazard identification involves identifying hazards (pathogens, chemical residue, vectors) that are potentially harmful, producing adverse consequences for the animal, human or country in question. The second component, the risk assessment, is itself comprised of four steps: release assessment, exposure assessment, consequence assessment and risk estimation (Figure 3.4). Release assessment identifies the pathways and likelihood that a specific identified hazard may enter a particular environment. The exposure assessment identifies the pathways and likelihood of exposure of an animal or human to the hazard released within the environment. The consequence assessment describes the consequences of hazard exposure (that is the alternate outbreak scenarios arising from pathogen

exposure) and the likelihood of each outbreak scenario to occur. These consequences may be direct, such as mortality and morbidity; or indirect, such as economic and environmental considerations. Risk estimation is the final step, when the likelihoods of release and exposure are combined with the consequence estimate to estimate the risk associated with the hazard.

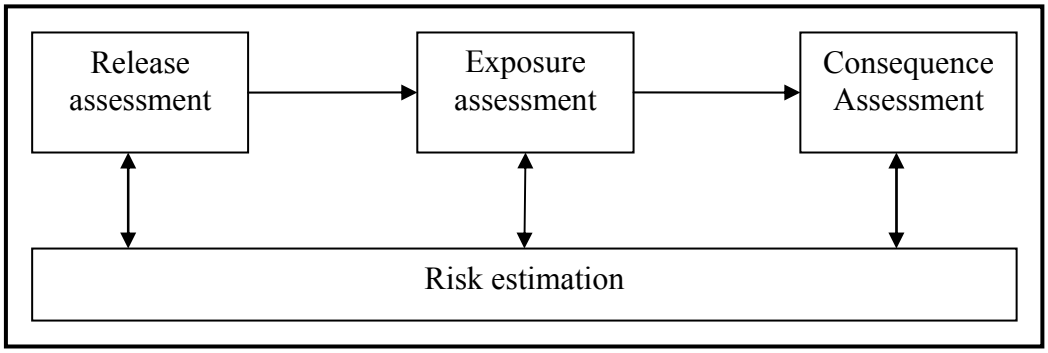

Figure 3.4. Four components of a risk assessment (OIE 2010).

The risk management component of risk analysis is also comprised of four steps (Figure 3.5). These include the risk evaluation, option evaluation, implementation, and monitoring and review. The risk evaluation compares the risk estimate to the appropriate level of protection determined by the country or territory. The appropriate level of protection for imports into Australia and New Zealand is a qualitative value of Very Low, which is based on the outcome of the overall risk estimation, which includes release, exposure and consequences assessments (Biosecurity Australia 2009). Some risk mitigation options common for imported products include pre-border quarantine arrangements, screening of vessels and imported products at the border, as well as the development of emergency response plans to reduce the consequences of an event occurring (Biosecurity Australia 2009). Identified options that lower the risk to the appropriate level of protection are then implemented, monitored and reviewed for effectiveness.

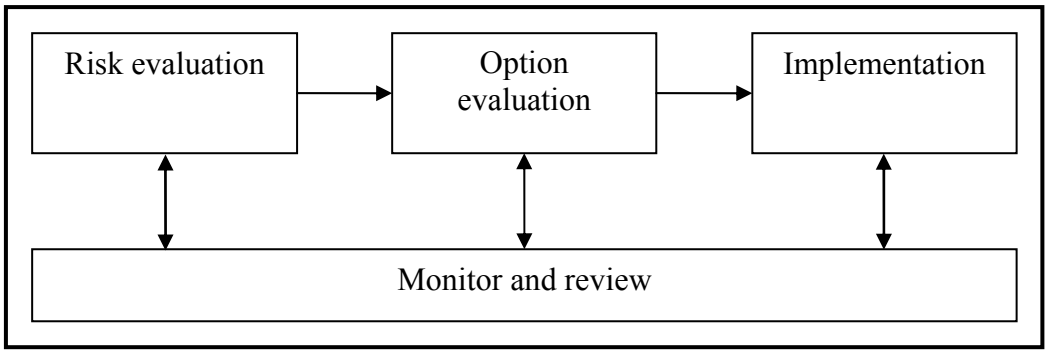

Figure 3.5. Four components of risk management (OIE 2010).

Risk communication is an all-encompassing component of the risk analysis process, involving the ongoing consultation of interested parties. All stakeholders involved in the issue assessed should be consulted and invited to review the risk analysis at different points in time, to ensure relevant information and opinions are considered and relevant parties remain informed.

Only the exposure assessment component of a risk analysis was undertaken in this thesis. In a very broad sense, the hazard identification component of a risk analysis was also undertaken in Chapter 2 and throughout the thesis by identifying those pathogens harmful to pigs that could be carried by the wild animals included in the study (European starlings, rodents and feral pigs). The exposure assessment conducted sought to identify the possible pathways through which a pig may be exposed to a sufficient dose of a pathogen from an infected wild animal to result in infection, as well as the likelihood of these exposure pathways occurring.

Risk assessments have been used recently to determine the likelihood of pathogen transmission from wildlife to livestock. Gallagher et al. (2003) used the OIE framework for risk assessment to determine the probability of the release of *Mycobacterium bovis* from badgers, the probability of cattle exposure to the pathogen from badgers, and the consequence of different pathogen dose responses to the cattle in the south west of England. Gallagher et al. (2003) reported that the first cow in a herd of 74 individuals will become infected after 102 days if there is a single badger excreting *Mycobacterium bovis* present. This was determined to vary given the number of excreting badgers, the routes of pathogen shedding and the likelihood of infection dependent on dose in cattle. Sutmoller et al. (2000) determined the probability of foot-and-mouth disease transmission to cattle in

Zimbabwe through a number of different scenarios. Transmission of foot-and-mouth disease from buffalo to antelope, which then gain access to the cattle holding areas by jumping the double fenced perimeter, was the most likely scenario. Other less likely scenarios of transmission involved aerosol transmission, and a break in the fence allowing buffalo access to cattle. The results of their studies provided transparent probability values with recommendations regarding the most important areas where mitigation strategies could be applied to reduce the risk of transmission.

Morgan et al. (2006) and Kilpatrick et al. (2009) also aimed to assess the transmission of pathogens from wildlife to livestock. However, neither implemented a standard risk assessment methodology and, consequently, their work does not provide a complete answer to their study hypotheses. Morgan et al. (2006) studied the likelihood of infection of saiga antelope with foot-and-mouth disease virus sourced from livestock: how long the virus could persist in the antelope population, and the likelihood of transmission of the virus back to livestock. However, the results obtained were unable to determine the likelihood of antelope being exposed to the virus from livestock and the reverse likelihood. Kilpatrick et al. (2009) aimed to determine the relative risk of *Brucella spp*. transmission from bison to cattle outside Yellow Stone National Park. Authors present results to answer this aim; however, quantitative and qualitative estimates are presented without definition of the qualitative risk estimates.

The aim of Chapter 7 of this thesis is to determine the quantitative likelihood of exposure of domestic pigs on commercial piggeries to pathogens from wild animals. As the pathogens investigated are endemic to Australia and present within regions of the piggeries included in this study, the release of the pathogen to the region was not required. A consequence assessment was beyond the scope of the current study. In addition, extensive information is available on the impact of the endemic diseases studied upon domestic pigs and the pig industry in Australia.

3.11 Summary

Pathogens can be transmitted in a number of different ways from wildlife to livestock. These modes of transmission include direct transmission by contact between animals and exchange of body fluids, and indirect methods, such as contamination of the environment,

food, water and air in the surroundings of livestock. Often a pathogen is not restricted to being transmitted via a single mode. The pig pathogenic agents studied in this thesis can be transmitted in either one or many of these pathways.

The bacteria *Salmonella spp*., *Escherichia coli*, *Campylobacter spp*., *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli* and *Lawsonia intracellularis* are generally transmitted through direct or indirect contact and consumption of infected faeces. *Brucella suis* and *Leptospirosis spp*. are more commonly transmitted through direct or indirect contact with body fluids, such as urine or reproductive fluids. Finally, the two pneumonia-causing pathogens, *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae,* can be transmitted through direct or indirect contact, including aerosols.

The likelihood of pathogen transmission from a wild animal to a domestic pig is affected by the pathogen prevalence, the period of time the wild animal is within proximity of a piggery, and the mode of transmission. The determination of the probability of endemic pathogen transmission from a wild animal to a domestic pig in Australia will assist in directing biosecurity practices to mitigate areas of high likelihood of exposure. Not only will this help to mitigate endemic pathogen transmission, but it can also be applied to help predict likely transmission patterns of transboundary and emerging diseases, for which there is little or no data available in Australia.

4 European starling biosecurity threat to piggeries

4.1 Introduction

The three most significant bird pests in the world include the European starling (*Sturnus vulgarus*), house sparrow (*Passer domesticus*) and the common pigeon (*Columba livia*) (Pimentel et al. 2001). These bird species are highly adaptable to urban environments and are primarily found in close association with humans and their practices. As such, these birds are common pests on agricultural enterprises, and may be present in large flocks on intensive farming practices due to the abundance of freely available food sources. As an example, a study in Scotland (Daniels et al. 2003) reported an average of 25 bird faeces deposits per metre squared of stored feed per month on a number of different cattle farms. This is of concern, as these three bird species are associated with over 40 pathogens, many of which are capable of being transmitted inter-species (Weber 1979). Most of these pathogens are present in bird excrement; therefore, faecal contamination of feed poses a risk of pathogen transmission to domestic animals (Morishita et al. 1999; Craven et al. 2000; Benskin et al. 2009). The contamination of food and water by *Salmonella spp*. in cattle feedlots in the United States was directly related to the number of starlings present (Carlson 2011a) and decreased when starlings were removed from the feedlot (Carlson 2011b). European starlings were one of the most frequently reported birds on piggeries in Australia (Chapter 2).

The majority of diarrhoea-causing illnesses in pigs result from infection with *Salmonella spp*., *Campylobacter spp*. or *Escherichia coli* (Jackson and Cockcroft 2007). Infection with these pathogens causes significant negative financial implications, mainly due to the reduction in feed conversion rate, increased medication expenses and the related mortality (Taylor 2006). Additionally, the contamination of food products, such as pork, with *Salmonella spp*., *Campylobacter spp*. and *Escherichia coli* account for over 90% of bacterial enteritis infections in humans globally (Thorns 2000). These bacterial enteropathogens also correspond with the most common bacteria isolated from wild birds (Benskin et al. 2009). It is important to determine the prevalence of these pathogens in

wild bird populations in the proximity of piggeries in order to assess potential biosecurity risks.

Although these pathogens have been previously isolated from birds in Denmark, the United States and England, studies in relation to the presence of bacteria in starlings have been limited, and no studies have been conducted in Australia. Additionally, the extent of the contribution of starlings to the transmission of pathogenic strains of the bacteria to pigs has not been determined internationally or in Australia. These three pathogens have important financial consequences for the pig industry as well as public health implications due to their zoonotic nature. People can get infected through the consumption of contaminated pig meat. The objective of the research presented in this chapter was to detect the presence of *Salmonella spp*., *Campylobacter spp*. and *Escherichia coli* in starlings captured on four large-scale commercial piggeries and the distribution of serotypes causing disease in pigs.

4.2 Materials and methods

4.2.1 Study location and sample size

Four piggeries in South Australia, de-identified as A, B, C and D as shown in Figure 4.1, were selected for capture and sampling of European starlings. Piggeries were purposively selected from those involved in an assessment of the use of the DRC-1339 avicide for control of starlings at intensive livestock production facilities. The DRC-1339 avicide project was in the initial stage of determining the starling population size on piggeries and as such would not have had any impact on the results obtained in the current study. Selected piggeries had a daily presence of starlings throughout summer, and the piggery owners were willing to participate in a confidential disease assessment.

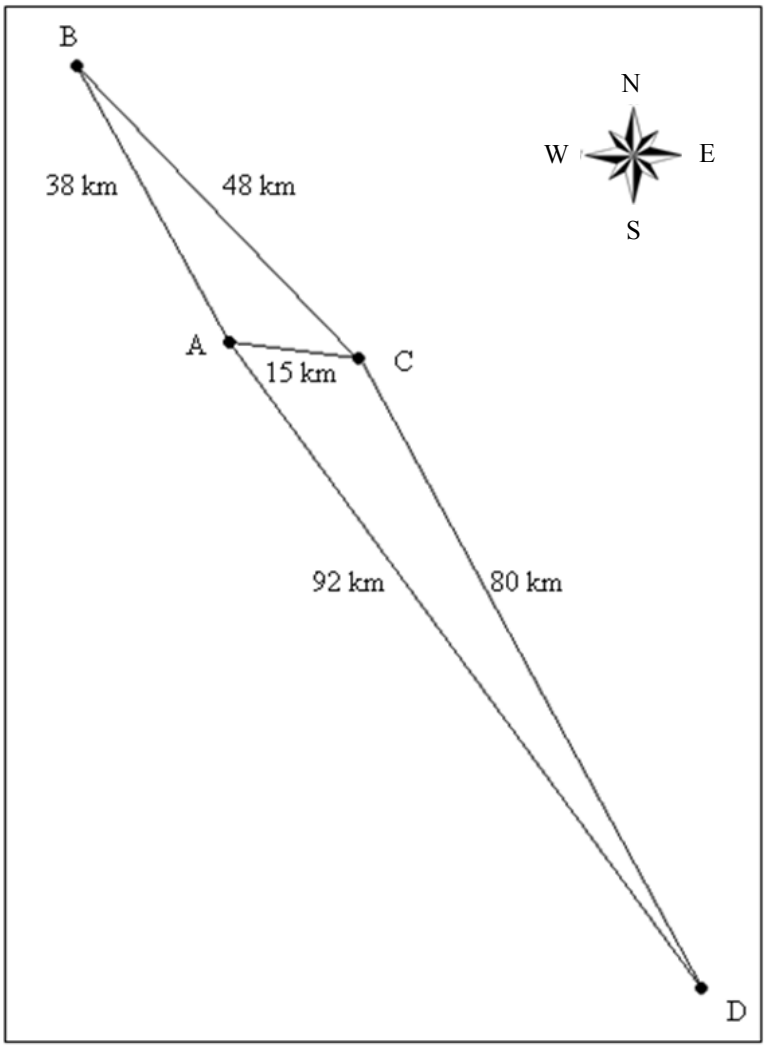

Figure 4.1. Relative locations of four piggeries in South Australia which participated in a study to detect the presence of *Salmonella spp*., *Campylobacter spp*. and *Escherichia coli* in European starlings.

In February 2008 and February 2009, a total of 473 starlings were captured on the four piggeries. The number of starlings sampled on each piggery is shown in Table 4.1

Table 4.1. The number of European starlings sampled on four different piggeries in South Australia to detect presence of pathogens at an expected level of ≥ 3.5%.

Piggery	Year	Sample size (*n*)
A	2008	84
B 2008	2008	88
B 2009	2009	100
C	2009	101
D	2009	100

The required sample size (n) of starlings per trapping for each piggery was based on calculations to detect pathogens in an infinite population (defined as >1000 animals) using the following formula from Dohoo et al. (2003; Eq 2.18.).

$$n = \frac{\ln \alpha}{\ln q} \quad \text{.....Equation 4.1}$$

where α = 0.05 (1 − 95% confidence level) and q = 1 − expected prevalence (expected prevalence: 3.5%). The expected prevalence of 3.5% was selected because of the prevalence of the pathogens of interest found in previous studies (Benskin et al. 2009; Gaulker et al. 2009; Carlson et al. 2011a).

4.2.2 Sampling the starling population

Starlings were trapped using 18 m mist nets (M. Nakamori & Co., Yokkaichi, Japan) suspended between two aluminium masts, within 50 cm of the windows of piggery buildings shown in Figure 4.2, where the greatest number of birds were previously observed by piggery workers. Nets were erected in advance of bird arrival on the piggery of a morning. The number of days taken to trap and sample starlings varied between piggeries. Piggery A, B in 2008 and C required 2 days each, piggery B in 2009 required 3 days and piggery D only required a single day. Birds were scared from the open side of the piggery building into the mist nets which covered the windows on the opposite side. Birds trapped in nets were untangled by hand and placed in white fabric bags in the shade until processing. Each bird was banded with one coloured band, each colour specific to the piggery the birds were caught on; and one brass band with a sequence number, for identification in the case of a recapture. A cloacal swab was taken from each bird using a minitip culturette (Interpath Services, Copan Innovation – sterile swab applicators in peel pouches) which was then stored in transport media and refrigerated before transportation to Gribbles laboratory for culturing each day. Each bird was released after they were banded and had the cloacal swab taken. All captive and handling procedures were approved by the Department of Primary Industries and Resources of South Australia Animal Ethics Committee (reference number # 04-07).

Figure 4.2. A mist net erected along the windows of an intensive piggery in South Australia to capture European starlings in 2009.

4.2.3 Piggery management and disease control history

Information on pig health status and disease and pest control programs implemented on each piggery was obtained through administration of a written questionnaire to each of the piggery managers after sampling of the starlings and laboratory tests were performed (Appendix 2). A single page letter outlining results obtained from the starlings captured on their piggery was also attached to the three page questionnaire. The questionnaire contained open questions covering pig disease history and pest control management. Questions on disease history focused on information regarding the history of the presence of the pathogens of interest in this study, including how the pathogen presence was confirmed, and the medication used to control illness. Questions pertaining to starling pest control focused on the control measures in place to manage birds. The general husbandry questions requested information on the herd size, piggery production type (e.g. intensive, free-range or ecoshelter) and any other potential food sources for starlings within a 20 km radius of the piggery. All pig health results were laboratory confirmed, and such were not

affected by any potential bias created by knowing the starling results prior to questionnaire completion.

4.2.4 Laboratory testing

Culture of *Salmonella spp*., *Campylobacter spp*. and *Escherichia coli* was done by Gribbles Veterinary Pathology Laboratory (Adelaide, South Australia). The culture methods followed those recommended by the Australia/New Zealand standards (Australian Standard 2004; Australian Standard 2006; Australian Standard 2009). A different culture kit was used to grow each bacterium. Methods follow the instructions on the kit inserts.

4.2.4.1 Transport Media

Once a cloacal sample was taken, the minitip culturette was placed in bacterial transport media. There were two types of transport media used depending on the bacteria being tested for. The swabs to be analysed for *Salmonella spp*. and *Escherichia coli* were placed in Stuart's medium (ThermoFisher, Oxoid© Australia Pty Limited) which was comprised of 0.1 g/L calcium chloride, 1 g/L sodium thioglycollate and 10 g/L di-sodium β-clycerophosphate (Remel 2003). The swabs to be analysed for *Campylobacter spp*. were placed in Aimes Charcoal media (ThermoFisher, Oxoid© Australia Pty Limited) which was comprised of 1.15 g/L di-sodium phosphate, 0.2 g/L monopotassium phosphate, 0.2 g/L potassium chloride, 3.0 g/L sodium chloride, 0.1 g/L magnesium chloride, 0.1 g/L calcium chloride, 1 g/L sodium thioglycollate, 10 g/L charcoal and 5g/L agar (Remel 2003).

4.2.4.2 Escherichia coli

The kit for culture was obtained from ThermoFisher (Oxoid© Australia Pty Limited). The media used for *Escherichia coli* culture was half Columbia Horse Blood Agar, half MacConkey No Salt Agar (ThermoFisher, Oxoid© Australia Pty Limited). The Columbia Horse Blood Agar was comprised of 23 g/L special peptone, 1 g/L starch, 9 g/L sodium chloride, 10 g/L agar No. 1 and 50 ml horse blood – defibrinated. The MacConkey No Salt Agar was comprised of 20 g/L peptone, 10 g/L lactose, 5 g/L bile salts, 0.075 g/L Neutral Red (indicator) and 12 g/L agar. Both sides of the plate were swabbed with the sample and incubated at 37 ºC (± 2 ºC) in an oxygen incubator for 24 h. *Escherichia coli* bacteria colonies appear pink on the MacConkey agar, due to acid production as a result of the fermentation of lactose. Pathogenic strains of the *Escherichia coli* are often haemolytic and will appear as creamy, grey, wet colonies on the Horse Blood Agar.

A Microbact gram-negative identification system was used to identify bacteria according to manufacturer's instructions (ThermoFisher, Oxoid© Australia Pty Limited). Briefly, 1 to 3 isolated colonies were selected and emulsified in 2.5 ml saline solution (0.85%). A test strip was inserted into the holding tray and 4 drops of the bacterial suspension were added to each well using a sterile pipette. Then, two drops of a mineral oil were added to the 3 black wells of the identification strip and the rows were sealed with an adhesive seal and incubated at 35 ºC (± 2 ºC) for 18 to 24 h. Reagents were added to appropriate wells and read within the time frame recommended. The results were found to be positive or negative by comparing them to the colour charts provided.

Serotyping of *Escherichia coli* isolates and determination of fimbrial antigens were performed at the National *Escherichia coli* Reference Laboratory (Bendigo, Victoria). The *Escherichia coli* isolates were tested via slide agglutination against the serotypes and associated fimbrial types pathogenic to pigs in Australia (Gyles 1994), shown in Table 4.2. The antisera for the different *Escherichia coli* serotypes were added to the *Escherichia coli* isolate on a slide under light and observed for agglutination. Agglutination occurring within 60 sec was regarded as a positive reaction.

Table 4.2. *Escherichia coli* O serotypes and associated fimbrial and capsule types considered to cause disease in pigs in Australia.

O serotype	Associated fimbrial types	Associated capsule types
O8	K88, K99, 987P	
O9	K88, K99, 987P	
O20	K88	
O64	K88, K99	
O101	K88, K99	
O8G7	K88	K87
O45	K88	K1
O98		
O138	F18	K81
O139		K82
O141	F18	K85ab, K85ac
O147	K88	K89
O149	K88, F18	K91
O157	K88, F18	KV17

4.2.4.3 Salmonella spp.

The kit for culture for *Salmonella spp*. was obtained from ThermoFisher (Oxoid© Australia Pty Limited). The cloacal swab sample was inoculated in a Mannitol Selenite enrichment broth. The broth was made of 0.010 g/L cystine, 4 g/L mannitol, 10 g/L sodium dihydrogen phosphate, 4 g/L sodium hydrogen selenite and 5 g/L tryptone. This was incubated for 18 to 24 h at 35 ºC in aerobic conditions. A subculture of the incubated undisturbed Selenite broth was obtained from the surface and swabbed onto an XLD (Xylose Lysine Desoxycholate) plate. The XLD plate was comprised of 3 g/L yeast extract, 5 g/L L-Lysine HCI, 3.75 g/L xylose, 7.5 g/L lactose, 7.5 g/L sucrose, 1 g/L sodium desoxycholate, 5 g/L sodium chloride, 6.8 g/L sodium thiosulphate, 0.8 g/L ferric ammonium citrate, 0.08 g/L phonol red and 12.5 g/L Agar No.1. The inoculated XLD plate was incubated for 24 to 48 h at 35 ºC (± 2 ºC), in aerobic conditions. *Salmonella spp*. bacteria colonies appear pink with a black centre caused by hydrogen sulphide. The Microbact gram-negative identification system was also used to identify bacteria, using the methods described above for *Escherichia coli*.

Serotyping of *Salmonella spp*. isolates were performed at the Australian Salmonella Reference Centre (Institute of Medical and Veterinary Science, Adelaide). The *Salmonella spp*. isolates were tested via slide agglutination against serotypes as per the Kauffman-White classification scheme (Murray et al. 2003). The *Salmonella spp*. O serotypes were firstly identified via agglutination, and associated fimbrial types were subsequently tested also via agglutination. There are over 2000 serotypes of *Salmonella spp*., which are continually updated by the World Health Organisation Collaborating Centre for Reference and Research on Salmonella (Popoff and Le Minor 2001).

4.2.4.4 Campylobacter spp.

Campylobacter spp. are microaerophyllic and their growth is inhibited by atmospheric oxygen levels (Kiggins and Plastridge 1956). As such, to grow the *Campylobacter spp*., specific atmospheric conditions were created for incubation. The kit for culture was obtained from ThermoFisher (Oxoid© Australia Pty Limited). The cloacal sample was inoculated into a saline suspension and a drop of this suspension was applied to the *Campylobacter spp*. agar plate. This plate was comprised of 23 g/L special peptone, 1 g/L starch, 5 g/L sodium chloride, 10 g/L Agar No.1, 0.25 g/L sodium pyruvate, 0.25 g/L sodium metabisulphite, 0.25 g/L ferrous sulphate, 0.01 g/L vancomycin, 0.005 g/L

trimethoprim, 40000 u/L colimycin and 50 ml horse blood defibrinated. The *Campylobacter spp*. agar plate was incubated for 48 h at 42 ºC in a microaerophylic environment created by an Oxoid© atmosphere generation system. A control culture of *Campylobacter spp*. was incubated with each sample batch. If the *Campylobacter spp*. control failed to grow, the culture procedure was repeated with a fresh control. Confirmation of *Campylobacter spp*. was done by Gram staining preparations with dilute carbol fuchsin and identifying gram-negative, oxidase positive "S"-shaped, gull winged or long spiral forms (Winn et al. 2006).

To identify the *Campylobacter spp*. serotype, the isolate was inoculated into a saline suspension and spread over the surface of a new *Campylobacter spp*. agar plate. A nalidixic acid disc and a cephalothin disc (ThermoFisher, Oxoid© Australia Pty Limited) were placed on the dried surface of the blood agar separately and incubated at 42 ºC for 48 h in a microaerophylic environment created by an Oxoid© atmosphere generation system. *Campylobacter jejuni* and *Campylobacter coli* are sensitive to nalidixic acid and will not grow within 6 mm of the disc, whereas they are both resistant to cephalothin, growing right up to the disc. To further differentiate between *Campylobacter jejuni* and *Campylobacter coli*, a hippurate hydrolysis test was performed. Sterile sodium hippurate (South Australian Research and Development Institute, Adelaide) was added at a quantity of 0.5 ml to a screw capped tube. A loop full of the *Campylobacter spp*. isolate was added and incubated at 37 ºC for 2 h along with an un-inoculated control tube. Ninhydrin solution (bioMérieux – Australia Pty. Ltd. Baulkham Hills), 0.2 ml, was added to the tubes. A positive result was indicated by the solution turning a deep purple colour within 5 min: only *Campylobacter jejuni* hydrolyses hippurate, resulting in this positive reaction (Winn et al. 2006).

4.2.5 Data analysis

4.2.5.1 Calculation to substantiate freedom from a pathogen and to estimate prevalence

The likelihood that a pathogen is present in a population of animals (or alternatively, to prove freedom from a pathogen), according to the results of the diagnostic tests for the pathogens studied, was calculated based on a hypergeometric exact probability formula by Cameron and Baldock (1998) using the FreeCalc version 2 software (Sergeant 2009b). This software allows you to conclude if a population is free from a pathogen according to the diagnostic test sensitivity and specificity, minimum expected prevalence, the

population size, the sample size, and the number of positive reactors. The null hypothesis (pathogen is present) is rejected when the probability of x positive reactors is ≤0.05, in which case you can conclude that the population is free from a pathogen at the minimum expected pathogen prevalence.

The apparent prevalence was calculated by dividing the test positive results by the total number of samples tested. The true prevalence (Tp) was estimated using the formula developed by Rogan and Gladen (1978) (Equation 4.2) based on the known sensitivity and specificity of the laboratory techniques used in laboratory testing.

$$Tp = \frac{\gamma + \beta - 1}{\alpha + \beta - 1} \quad \text{.....Equation 4.2}$$

where γ is the apparent prevalence, α is the test sensitivity and β the test specificity.

The sensitivity and specificity of the laboratory tests used are summarised in Table 4.3. The sensitivity and specificity of the different culture methods used for the various pathogens being studied were not determined by the diagnostic laboratory where the samples were processed. Furthermore, sensitivity of the culture techniques used has not been determined in past studies. As such, a range of sensitivities were adapted from references that used similar methods or parts thereof. Sensitivities for *Escherichia coli* detection ranged from 49 to 98% (Eriksson and Aspan 2007); *Salmonella spp*. detection ranged from 86 to 100% (Mugg and Hill 1981); and *Campylobacter spp*. detection ranged from 63 to 100% (Patton et al. 1981).

Table 4.3. A summary of the sensitivity and specificity of culture methods used to determine true prevalence of bacteria species in European starlings trapped and sampled on piggeries in Australia in 2008 and 2009.

Bacteria species	Sensitivity range			Reference	Specificity	Reference
Escherichia coli	60%	90%	100%	Adapted from Eriksson and Aspan (2007)	100%	Eriksson and Aspan (2007)
Salmonella spp.	60%	90%	100%	Adapted from Mugg and Hill (1981)	98%	Microbact™ Gram-Negative identification system booklet.
Campylobacter spp.	60%	90%	100%	Adapted from Patton et al. (1981)	100%	Oxoid© Technical specification

The 95% confidence intervals around the true prevalence estimate were calculated using the Epitools epidemiological calculator for estimated true prevalence and predictive values from survey testing (Sergeant 2009a). From the confidence intervals generated by this calculator, the Blaker (2000) method values are reported in this study, because the Blaker method provides exact two-sided confidence intervals that are restricted by 0 and 100 and are more accurate than values generated using other methods when true prevalence is <20% and >80% (Reiczigel et al. 2010).

4.3 Results

4.3.1 Probability of presence of pathogens in the European starling populations.

Escherichia coli was found statistically to be present in starling populations on all four piggeries with probabilities shown in Table 4.4.

Table 4.4. Probability of presence of *Escherichia coli* in the European starling populations at four piggeries in South Australia in 2008 and 2009.

		Probability of presence of the pathogen in the starling population at different test sensitivity (Se) and specificity (Sp)[a]		
Piggery	*Escherichia coli* Positive/tested	Se 60% Sp 100%	Se 90% Sp 100%	Se 100% Sp 100%
A	22/84	1.0	1.0	1.0
B 2008	46/88	1.0	1.0	1.0
B 2009	27/100	1.0	1.0	1.0
C	10/101	1.0	1.0	1.0
D	68/100	1.0	1.0	1.0

[a] Probability that pathogen is present at the specified minimum expected prevalence (probability ≤0.05 indicates population is free from the pathogen at the minimum expected pathogen prevalence). Probability obtained using the hypergeometric formula described by Cameron and Baldock (1998) implemented with the FreeCalc version 2 software (Sergeant 2009b). Parameters used: Population size of 2,000 on each piggery; Minimum expected prevalence of 3.5%; Estimated values for test sensitivity and specificity based on culture technique.

Salmonella spp. and *Campylobacter jejuni* were found statistically to be present in the starling population sampled on piggery D with probabilities shown in Table 4.5 and 4.6, respectively. The absence of *Campylobacter jejuni* in the starling populations of piggeries A, B and C was statistically confirmed using a laboratory method sensitivity value of 100%; however, when sensitivity used was 60% and 90%, absence of this pathogen in the bird populations could not be confirmed (Table 4.6).

Table 4.5. Probability of presence of *Salmonella spp*. in the European starling populations at four piggeries in South Australia in 2008 and 2009.

		Probability of presence of the pathogen in the starling population at different test sensitivity (Se) and specificity (Sp)[a]		
Piggery	*Salmonella spp*. Positive/tested	Se 60% Sp 98%	Se 90% Sp 98%	Se 100% Sp 98%
A	0/84	**0.031**	**0.012**	**0.009**
B 2008	0/88	**0.026**	**0.01**	**0.007**
B 2009	0/100	**0.022**	**0.008**	**0.006**
C	0/101	**0.021**	**0.008**	**0.006**
D	6/100	0.92	0.82	0.777

[a] Probability that pathogen is present at the specified minimum expected prevalence (probability ≤0.05, shown in bold font, indicates population is free from the pathogen at the minimum expected pathogen prevalence). Probability obtained using the hypergeometric formula described by Cameron and Baldock (1998) implemented with the FreeCalc version 2 software (Sergeant 2009b). Parameters used: Population size of 2,000 on each piggery; Minimum expected prevalence of 3.5%; Estimated values for test sensitivity and specificity based on culture technique.

Table 4.6. Probability of presence of *Campylobacter jejuni* in the European starling populations at four piggeries in South Australia in 2008 and 2009.

		Probability of presence of the pathogen in the starling population at different test sensitivity (Se) and specificity (Sp)[a]		
Piggery	*Campylobacter jejuni* Positive/tested	Se 60% Sp 100%	Se 90% Sp 100%	Se 100% Sp 100%
A	0/84	0.164	0.065	**0.047**
B 2008	0/88	0.151	0.057	**0.041**
B 2009	0/100	0.158	0.061	**0.044**
C	0/101	0.155	0.059	**0.043**
D	3/100	0.896	0.717	0.648

[a] Probability that pathogen is present at the specified minimum expected prevalence (probability ≤0.05, shown in bold font, indicates population is free from the pathogen at the minimum expected pathogen prevalence). Probability obtained using the hypergeometric formula described by Cameron and Baldock (1998) implemented with the FreeCalc version 2 software (Sergeant 2009b). Parameters used: Population size of 2,000 on each piggery; Minimum expected prevalence of 3.5%; Estimated values for test sensitivity and specificity based on culture technique.

4.3.2 Prevalence of pathogens in the starling populations.

Table 4.7 shows apparent and true prevalence of pathogens included in this study among the starling population sampled on the different piggeries. *Escherichia coli* was present at

the highest apparent prevalence on piggery D (68.0%), with a corresponding true prevalence ranging from 68% to 100%, depending on the sensitivity of the diagnostic test (sensitivity of 100% and 60%, respectively). The lowest *Escherichia coli* true prevalence was found on piggery C, ranging from 9.9% to 16.5% for a test sensitivity of 100% and 60%, respectively. *Salmonella spp*. were present on piggery D with an apparent prevalence of 6% and true prevalence from 4.1% (sensitivity 100%) to 6.9% (sensitivity 60%). The six *Salmonella spp*. serotypes isolated on piggery D were: *Salmonella kottbus*, *Salmonella muenster*, two *Salmonella bredeney*, *Salmonella anatum* and *Salmonella oranienburg*. *Campylobacter jejuni* was present on piggery D at an apparent prevalence of 3% and a true prevalence ranging from 3.0% (sensitivity 100%) to 5.0% (sensitivity 60%).

Table 4.7. Apparent prevalence and true prevalence of *Escherichia coli*, *Salmonella spp*. and *Campylobacter jejuni* determined by culture of cloacal samples, in European starling populations at four different piggeries sampled in 2008 and 2009 in South Australia.

Piggery	Positive /tested	Apparent prevalence (%) (95% CI)	True prevalence (% along with 95% CI) of pathogens in the starling populations at different test sensitivities (Se)		
			Se 60%	Se 90%	Se 100%
Escherichia coli					
A	22/84	26.2 (18–36.5)	43.7 (29.2–61.3)	29.1 (19.5–40.9)	26.2 (17.5–36.8)
B 2008	46/88	52.3 (42–62.4)	87.1 (69.0–100.0)	58.1 (46.0–69.6)	52.3 (41.4–62.6)
B 2009	27/100	27.0 (19.3–36.4)	45.0 (31.2–60.7)	30.0 (20.8–40.4)	27.0 (18.7–36.4)
C	10/101	9.9 (5.5–17.3)	16.5 (8.5–28.4)	11.0 (5.6–19.0)	9.9 (5.1–17.1)
D	68/100	68.0 (58.3–76.3)	100.0 (96.8–100.0)	75.6 (64.5–85.3)	68.0 (58.1–76.7)
Salmonella spp.					
A	0/84	0 (0–4.4)	0[a] (0–3.9)	0 [a] (0–2.6)	0 [a] (0–2.3)
B 2008	0/88	0 (0–4.2)	0 [a] (0–3.5)	0 [a] (0–2.3)	0 [a] (0–2.1)
B 2009	0/100	0 (0–3.7)	0 [a] (0–2.7)	0 [a] (0–1.8)	0 [a] (0–1.6)
C	0/101	0 (0–3.7)	0 [a] (0–2.6)	0 [a] (0.1–1.7)	0 [a] (0–1.6)
D	6/100	6.0 (2.8–12.5)	6.9 (1.1–17.6)	4.5 (0.7–11.6)	4.1 (0.7–10.4)
Campylobacter jejuni					
A	0/84	0 (0–4.4)	0 (0–7.1)	0 (0–4.7)	0 [a] (0–4.2)
B 2008	0/88	0 (0–4.2)	0 (0–6.8)	0 (0–4.5)	0 [a] (0–4.1)
B 2009	0/100	0 (0–3.7)	0 (0–5.9)	0 (0–4.0)	0 [a] (0–3.6)
C	0/101	0 (0–3.7)	0 (0–5.9)	0 (0–3.9)	0 [a] (0–3.5)
D	3/100	3.0 (1–8.5)	5.0 (1.4–13.6)	3.3 (0.9–9.1)	3.0 (0.8–8.2)

Confidence intervals (CI) for true prevalence determined using Blakers analysis. Specificity = 100% for *Escherichia coli* and *Campylobacter spp*.; 98% for *Salmonella spp*.

[a] Statistically confident that the pathogen was determined to be absent from the starling population.

Piggery D had the highest level of pig pathogenic serotypes of *Escherichia coli*, with an apparent prevalence of 12.0%, as shown in Table 4.8. The true prevalence of these pathogenic serotypes in starling populations on piggery D was 12.0% (CI 6.6–19.7%), followed by the 8.0% prevalence among starlings sampled in piggery B in 2008 (CI 3.6–15.6%). Piggery C had the lowest true prevalence (1%; CI 0–5.1%).

Table 4.8. Apparent prevalence and true prevalence of pig pathogenic serotypes of *Escherichia coli* determined by culture of cloacal samples, in European starling populations at four different piggeries sampled in 2008 and 2009 in South Australia.

Piggery	Positive/tested	Apparent prevalence %	95% confidence interval	True prevalence %	95% confidence interval
A	3/84	3.6	1.2–10	3.6	1–9.7
B 2008	7/88	8	3.9–15.5	8	3.6–15.6
B 2009	4/100	4	1.6–9.8	4	1.4–9.7
C	1/101	1	0.2–5.4	1	0–5.1
D	12/100	12	7–19.8	12	6.6–19.7

Confidence intervals for true prevalence determined using Blakers analysis. Sensitivity = 100%, specificity = 100%.

A number of different pig pathogenic serotypes of *Escherichia coli* were identified among samples from starlings including 987P serotype O:9, K88 serotype O:8 and O:141 K85ab, as shown in Table 4.9. Some serotypes differed among piggeries, while K88 fimbrial type was common on piggery A, C and D.

Table 4.9. Pig pathogenic serotypes of *Escherichia coli* isolated from European starling populations at four different piggeries sampled in 2008 and 2009 in South Australia.

Piggery	Serotype
A	**3** K88 fimbrial types with non recognised serotype.
B 2008	**5** O9 – 987P serotypes. **1** 987P and 1 K99 fimbrial types with non recognised serotype.
B 2009	**4** O9 – 987P serotype.
C	**1** K88 fimbrial types with non recognised serotype.
D	**3** O8 – K88 serotypes. **1** O8 – 987P serotype **1** O141 – K85ab **6** K88 and 1 987P fimbrial types with non recognised serotype.

4.3.3 Disease and pest control history of piggeries

A description of the pig disease and pest control history of the piggeries included in this study is shown in Table 4.10. All four piggeries had persistent *Escherichia coli* infection in their pig herds, according to regular testing of faecal samples. *Salmonella spp*. and *Campylobacter spp*. were only detected on piggery D, where significant outbreaks of both pathogens had occurred in 2008 and 2009. Piggery A and C were both ecoshelter piggeries and, as such, practiced an all-in all-out production system, whereas piggeries B and D were both intensive piggeries practicing continuous flow production. All piggeries had other piggeries within a 20 km radius. Other facilities located within 20km included feed mills, feed lots, poultry units and garbage dumps.

Table 4.10. Health status and pest control for four piggeries in South Australia from 2007 to 2009.

	Piggery A	Piggery B	Piggery C	Piggery D
Piggery type	Ecoshelter	Intensive	Ecoshelter	Intensive
Production system	All-in all-out	Continuous flow	All-in all-out	Continuous flow
Food storage	Silos	Silos	Silos	Silos
Clothing/boot change	Clothing and boots. Not changed between shelters	Clothing and boots. Not changed between shelters	No clothing and boots provided	Clothing and boots. Not changed between shelters
Starling control	Window netting. Easily damaged. Considered ineffective	Nil	Nil	Nil
Other facilities within 20 km of the piggery	Two piggeries	Multiple piggery and poultry units; feedlots; feed mills; garbage dump	Multiple piggeries; olive and grape orchards; feed mills	Multiple piggery and poultry units; feed mills; garbage dump
***Escherichia coli* presence**	Yes, laboratory confirmed in 2008	Yes, laboratory confirmed. Outbreak in 2007	Yes, laboratory confirmed in 2008 and 2009	Yes laboratory confirmed 2008: 5–15% stock. 2009: 5% stock
***Escherichia coli* control**	Short acting penicillin for affected pigs	ECOvac[a] vaccination 2007. Short acting penicillin for affected pigs	Affected piglets treated with antibiotics in feed	Neomycin[b] injection to affected piglets
***Salmonella spp.* presence**	Not present, laboratory confirmed at slaughter in 2008	No – no clinical symptoms	No – no clinical symptoms	Yes laboratory confirmed 2008 – 25% stock 2009 – 20% stock
***Salmonella spp.* control**	Pressure clean and disinfect. Pig health monitoring scheme[c]	Nil	Nil	Amoxil[d] and Lincomycin[e] in water to affected pigs
***Campylobacter spp.* presence**	No – no clinical symptoms	No – no clinical symptoms	No – no clinical symptoms	Yes laboratory confirmed 2008 – 25% stock 2009 – 25% stock
***Campylobacter spp.* control**	Pressure clean and disinfect. Pig health monitoring scheme	Dimetradiazole in feed	Nil	Amoxil, Lincomycin and Dimetradazole[f] in feed

[a] Inactivated *Escherichia coli* vaccine; [bdef] antibiotics; [c] The scheme involves the examination of pig carcases, tissues and organs at slaughter. Disease reports are then sent to producers.

4.4 Discussion

Escherichia coli, *Salmonella spp*. and *Campylobacter spp*. are three bacterial pathogens of significance to the Australian pork industry. Previous studies have shown that birds can carry these bacterial pathogens and excrete them in their faeces (Morishita et al. 1999; Tizard 2004; Benskin et al. 2009). Starlings carrying these pathogens have been detected on intensive agricultural farms, including intensive poultry farms (Craven et al. 2000) and cattle feedlots in the United States (Benskin et al. 2009; Gaukler et al. 2009). However, the risk of transmission of these pathogens from starlings to pigs has not previously been investigated, nor have the pathogens been investigated in starling populations in Australia.

The presence of *Escherichia coli*, *Salmonella spp*. and *Campylobacter spp*. in starlings are not only important to the health of pigs, but also to the health of humans and other livestock. They are the predominant enteric pathogens in humans reported to the National Notifiable Disease Surveillance System in Australia (Blumer et al. 2003). The rate of infection for Salmonellosis has ranged from 33.2 to 90.7 infections per 100,000 individuals per month in 2011 in Australia (DHA 2012c). The rate of infection of *Campylobacter spp*. over this same period has ranged from 100.7 to 149.7 infections per month in 100,000 people (DHA 2012b). *Campylobacter jejuni* is the most common bacterial cause of food-borne disease in developed countries (Allos 2001). The consumption of contaminated pork is a proven route of infection (Beran 1995).

The current study detected these three pathogens in starling populations around four piggeries in the south of Australia. *Escherichia coli* were detected in starlings on all four piggeries, while *Salmonella spp*. and *Campylobacter spp*. were only detected on one piggery. The sample size was sufficiently large to detect the presence of these pathogens in the starling populations at the minimum expected prevalence of 3.5%. This minimum prevalence level was based on detection of these pathogens within birds from past studies. The sensitivity of the laboratory techniques used to determine the true prevalence of the pathogens was not determined for the culture techniques used in the current study, and thus were based on assumptions. Consequently, the resulting true prevalence values must be provided as a range, making comparison with other studies less accurate. A range of sensitivity values were adapted from literature, though the sensitivity value of 60% was

probably much lower than the actual value, as culture techniques are one of the most accurate methods available for pathogen detection (Eriksson and Aspan 2007).

The true prevalence of *Escherichia coli* varied greatly between piggeries, with the highest prevalence of *Escherichia coli* isolates, as well as pig pathogenic serotypes, found on the same piggery as the *Salmonella spp*. and *Campylobacter spp*. infected starlings (piggery D). Two previous studies identified *Escherichia coli* in starlings on cattle farms (Gaulker et al. 2009; Nielsen et al. 2004). While Nielsen et al. (2004) only identified *Escherichia coli* in a single starling out of an unknown number of total starlings sampled on a Danish cattle farm, Gaulker et al. (2009) reported a similar prevalence of these bacteria to the current study in starlings on a cattle feedlot in the United States (ranging from 38.0 to 83.3%). Morishita et al. (1999) determined an overall 21.4% prevalence of *Escherichia coli* in starlings in agricultural and urban settings, which was higher than that found among other species of birds, though within the prevalence range identified in the current study.

Prevalence of *Escherichia coli* in piggery B differed among birds caught in 2008 and 2009. Similar variation between years in *Escherichia coli* prevalence in starlings was observed by Gaulker et al. (2009); however, this difference was attributed to the different sampling methods used over the two years. The same sampling method was used in 2008 and 2009 in the current study. This shows that *Escherichia coli* level can vary greatly at a single location, and may be attributed to a number of different factors, including *Escherichia coli* level of infection in the pigs on the piggery. Piggery B experienced a diarrhoea outbreak related to *Escherichia coli* infection in late 2007, which aligns with the higher prevalence of these bacteria in starlings in 2008 compared to 2009. The level of infection of *Escherichia coli* in other livestock in the proximity of the piggery of study could also influence the prevalence of these bacteria in starlings.

Season also impacts upon the pathogen prevalence. Gaulker et al. (2009) determined that faecal material collected from starlings during summer was four times more likely to be positive for *Escherichia coli* than during winter. Starlings in the current study were only sampled during summer for both years. Assuming the summer temperature conditions and the faecal material sampling technique by Gaulker et al. (2009) are equivalent to the conditions and cloacal sample in the current study, the reported prevalence in the current study may be the highest level of infection and seasonal effects might not apply. Seasonal

affects on the prevalence of these pathogens in starlings is an area that may require further investigation.

Additional to the overall prevalence of *Escherichia coli*, the prevalence of pig pathogenic serotypes was also determined. Prevalence of pathogenic serotypes also differed by year. The common pathogenic serotypes O9;987P, O8;K88 and O8;987P are associated with diarrhoea in sucker pigs in Australia, while O141;K85ab is associated with diarrhoea in sucker and weaner pigs (Gyles, 1994). The remainder of the isolates were identified as the fimbrial types K88, K99 or 987P without recognised pig pathogenic O serotypes attached. These fimbrial types enable adherence and colonisation of epithelia in pigs, and are significantly correlated with pathogenicity and disease in pigs (Klemm 1985). Therefore, it can be assumed these isolates are also likely to be pathogenic despite the O serotype attachments not being identified as those most commonly isolated from pigs. Based on this information, the fimbrial types have been treated as pathogenic *Escherichia coli* in prevalence estimates in the results. This confirms that starlings are carrying a range of *Escherichia coli* serotypes that are infective and detrimental to pig health and production efficiencies in Australia.

Salmonella spp. have been frequently isolated from birds in the past 40 years, this is considered to have coincided with the increase of artificial feeding of birds using backyard bird feeders (Tizard 2004). *Salmonella spp*. have been isolated from starlings at a prevalence range of 0.7 to 8.8% in a number of different serological studies since the 1960s, none of which were conducted in Australia (Snoeyenbos et al. 1967; Morishita et al. 1999; Kirk et al. 2002; Gaukler et al. 2009; Carlson et al. 2011a). A similar prevalence of *Salmonella spp*. was found in the starling population sampled at piggery D in the current study (4.1 to 6.9%). In the same piggery, 20 to 25% of pigs were reported to be infected with *Salmonella spp*. during the time of bird sampling. The serotypes of *Salmonella spp*. infecting pigs on this piggery were unknown, and as such could not be compared with those in the starlings. This could have been rectified by analysing samples from domestic pigs at the time of starling sampling. The fact that the other piggeries (A, B and C) did not report *Salmonella spp*. infection in pigs and the starling populations were also negative for these bacteria, seems to indicate that the level of infection in starling populations around piggery D is in some way related to the infection in pigs. Carlson et al. (2011a) reported the same correlation on cattle feedlots in the United States: *Salmonella spp*. infection in

cattle and starlings on the same farms were reported. They took the serological study further and identified that removal of starlings resulted in a subsequent drop in *Salmonella spp*. contamination of cattle feed and water (Carlson et al. 2011b).

There were five *Salmonella spp*. serotypes isolated from the starlings on piggery D, one serotype isolated from two different starlings: *Salmonella kottbus*, *Salmonella muenster*, *Salmonella anatum*, *Salmonella oranienburg*, and two *Salmonella bredeney* isolates. Three of these serotypes (*Salmonella anatum, Salmonella bredeney* and *Salmonella kottbus*) have been isolated from pig carcasses at abattoirs in Australia (Hamilton et al. 2007). This suggests there is a correlation between serotypes present in starlings and pigs. Although the origin of the infection is unknown, these findings suggest there is a potential risk for pathogen transmission between pigs and starlings, with a subsequent public health implication.

Campylobacter spp. have been isolated from many wild bird species with a very wide prevalence range (3.0 to 90.0%; Benskin et al. 2009). Limited information is available on the prevalence of *Campylobacter spp*. in starlings. A previous study in Sweden (Palmgren et al. 1997) reported a 3.2% prevalence of this bacteria among migrating starling populations in urban environments. Craven et al. (2000) found a single positive result from five intestinal and two cloacal samples from starlings at a broiler chicken farm in Georgia (US). The current study provides an insight into the presence and approximate prevalence of infection of starling populations with *Campylobacter spp*. around piggeries in the south of Australia.

Campylobacter jejuni was present in starlings at a true prevalence range of 0.8 to 13.6% on piggery D, depending on the different assumed sensitivities of the diagnostic tests. Similar to the case for *Salmonella spp*., pigs from piggery D were reported to be infected with *Campylobacter spp*. during the time of the current study. As such, the level of infection in starling populations around piggery D seems to be related to the infection in pigs. Additionally, the absence of the pathogen in starlings on piggeries A, B and C, although likely, could not be confirmed when the sensitivity used to determine the true prevalence was less than 100% due to the potential for false negatives. As a result the two lower sensitivities of 60 and 90% provided p-values greater than 0.05, which meant the pathogen could not be statistically confirmed to be absent from the population. However it is likely

that the sensitivity of the culture method used in this work would be greater than 90%, based on slightly different culture techniques used for *Campylobacter spp*. compared to those reported in the literature (Patton et al. 1981). As such, the likelihood that the pathogen is present in piggeries A, B and C is low.

Results from the current study show that the prevalence of *Campylobacter spp*. in starlings is lower than in other wild birds. An average 37.0% prevalence of *Campylobacter spp*. among gull species (*Larus*), obtained from results of nine different studies, was reported in a review by Benskin et al. (2009). Despite the lower prevalence in starling populations, these birds should still be considered reservoirs of *Campylobacter jejuni* and a potential source of infection to pigs.

The starling population in piggery D had an overall higher presence of enteropathogens than starlings in the other three piggeries. Piggery D was located at a minimum of 80 km from the other piggeries, while piggery A, B and C were in closer proximity to each other. Other facilities located within 20 km of piggery D were similar to those reported for the other piggeries in this study, such as other piggeries, poultry farms, feed mills and garbage dumps. There is the potential for movement of starlings between piggeries and the other facilities in close proximity to the piggeries. LeJeune et al. (2008) demonstrated that starlings move between dairy farms in the United States, feeding and associating with cattle on the different farms. The starlings shared the same communal night-time roost, and travelled at least 20 km each day between the dairy farms and their night-time roost. The movement of starlings between piggeries and other facilities and the implications this might have for transmission of disease between facilities has not been studied in Australia.

The piggery type and production system may have been correlated to the prevalence of *Escherichia coli* burden in starlings. Piggery B and D, which had a higher prevalence of *Escherichia coli*, were both intensive piggeries, with a continuous flow of pig production, while piggery A and C were ecoshelters with an all-in all-out system. Continuous flow production is associated with an increased risk of pig infection with certain pathogens (Collins and Love 2003). The results of this study suggest that intensive piggeries may have a higher prevalence of enteropathogens among the pig population and associated starling population. A recent pig infection is also likely to be linked with the pathogen in

starlings. The increased pathogen prevalence in starling populations could interfere with control measures for diseases on these piggeries.

The detection of the three pathogens in starlings seemed to correspond to the presence of the pathogens in pigs. Consequently, any methods implemented to control infection within domestic pigs should also include management of starling numbers. Removal of starlings from agricultural enterprises, or prevention of starling access to animal feed and water, could substantially reduce the risk of transmission of enterobacterial pathogens from starlings to livestock. Unfortunately there is no method currently available in Australia to substantially reduce starling numbers. The avicide, DRC-1339 is currently being used in the United States successfully, as demonstrated by Carlson et al. (2011b), on cattle feedlots. Until starling populations and access to piggery interiors can be effectively restricted, control of infection in pigs from these and other pathogens will be impaired. The magnitude of the risk of transmission of these pathogens from starlings to pigs has been evaluated in Chapter 7.

5 Rodent biosecurity threat to piggeries

5.1 Introduction

Rodents are well recognised as the source of infection for major outbreaks in humans and livestock of a number of infectious diseases, including Bubonic plague and Leptospirosis (Alderton 1996; Smythe et al. 2000). In Australia, rodents have been shown to be reservoirs of a large number of pathogens, including bacteria and parasites (Le Moine et al. 1987; Amass and Clark 1999; Henderson 2009). As rodents thrive in locations where food is readily available, such as intensive livestock farms, their presence in substantial numbers can impact health and economic return. Their economic significance for farm enterprises is due to infrastructural damage, food loss and production loss. The presence of rodents also poses a risk of inter-species pathogen transmission. It is documented that each rodent can produce an average of 50 faecal deposits a day (Drummond, 2001) and one study recorded an average of 80 rodent faeces deposits per metre squared of stored feed per month on a number of different cattle farms in Scotland (Daniels et al. 2003). This contamination of food and the environment is of concern due to the presence of disease-causing organisms in rodent excrement (Twigg 1975). Rats (Black rat: *Rattus rattus* and Brown/Norway rat: *Rattus norvegicus*) and house mice (*Mus musculus*) have been shown to be reservoirs of many pathogens that can infect pigs, including endemic production-limiting pathogens as well as zoonotic pathogens (Le Moine et al. 1987; Amass, 1999; Henderson, 2009). As such, they pose a biosecurity threat to piggeries for the introduction, persistence, and reintroduction of a number of important pig pathogens.

The four bacteria *Brachyspira hyodysenteriae*, *Lawsonia intracellularis*, *Salmonella spp*. and *Brachyspira pilosicoli* cause illnesses associated with gastrointestinal disruption leading to diarrhoea and a multitude of other symptoms that can negatively affect piggery productivity, and in some cases result in mortality. *Brachyspira hyodysenteriae* and *Lawsonia intracellularis* are arguably the two most important pathogens in Australian piggeries. *Brachyspira hyodysenteriae* is the causative pathogen of swine dysentery in pigs (Taylor 2006). This disease is one of the most economically important pig diseases in Australia (Cutler and Gardiner 1988). Polson et al. (1992) showed a difference in profit

margin of US $5.77 per 100 kg live weight pig between a swine dysentery infected piggery and a non-infected piggery. Despite it being known that rodents can be infected with this pathogen, the role that rats and mice have in the maintenance and reintroduction of swine dysentery on piggeries is poorly understood.

Proliferative enteropathy, the disease caused by the pathogen *Lawsonia intracellularis*, is another economically important pig disease, which is present in the majority of Australian pig herds (Holyoake et al. 2010b). Holyoake et al. (2010a) estimated the cost of a subclinical and clinical infection of *Lawsonia intracellularis* to be AU $8.33 and AU $13.00 per slaughtered pig, respectively. This pathogen has been isolated from wild rodents in a piggery in the Czech Republic (Friedman et al. 2008). *Lawsonia intracellularis* has not been investigated previously in wild rodents in Australia, and limited information is available on prevalence of this pathogen in wild rodents in piggeries.

The first objective of this study was to detect the presence of the pathogen *Brachyspira hyodysenteriae* in rat and mice populations on three large-scale commercial piggeries that had previously applied control methods for eradication of swine dysentery in their pig herds. Finding an infected rodent would indicate reinfection of pigs from a rodent source could be possible. The second objective was to detect the presence of the gastrointestinal pathogens *Lawsonia intracellularis*, *Salmonella spp*. and *Brachyspira pilosicoli* in rats and mice on these three piggeries.

5.2 Materials and methods

5.2.1 Study location and sample size

Three piggeries were selected using a non-random method based on the method implemented for control of swine dysentery in pig populations. Straw et al. (2006) provides an outline of the effective control measures for swine dysentery, which loosely fit into three main categories: control via medication without piggery depopulation, and control with full or partial depopulation (Swiss depopulation). Control via medication involves the administration of one of four drugs: Tiamulin, Valnemulin, Tylosin and Lincomycin, which are often administered in feed or in drinking water to adult pigs. Total depopulation involves removal of all animals from the piggery, disinfection of the piggery, and repopulation from uninfected stock. The Swiss depopulation method involves the

removal of only the high risk animals, such as the adult breeding stock, medication of remaining stock, and reintroduction of medicated animals and replacement stock from swine dysentery-free herds.

Two Victorian piggeries were chosen: a piggery (A) with medication against the pathogen, and a piggery (B) where a total depopulation and repopulation was applied 18 months prior to rat capture. The third piggery (C), which was located in South Australia, had undergone a Swiss depopulation 3 months prior to rat capture. No mice were detected on any of the piggeries included in this study, so all subsequent methods and results relate to rats.

Sixty rats were obtained from piggery A to detect the presence of pathogens, assuming a minimum expected prevalence in the rat population of 5%. One hundred and twenty rats were obtained from each of the piggeries B and C to detect the presence of pathogens, assuming a minimum expected prevalence in the rat population of 2.5%. The minimum expected prevalence used for piggery A was higher compared to piggeries B and C as this piggery was known to be swine dysentery infected and using in-feed medication.

The sample size was calculated using the same methods shown in Chapter 4, Section 4.2.1.

5.2.2 Sampling the rat population

Between September and December 2009, a total of 300 rats were trapped or shot on the three piggeries included in the study as part of routine rodent control practices on each piggery. Live traps were laid inside piggeries in the walking aisles and along beams on walls for ten nights of trapping, prior to shooting. The live traps were baited with a mixture of peanut butter, oats, flour, honey and water (recommended by Luke Leung, University of Queensland). Traps were checked daily and the bait was refreshed every second day. Rats caught in live traps were euthanised humanely. Rats were shot inside the piggeries over one or two nights using .22 rifles and rat shot ammunition. All rats obtained in this capture exercise were frozen at −20 ºC immediately after removal from the piggery.

5.2.3 Piggery management and disease control history

Information on pig health status and disease and pest control programs implemented on each piggery was obtained through administration of a written questionnaire to each of the piggery managers after sampling of the rats and laboratory tests were performed (Appendix

3). A single page letter outlining results obtained from the wild rats captured on their piggery was also attached to the four page questionnaire. The questionnaire contained open questions covering pig disease history and pest control management. Questions on disease history focused on information regarding the history of the presence of the pathogens of interest in this study, including how the pathogen presence was confirmed, and the medication used to control illness. Moreover, specific questions on the methods of control used for swine dysentery were also asked. Questions on rodent pest control obtained information about differences in the measures applied before and after swine dysentery disease control. The general husbandry questions requested information on the herd size, piggery production type (e.g. intensive, free-range or ecoshelter) and any other potential food sources for rodents within a 5 km radius of the piggery. The majority of pig health results were laboratory confirmed, and such were not affected by any potential bias created by knowing the rat results prior to questionnaire completion.

5.2.4 Laboratory testing

5.2.4.1 DNA extraction

Just prior to laboratory testing, rats were thawed and the large intestine was removed. Intestinal lining material and faecal material were obtained from the intestinal wall using a scalpel. Approximately 0.2 g of this intestinal material was placed in a 2 ml eppendorf tube and refrigerated prior to DNA extraction.

A QIAamp DNA Stool Mini Kit (Qiagen), supplying all necessary preparations and materials, was used for isolation of DNA from stool for pathogen detection according to the manufacturer's instructions. In summary, 0.18 to 0.22 g stool samples were homogenised and lysed in Buffer ASL for one min, incubated for five min at 70 ºC, homogenised for a further 15 sec, then centrifuged for one min at 20,000 × gravitational force (G). An inhibitEX Tablet was added to 1.2 ml of the supernatant and vortexed continuously for one min to absorb inhibitors. The sample was centrifuged for three min at 20,000 × G, the pellet discarded and the supernatant centrifuged for a further three min on 20,000 × G. Proteinase K (15 µl) was added to 200 µl of the supernatant, followed by 200 µl Buffer AL, vortexed for 15 sec and incubated at 70 ºC for 10 min. Ethanol (200 µl) was then added, the mixture briefly vortexed, and then the mixture was applied to a QIAamp spin column (with a membrane inside) and centrifuged at 20,000 × G for one min. The sediment retained by the membrane was washed with two buffer solutions, Buffer AW1

(500 µl) and Buffer AW2 (500 µl), each followed by a centrifuge step. To eluate, Buffer AE (200 µl) was added to the QIAamp membrane and centrifuged at 20,000 × G for one min. The eluate was pipetted back onto the QIAamp membrane and centrifuged for a further one min at 20,000 × G. The 200 µl solution containing DNA was stored at 4 ºC prior to PCR analysis for a period no longer than 4 weeks.

5.2.4.2 Multiplex PCR

A multiplex PCR that detects the four pathogens, *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli*, *Salmonella spp*., and *Lawsonia intracellularis* was used, following the methodology previously described by Elder et al. (1997). The primer sequences used are shown in Table 5.1.

Table 5.1. Primers used for a multiplex PCR amplification of *Brachyspira hyodysenteriae*, *Lawsonia intracellularis, Salmonella spp*. and *Brachyspira pilosicoli* (modified from Elder et al. 1997).

Agent	Primer sequences	Product size (bp)	Reference
Brachyspira hyodysenteriae	F 5' GGTACAGGCGGAAACAGAC 3' R 5' TCCTATTCTCTGACCTACTG 3'	1557	Elder et al. 1994
Lawsonia intracellularis	F 5' TATGGCTGTCAAACACTCGG 3' R 5' TGAAGGTATTGGTATTCTCC 3'	319	Jones et al. 1993
Salmonella spp.	F 5' TGCCTACAAGCATGAAATGG 3' R 5' AAACTGGACCACGGTGACAA 3'	457	Stone et al. 1994
Brachyspira pilosicoli	F 5' CATAAGTAGAGTAGAGGAAAGTTTTT 3' R 5' CTCGACATTACTCGGTAGCAACAG 3'	930	Fellstrom et al. 1997

Reactions were performed in a 25 µl total reaction volume, containing 12.5 µl of Qiagen Multiplex HotStart Mastermix Taq, 6.5 µl of nuclease-free water (Qiagen), 0.5 µl of both forward and reverse primers (Sigma Aldrich; *Brachyspira hyodysenteriae* primers final concentration 0.1 µM, other primers final concentration 0.2 µM) for *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli*, *Salmonella spp*., and *Lawsonia intracellularis.* Two µl of extracted DNA sample was then added. For the negative control 2 µl of nuclease-free water was added.

The PCR cycling involved an initial Taq polymerase activation step of 15 min at 95 ºC followed by 30 cycles of 45 sec at 94 ºC, 90 sec at 60 ºC, and 2 min at 72 ºC. A final extension step was performed for 15 min at 68 ºC. Eight µl of PCR product and 2 µl of

loading buffer were analysed by electrophoreses on a 1% agarose gel (ProBiogen) stained with ethidium bromide (Astral Scientific, Ohio, USA).

5.2.4.3 Nox PCR

A NADH (Nicotinamide adenine dinucleotide) oxidase gene (nox) PCR was used to specifically detect *Brachyspira hyodysenteriae* and confirm the multiplex PCR finding for this pathogen (Atyeo et al. 1999). This method is also able to correctly identify some non-pathogenic *Brachyspira spp.* that may be classified as a pathogenic *Brachyspira spp*., such as *Brachyspira hyodysenteriae* using the multiplex PCR method. The use of this nox PCR method in addition to the standard PCR method is recommended by Atyeo et al. (1999). The primers used for specific amplification of *Brachyspira hyodysenteriae* are as follows: F 5' TTAAAACAAGAAGGAACTACT 3'; R 5' CTAATAAACGTCTGCTGC 3' (Atyeo et al. 1999).

Reactions were performed in a 25 µl total reaction volume, containing 12.5 µl of Qiagen Multiplex HotStart Mastermix Taq, 6.0 µl of nuclease-free water (Qiagen), 1.0 µl of both forward and reverse primers (Sigma Aldrich). Two and a half µl of extracted DNA sample was then added. For the negative control 2.5 µl of nuclease-free water was added.

The PCR cycling involved an initial Taq polymerase activation step of 15 min at 95 ºC followed by 30 cycles of 30 sec at 94 ºC, 60 sec at 45 ºC, and 2 min at 72 ºC. A final annealing step was performed for 60 sec at 48 ºC, followed by an extension step for 10 min at 72 ºC. Eight µl of PCR product and 2 µl of loading buffer were analysed by electrophoreses on a 1% agarose gel (ProBiogen) stained with ethidium bromide (Astral Scientific, Ohio, USA).

5.2.4.4 Quantitative real-time PCR for Lawsonia intracellularis

All rat DNA samples (n = 299) were subject to an additional real-time qPCR (Collins et al. 2011), using published primers and a Taq Man probe from Nathues et al. (2009), for the detection of *Lawsonia intracellularis*. Methods are described in full in Collins et al. (2011).

5.2.5 Data analysis

5.2.5.1 Calculation to substantiate freedom from pathogen and to estimate prevalence

Calculations to substantiate freedom from a pathogen and to estimate true prevalence were conducted as for Chapter 4, Section 4.2.5.1.

The sensitivity and specificity for the laboratory tests used are summarised in Table 5.2. The sensitivity and specificity of the multiplex PCR method used was not determined by the diagnostic laboratory. Therefore, as the methods used in this study were based on those developed by Elder et al. (1997), test sensitivity (100%) and specificity (98.2%) of the multiplex PCR for all pathogens were assumed to be the same as reported by these authors. As the sensitivity and specificity of the nox PCR method were unknown and not available in the literature, two different values (95% and 100%) of sensitivity and specificity were used to calculate freedom from the pathogen, based on values used for the multiplex PCR method. The sensitivity and specificity of the *Lawsonia intracellularis* real-time qPCR were 99% and 97%, respectively (Collins et al. 2011).

Table 5.2. Specifications of the diagnostic tests used to determine the true prevalence of different pathogens in the rat population in two piggeries in Victoria and one piggery in South Australia in 2009.

Pathogen	Diagnostic test	Sensitivity	Specificity	Reference
Brachyspira hyodysenteriae, *Brachyspira pilosicoli*, *Salmonellae spp*. and *Lawsonia intracellularis*	Multiplex PCR	100%	98.2%	Elder et al. (1997)
Brachyspira hyodysenteriae,	nox PCR	95%, 100%	95%, 100%	Based on Multiplex PCR
Lawsonia intracellularis	Real-time qPCR	99%	97%	Collins et al. 2011

nox – Nicotinamide adenine dinucleotide oxidase genes

5.3 Results

No mice were detected on any of the piggeries included in this study. A total of 300 rats were sampled and intestinal material from 299 rats was analysed, as the DNA sample from one rat could not be used.

5.3.1 Probability of presence of pathogens in the rat populations.

5.3.1.1 Multiplex PCR

Brachyspira hyodysenteriae and *Brachyspira pilosicoli* were not detected in any of the rat samples collected on the three piggeries using the multiplex PCR method. The absence of these pathogens from the rat populations was statistically confirmed (Table 5.3). *Lawsonia intracellularis* and *Salmonella spp*. were present in the rat populations on all piggeries, except for *Lawsonia intracellularis* on piggery C ($P = 0.005$; Table 5.3).

Table 5.3. Probability of presence of *Lawsonia intracellularis*, *Salmonella spp*, *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* in the rat populations at three piggeries in Australia in 2009. Rat samples were analysed using a Multiplex PCR.

Piggery	Positive/tested	Probability of presence of pathogen in the rat population [a]
Lawsonia Intracellularis		
A	1/60	0.080
B	11/119	0.996
C	0/120	**0.005**
Salmonella spp.		
A	5/60	0.788
B	2/119	0.110
C	2/120	0.107
Brachyspira hyodysenteriae		
A	0/60	**0.015**
B	0/119	**0.005**
C	0/120	**0.005**
Brachyspira pilosicoli		
A	0/60	**0.015**
B	0/119	**0.005**
C	0/120	**0.005**

[a] Probability that pathogen is present at the specified minimum expected prevalence (probability ≤0.05, shown in bold font, indicates population is free from pathogen at the minimum expected pathogen prevalence). Probability obtained using the hypergeometric formula described by Cameron and Baldock (1998) implemented with the FreeCalc version 2 software (Sergeant 2009b). Parameters used: Population size of 2,000 on each piggery; Minimum expected prevalence of 5% on piggery A and 2.5% on piggery B and C; Test sensitivity and specificity of 100% and 98.2%, respectively.

5.3.1.2 Nox PCR Brachyspira hyodysenteriae

Brachyspira hyodysenteriae was not detected using the nox PCR method in any of the rat samples collected on the three piggeries. The absence of this pathogen from the rat populations was statistically proven depending on the sensitivity and specificity of the test used. As shown in Table 5.4, when the sensitivity and specificity of the diagnostic test were assumed 95% and 100% respectively, freedom from the pathogen could not be proved.

Table 5.4. Probability of presence of *Brachyspira hyodysenteriae* in the rat populations at three piggeries in Australia in 2009. Rat samples were analysed using a nox PCR.

		Probability of presence of the pathogen in the rat population at different test sensitivity (Se) and specificity (Sp)[a]			
Piggery	*Brachyspira hyodysenteriae* Positive/tested	Se 95% Sp 95%	Se 95% Sp 100%	Se 100% Sp 95%	Se 100% Sp 100%
A	0/60	**0.002**	0.052	**0.002**	**0.044**
B	0/119	**<0.001**	0.053	**<0.001**	**0.045**
C	0/120	**<0.001**	0.051	**<0.001**	**0.044**

[a] Probability that pathogen is present at the specified minimum expected prevalence (probability ≤0.05, shown in bold font, indicates population is free from pathogen at the minimum expected pathogen prevalence). Probability obtained using the hypergeometric formula described by Cameron and Baldock (1998) implemented with the FreeCalc version 2 software (Sergeant 2009b). Parameters used: Population size of 2,000 on each piggery; Minimum expected prevalence of 5% on piggery A and 2.5% on piggery B and C; Estimated values for test sensitivity and specificity based on Multiplex PCR.

5.3.1.3 Quantitative real-time PCR Lawsonia intracellularis

Lawsonia intracellularis was found statistically to be present on all three piggeries using the real-time qPCR (Table 5.5). Methods and results for the detection of this pathogen using the real-time qPCR have been published by Collins et al. (2011).

Table 5.5. Probability of presence of *Lawsonia intracellularis* in the rat populations at three piggeries in Australia in 2009. Rat samples were analysed using a quantitative real-time PCR.

Piggery	*Lawsonia intracellularis* Positive/tested [a]	Probability of presence of pathogen is the rat population [b]
A	50/60	1.000
B	84/119	1.000
C	5/120	0.363

[a] Positive/tested results from reference: Collins et al. (2011). Colonisation and shedding of *Lawsonia intracellularis* in experimentally inoculated rodents and in wild rodents on pig farms. Veterinary Microbiology, 150, p 384–388.

[b] Probability that pathogen is present at the specified minimum expected prevalence (probability ≤ 0.05 indicates population is free from pathogen at the minimum expected pathogen prevalence). Probability obtained using the hypergeometric formula described by Cameron and Baldock (1998) implemented with the FreeCalc version 2 software (Sergeant 2009b). Parameters used: Population size of 2,000 on each piggery; Minimum expected prevalence of 5% on piggery A and 2.5% on piggery B and C; Test sensitivity and specificity of 99% and 97%, respectively.

5.3.2 Prevalence of pathogens in the rat populations.

5.3.2.1 Multiplex PCR

The apparent and true prevalence of *Lawsonia intracellularis* and *Salmonella spp*. is shown in Table 5.6. *Lawsonia intracellularis* was detected on piggery A and B with a true prevalence of 0% (CI 0–6.9%) and 7.6% (CI 3.1–14.2%), respectively. *Salmonella spp*. were detected in rats caught on all piggeries with a true prevalence of 6.7% (CI 1.6–16.4%) among rats on piggery A and 0% (CI 0–3.9%) on piggery B and C.

Table 5.6. Apparent prevalence and true prevalence of *Lawsonia intracellularis* and of *Salmonella spp*. determined by multiplex PCR reaction assay, in rat populations at three different piggeries sampled in 2009 in Australia.

Piggery	Positive/tested	Apparent prevalence %	95% confidence interval	True prevalence %	95% confidence interval
Lawsonia Intracellularis					
A	1/60	1.7	0.3–8.9	0	0–6.9
B	11/119	9.2	5.2–15.8	7.6	3.1–14.2
C	0/120[a]	0	0–3.1	0	0–1.2
Salmonella spp.					
A	5/60	8.3	3.6–18.1	6.7	1.6–16.4
B	2/119	1.7	0.5–5.9	0	0–3.9
C	2/120	1.7	0.5–5.9	0	0–3.9

Confidence intervals for true prevalence determined using Blakers analysis. Sensitivity = 100%, specificity = 98.2%;

[a] Statistically confident that the pathogen was determined to be absent from the rat population.

5.3.2.2 Quantitative real-time PCR Lawsonia intracellularis

Lawsonia intracellularis was detected in rats caught on all piggeries using a real-time PCR assay (Table 5.7). It was detected at a true prevalence of 83.7% for piggery A, 70.4% for piggery B and 1.2% for piggery C.

Table 5.7. Apparent prevalence and true prevalence of *Lawsonia intracellularis* determined by real-time PCR assay in rat populations at three different piggeries sampled in 2009 in Australia.

Piggery	Positive/tested[a]	Apparent prevalence %	95% confidence interval	True prevalence %	95% confidence interval
Lawsonia Intracellularis					
A	50/60	83.3	72–90.7	83.7	71.9–92.1
B	84/119	70.6	61.9–78	70.4	61.3–78.5
C	5/120	4.2	1.8–9.4	1.2	0–6.5

[a] Positive/tested results from reference: Collins et al. (2011). Colonisation and shedding of *Lawsonia intracellularis* in experimentally inoculated rodents and in wild rodents on pig farms. Veterinary Microbiology, 150, p 384–388.

Confidence intervals for true prevalence determined using Blakers analysis. Sensitivity = 99%, specificity = 97%.

5.3.2.3 A comparison of Lawsonia intracellularis true prevalence determined by enteric multiplex PCR and real-time PCR.

The true prevalence of *Lawsonia intracellularis* in the rat population using the quantitative real-time PCR method was greater than that obtained using the multiplex PCR for the three piggeries. Figure 5.1 shows the true prevalence of this pathogen obtained using both diagnostic tests. The real-time PCR detected a true prevalence 83.7%, 62.8% and 1.2% higher than the multiplex PCR for piggery A, B and C, respectively.

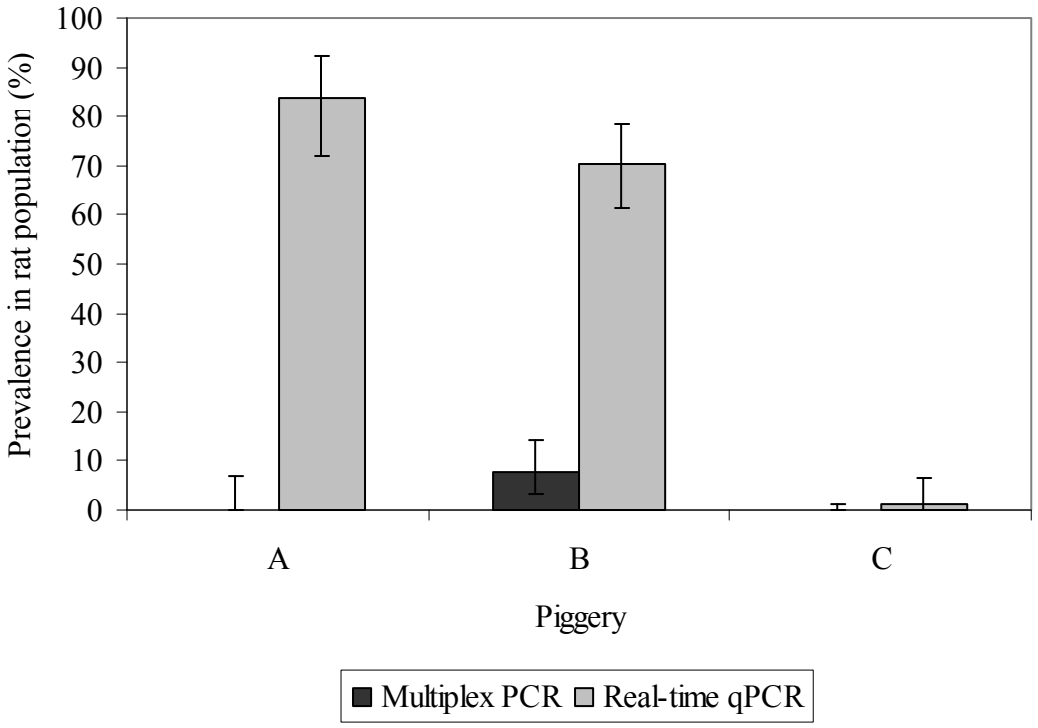

Figure 5.1. True prevalence of *Lawsonia intracellularis* in the rat population in three piggeries determined using a Multiplex PCR method (sensitivity = 100%, specificity = 98.2%) and a Quantitative real-time PCR method (sensitivity = 99%, specificity = 97%).

5.3.3 Piggeries health management and disease control

Piggery characteristics in relation to health management and disease control are shown in Table 5.8. All piggeries utilised a continuous flow production system and had other food sources within a 5km radius. All three piggeries utilised baiting and occasional shooting to control rat populations.

All three piggeries had confirmed swine dysentery in the past and chosen to undertake a control method to reduce the impacts of the disease in their piggery. Piggery A controlled swine dysentery in their pig herd from 2003 with dimetradazole (nitroimidazole antibiotic) in-feed. Piggery B applied a total depopulation technique spanning over 2007 and 2008 for control of swine dysentery in their pig herd. Piggery C applied a Swiss depopulation technique spanning over 2008 and 2009 for control of swine dysentery in their pig herd.

On piggery A, *Salmonella spp*. were present in the pigs determined by pig isolates prior to swine dysentery control and during the time of rat collection. *Lawsonia intracellularis*, causative bacteria for proliferative enteropathy, was present in pigs on the piggery at the time of rat collection despite using in-feed medication with the antibiotics tiamulin, chlortetracycline or amoxycillin. This was confirmed by histopathology in thickened ileal mucosa of pigs at the abattoir prior to and after swine dysentery control. *Brachyspira pilosicoli* was assumed to be present in the pigs based on clinical symptoms and colitis prior to swine dysentery control and during the time of rat collection.

Salmonella spp. were not detected on piggery B from pig isolates prior to or at the time of rat capture; however, a confirmed outbreak of Salmonella occurred on this piggery shortly afterwards (January 2010). Clinical proliferative enteropathy was present in pigs on the piggery at the time of rat collection despite using in-feed medication with tiamulin, chlortetracycline or amoxicillin. *Lawsonia intracellularis* was confirmed in randomly collected faeces from grower and finisher pigs via PCR prior to rat capture. *Brachyspira pilosicoli* was not detected on farm by piggery managers or veterinarian, based on the absence of clinical symptoms prior to or at the time of rat capture.

Salmonella spp. were confirmed to be present in pigs on piggery C based on pig isolates prior to swine dysentery control and during the time of rat collection. There were no clinical symptoms of clinical proliferative enteropathy present in pigs on the piggery at the time of rat collection. *Brachyspira pilosicoli* was assumed to be present in the pigs by the piggery manager and veterinarian, based on clinical symptoms prior to swine dysentery control and during the time of rat collection.

Table 5.8. Health status and pest control for three piggeries: two located in Victoria, and one located in South Australia from 2003 to 2009.

	Piggery A	Piggery B	Piggery C
Intensive piggery size (breeding sows)	2,500	11,600	7,500
Production system	Continuous flow	Breeder continuous, all-in all-out by shed	Breeder continuous, all-in all-out by shed
Rat control	Bait and shoot	2 hours per week dedicated to baiting. Occasional shooting	Baits were checked twice a week prior to Swiss depopulation. Baiting became irregular post Swiss depopulation. Shooting
Other food sources within 5 km radius	Multiple intensive piggery and poultry units. One swine dysentery positive piggery within 7 km	Multiple intensive poultry units.	One feed mill
Time since last confirmed *Brachyspira hyodysenteriae*	February 2003	October 2005	2005
Date of control implementation for *Brachyspira hyodysenteriae*	March 2003	Start 18th October 2007. Restock 24th March 2008	February 2008 – July 2009.
Control method	Medication	Total depopulation	Swiss depopulation
***Salmonella spp.* presence prior to and at time of rat collection (y/n)**	Yes, laboratory confirmed in 2003 and 2009	No	Yes, laboratory confirmed in 2008 and 2009
***Lawsonia intracellularis* presence (y/n)**	Yes – clinical, PCR verified in 2009	Yes – clinical, PCR verified in 2009	Yes – sub clinical, PCR verified in 2008 and 2009
***Brachyspira pilosicoli* presence (y/n)**	Suspected – clinical symptoms in 2009	No – no clinical symptoms	Suspected – clinical symptoms in 2008 and 2009

5.4 Discussion

Previous studies have identified that rats can be infected with a number of important pathogens that can affect pigs (Hampson et al. 1991; Friedman et al. 2008; Joens 1980). However, the risk of transmission of these pathogens from rodents to pigs has not previously been investigated in detail. The foundation knowledge required to assess this risk includes identifying the biosecurity threat rats pose to piggeries for the introduction, persistence and reintroduction of pathogens; as well as identifying the prevalence of these pathogens in the rat populations on piggeries.

To further previous research, the current study investigated the presence of *Brachyspira hyodysenteriae* in the rat population on three piggeries that had undertaken various procedures to control and eradicate swine dysentery from the pigs. *Brachyspira hyodysenteriae* was not detected in the large intestine of any of the 299 rats obtained from the three piggeries included in this study. The results of the multiplex PCR were confirmed by the use of the nox PCR method which did not detect any *Brachyspira hyodysenteriae* pathogens either.

The nox PCR was developed to identify only the pathogenic *Brachyspira spp*. serotype *hyodysenteriae*, whereas the multiplex PCR may identify other *Brachyspira spp*. It is also sensitive enough to detect as few as 10^{-10} *Brachyspira hyodysenteriae* organisms per gram of faeces (Atyeo et al. 1999) whereas the multiplex PCR has been proven to be sufficiently sensitive to detect between 0.1 and 1 *Brachyspira hyodysenteriae* organisms per gram of faeces (Elder, 1994). The only instance in which the likelihood of the pathogen being absent from the population was not statistically confirmed was if the nox PCR method had a sensitivity of 95% and a specificity of 100%, although the P-values were very close to 0.05. As the nox PCR is able to detect fewer *Brachyspira hyodysenteriae* organisms per gram of faeces than the multiplex PCR, the sensitivity is likely to be close to 100%. As such, considering the estimated population size, the number of rats sampled on each piggery, and the sensitivity and specificity of the diagnostic tests, the results indicate that the rat population was free of *Brachyspira hyodysenteriae* at the minimum expected prevalence of 5% on piggery A and 2.5% on piggery B and C.

Previous studies have reported the presence of *Brachyspira hyodysenteriae* in one rat on an Australian piggery out of 44 sampled (Hampson et al. 1991) and mice on piggeries in the United States (Joens and Kinyon 1982) at a prevalence of 2.5%, and in Sweden at a prevalence of 37.5% (Fellström et al. 2004). The sample size in the current study was sufficiently large to prove freedom from the pathogen in the rat population at the minimum expected prevalence with 95% confidence. Although the study cannot prove pathogen freedom if the pathogen is present at a lower prevalence, previous studies support the minimum expected prevalence chosen for the sample size calculations. Moreover, only a low level of infection in the rat populations in our study would have been needed for detection of the pathogen using both PCR diagnostic tests.

Joens (1980) has shown that *Brachyspira hyodysenteriae* exists in the intestines of a mouse for 180 days after infection. This carrier state duration has not been estimated for rats. Negative detection of *Brachyspira hyodysenteriae* in the current study suggests that the pathogen, if present in the rat population before swine dysentery control in pigs, has not replicated and maintained in the rat population over the period of time since its control, or alternatively, the pathogen in rats has been controlled and eliminated by the medication given to pigs on piggery A and C.

Brachyspira pilosicoli was also not detected in any rats caught on the three piggeries in this study. The presence of *Brachyspira pilosicoli* in rats has not been previously detected, though it has been detected in other animal species, including 18% of exotic waterbirds in a botanical garden in Perth, Australia (Oxberry et al. 1998) and 8% of wild ducks captured at a piggery in Australia (Oxberry and Hampson, 2003). The presence of this pathogen in the domestic pigs on the piggeries at the time of rat collection was also unconfirmed. This could have been rectified by analysing samples from the domestic pigs at the time of rat sample analysis.

Rat populations on piggeries A, B and C were identified as positive for *Lawsonia intracellularis* via the real-time qPCR at different true prevalence levels. The prevalence was higher on piggery A and B where the pigs were exhibiting clinical symptoms of proliferative enteropathy, unlike piggery C where the pigs were not exhibiting symptoms. The prevalence of this pathogen on piggery A and B was notably higher than the 17% reported for rats caught on piggeries in the Czech Republic, where pigs had proliferative

enteropathy (Friedman et al. 2008). However, this prevalence was obtained by a single positive rat sample out of six rats caught and sampled from four different piggeries. Despite the low number of rats sampled by Friedman et al. (2008), the presence of *Lawsonia intracellularis* in rats from infected piggeries was confirmed. Where clinical disease is present in a piggery, it seems likely that infected rats will be identified.

The real-time qPCR was also capable of determining the amount of bacteria being excreted in faeces by the rats. Of the infected rats on piggery A, 40% were excreting greater than 10^5 *Lawsonia intracellularis* organisms per gram of faeces, and on piggery B this proportion was 18% (Collins et al. 2011). One rat was even excreting as many as 10^{10} organisms per gram of faeces. Pigs dosed with 10^5 *Lawsonia intracellularis* organisms develop infection (Collins and Love 2007). Due to this high amount of bacterial excretion in rats, a single faecal deposit from approximately 40% of rats on a piggery with proliferative enteropathy would be sufficient to infect pigs. This has implications on control of *Lawsonia intracellularis* in piggeries. Control of the disease through destocking or medication can not be a gradual process as infected rats can retransmit the infection to naïve pigs, recirculating the infection.

The 299 rat DNA samples were also subjected to an older detection method, the multiplex PCR. This method was not capable of determining the quantity of bacteria in rat excrement as with the real-time qPCR. Using the multiplex PCR *Lawsonia intracellularis* was found statistically to be present in rats on piggeries A and B at a much lower true prevalence than that found using the real-time qPCR method. The reason for this variation in prevalence estimates between these two methods may be for two different reasons: the differences in the level of detection of the organism per gram of faeces, or the performance of the multiplex PCR below published standards.

The sensitivity and specificity of the two PCR methods are quite similar (sensitivity 100% and specificity 98.2% for the multiplex PCR; 99% sensitivity and specificity 97% for the real-time qPCR); however, the detection levels are different. The multiplex PCR was shown to detect 10^3 organisms per gram of faeces (Jones et al. 1993), while the new real-time qPCR method to date has been tested at a detection level of 10^8 to 10^4 organisms per gram of faeces (Collins et al. 2011). The real-time qPCR method may be able to detect fewer than the 10^3 organisms per gram of faeces that the multiplex PCR is capable of

detecting. It is recommended that detection levels in the range of 10^1–10^4 organisms per gram of faeces should be assessed for the real-time qPCR. Additional to this disparity in detection levels and likely of higher importance is the possibility that the multiplex PCR in the current study was not performing to the standard sensitivity and specificity levels described in published literature. To determine whether this is an issue, further validation within the laboratory may be necessary.

The rat populations from the three piggeries were positive for *Salmonella spp*. However, the true prevalence for this pathogen was higher in piggery A (1.6–16.4%) than piggery B and C (0–3.9%). Wild rats have previously been confirmed as carriers of *Salmonella spp*. One of the first studies confirming the presence of *Salmonella spp* in rats was in a piggery near Baltimore in the United States, where two positive rats were detected (Davis, 1948). In Brittany, west of France, *Salmonella spp*. were isolated from the faeces of one out of 40 rats captured on 15 different piggeries (Le Moine et al. 1987). The health of the pigs in these piggeries was not determined, though the piggeries were thought to be representative of the size and management of intensive piggeries in the region.

Information collected from the piggeries consulting veterinarians around the time of rat collection indicated that clinical proliferative enteropathy was present in pigs on piggery A and B, and sub-clinical proliferative enteropathy, which does not exhibit clinical signs, was confirmed to be present on piggery C from laboratory analysis. Piggery B also experienced unexplained development of severe *Lawsonia intracellularis* infections in individual pigs in pens. The history of *Lawsonia intracellularis* in pigs aligns with the prevalence detected in the rat population in these piggeries, especially when results from the real-time qPCR are considered. Similarly, the history of *Salmonella spp*. infection in pigs also aligns with the findings in the rat populations. Pigs at piggeries A and C were PCR positive at the time of rat sampling, while piggery B was negative. However, pigs from Piggery B had *Salmonella spp*. detected and confirmed via laboratory testing and post-mortem examination 4 months after detection of this pathogen in rats. The true prevalence of this pathogen in rats on piggery B and C was zero, though it was not statistically proven to be absent from the rat population. As such, assuming the two positive results in rats for piggeries B and C were not false positives, it could be determined that rats may be able to contribute to the maintenance of *Salmonella spp*. within a piggery, cycling it among rats and from rats to pigs and pigs to rats. There is also the real possibility that the pathogen

could have been transmitted to the domestic pigs on piggery B by the infected and shedding rats.

All piggeries in this study were experiencing a large burden of rats, irrespective of the different rodent control programs applied in each piggery. This might be due to the level of effectiveness of the different methods for rodent control. Anticoagulant poison baits are the most common technique used for control of rodents in Australian piggeries (Chapter 2) as well as internationally (Corrigan et al.1992; Endepols et al. 2003). This was also the main method used in the study piggeries. However, this method is not effective in situations where a large amount of alternative food to the poison bait is freely available (Quy et al. 1992; Gomez et al. 2001; Leung and Clark 2005).

The three piggeries involved in the current study had alternate sources of food within a 5 km radius. Piggery A had three piggeries within a close proximity, one 3.5 km away, one 5 km away, and a swine dysentery-infected piggery 7 km away. Rats generally have small home ranges in the presence of a food source. The minimum home range of *Rattus rattus* is estimated to be 17 m in piggeries with sufficient food sources (Leung and Clark 2005). Long-range movements are mainly as a result of translocation or food removal. *Rattus rattus* translocated from their home territory have been recorded travelling up to 8 km to return (Pippin 1961). In the case of food resource removal, *Rattus norvegicus* have been recorded travelling a 3.3 km round trip in a single day in search of a new food source in the United Kingdom (Taylor and Quy 1978). For *Brachyspira hyodysenteriae*, it is known that mice shed sufficient quantities to infect pigs. Pigs may be infected with doses between 10^7 and 10^9 *Brachyspira hyodysenteriae* per gram (Jacobson et al. 2004), while mice have been recorded shedding 10^7 to 2.2×10^8 *Brachyspira hyodysenteriae* per gram of faeces, with four out of four pigs becoming infected when exposed to the infected mice in this same study (Joens 1980). The quantity shed by rats has not been studied, and as such, quantification via real-time qPCR is recommended to assess the risk posed by migrating rats from one piggery to another in the event of a piggery closure. With this additional information the role of rodents as a potential means of reinfection of piggeries would be better understood.

Results from this study indicate that the rat populations of the study piggeries were free from *Brachyspira hyodysenteriae*; however, the proximity of other sources of food could

pose a risk of contact with other rat populations that may carry the pathogen. This study also provides accurate information on the level of infection in the rat population in piggeries of different pathogens of economical importance for the pig industry. The study highlights the importance of including rodent control techniques on piggeries in conjunction with health management programs, particularly for *Lawsonia intracellularis*, to reduce the possibility of transmission and spread of disease through rodents and subsequently improve efficiency of the health programs.

6 Feral pig biosecurity threat to piggeries

6.1 Introduction

Feral pigs are a wild animal reservoir responsible for several major exotic disease outbreaks in livestock internationally, including classical swine fever (Fritzemeier et al. 2000), African swine fever (Penrith and Vosloo 2009) and pseudorabies (Hahn et al. 2010). In Australia, feral pigs are known reservoirs of important endemic pathogens, many of which are transmissible to other species including humans, and all of which are transmissible to domestic pigs (Pavlov et al. 1992; Godfroid 2002; Phillips et al. 2009). As feral pigs move through their home ranges they may come within close proximity of commercial piggeries (Wu et al. 2011). Feral pigs have been shown to move within 100 m of domestic piggeries in the United States, suggesting that contact between wild and domestics pigs is possible (Wyckoff et al. 2009). Most pathogen prevalence studies in feral pigs to date have aimed to detect pathogens subject to mandatory notification and zoonotic pathogens of general risk to humans and livestock. In contrast, Baker et al. (2011) and Phillips et al. (2009) investigated the prevalence of domestic pig production-limiting pathogens in feral pigs. However, neither study investigated the presence of these pathogens in feral pig populations near piggeries.

Despite the acknowledgement of the potential for production-limiting and notifiable pathogen transmission from feral pigs to domestic pigs in Australia, no prevalence surveys have been conducted in the vicinity of piggery facilities. The first objective of the research presented in this chapter was to detect the presence of the notifiable pathogen *Brucella suis* and the four production-limiting bacterial pathogens *Leptospira spp*., *Lawsonia intracellularis*, *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*, in a feral pig population within a 10 km radius of two large-scale commercial piggeries in Southern Queensland. The second objective was to investigate the movement pattern of individual pigs within the feral population in the vicinity of these two piggeries.

6.2 Materials and methods

6.2.1 Study location and sample size

Southern Queensland was purposively selected based on the presence of both feral pigs and commercial piggeries and the conduct of a feral pig ecological damage study in this region. The ecological damage study by the Queensland Murray Darling Committee (QMDC) aimed to assess the impact of feral pigs on agriculture and natural ecology, and determine whether management of feral pig populations reduced these impacts (Gentle et al. 2011). Feral pigs were captured or shot during the QMDC study which enabled serological samples to be obtained for this current study. The two piggeries involved in the study incorporated all pig production facility types: the first piggery was a free-range piggery shown in Figure 6.1, while the second piggery was an intensive piggery with some ecoshelter units shown in Figure 6.2 (piggery identification is confidential). The intensive piggery had three different units separated from each other for disease management purposes, including 20 ecoshelters separated from the two intensive housing units. The current study considered that the feral pigs in the study region (including both piggeries) constituted a single population.

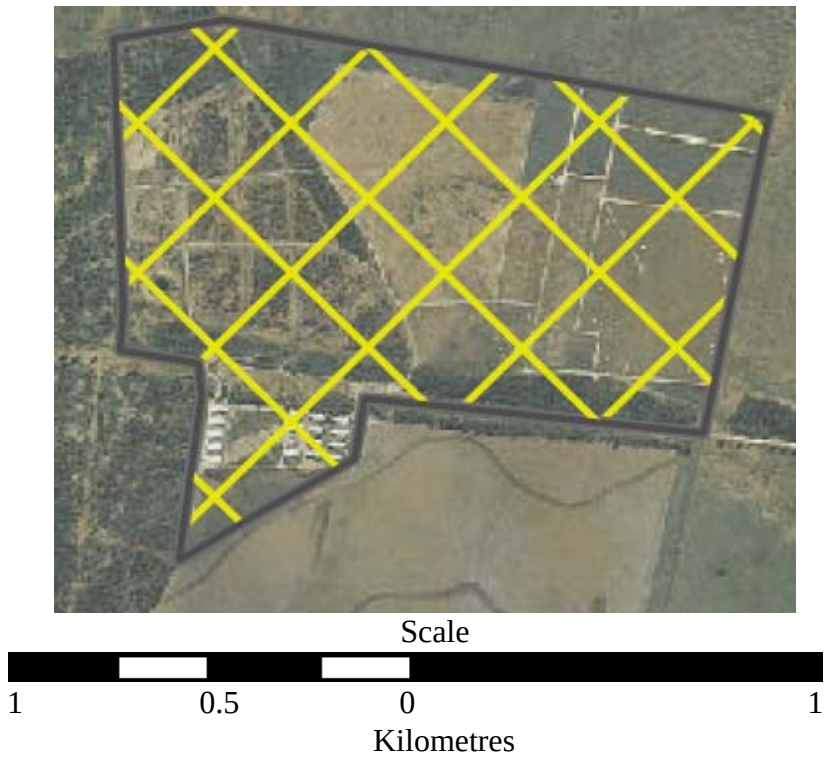

Figure 6.1. Free-range piggery included in a feral pig pathogen detection survey in 2010 and 2011 in Southern Queensland, Australia.

Figure 6.2. An intensive piggery and ecoshelters included in a feral pig pathogen detection survey in 2010 and 2011 in Southern Queensland, Australia.

A minimum of 27 feral pigs needed to be sampled to detect the presence of pathogens at a prevalence of 10% or greater in the feral pig population surrounding the piggeries. The

sample size (n) was calculated to detect a pathogen in a finite population using the following formula from Dohoo et al. (2003; Eq 2.17):

$$n = \left(1 - \alpha^{1/D}\right)\left(N - \frac{D-1}{2}\right) \quad \text{.....Equation 6.1}$$

where: α = 0.05 (1-confidence level);

N = 175.5 (population size). The feral pig population size ranged from 0.34–3.51 pigs km^{-2} (Gentle et al. 2011). To ensure sufficient feral pigs were sampled, the highest population density estimate of 3.51 pigs km^{-2}, equalling 175.5 pigs within a 50 km radius of piggeries, was used;

D = N multiplied by minimum expected prevalence (10%).

In addition, sows from the intensive piggery were also sampled to detect *Leptospira spp*., *Lawsonia intracellularis* and *Mycoplasma hyopneumoniae*. A minimum of 59 sows needed to be sampled to detect the presence of pathogens at a prevalence of 5% or greater in the domestic sow population. The prevalence of some pathogens was expected to be lower in the domestic sows (5%) than in the feral pigs (10%), necessitating a larger sample size to detect pathogen presence. Consent to sample pigs from the free-range piggery was not granted.

The sample size for domestic sows was calculated using Equation 4.1 for an infinite population, shown in Chapter 4, Section 4.2.1.

6.2.2 Sampling in the feral pig population

Blood samples were collected from 83 feral pigs, and lung samples from 72 of these animals. Feral pigs were captured within 10 km from each piggery, in agreement with feral pig movement capabilities (Saunders and Kay 1996; Dexter 1999; Mitchell et al. 2009). A proportion of the feral pigs sampled were shot from a helicopter as a part of the feral pig ecological damage study (n=33); the remainder were trapped within 5 km of either piggery (n=50) using swing door box pig traps (BLASK Engineering, Australia) and 43 were shot. The sex and approximate age of all sampled animals was recorded. Their age was approximated based on their body size as defined by Phillips et al. (2009): weaners were <10 weeks old and <10 kg; adults >1 year old and >35 kg; and juveniles between the two

age groups. All capture and handling procedures were approved by the Department of Primary Industries and Fisheries Animal Ethics Committee (reference number CA 2009/10/386).

A blood sample was collected from the jugular of all euthanised feral pigs (n=76) within 5 min of euthanasia in an 8 ml vacuette with clot separator tube (Interpath). One quarter of lung from the right, dorsal lobe was collected from 72 of the euthanised animals. Samples were kept chilled in eskies throughout the day. Serum was removed from the blood sample each afternoon and divided into three 2 ml eppendorf tubes using disposable pipettes, then frozen along with the lung samples at −20 ºC.

Of the 50 feral pigs trapped, seven were sedated with either Zoletil (4–9 mg/kg) or Xylazil (1.1–2.2 mg/kg) via intramuscular injection into the muscle of the hind leg in order to be fitted with a radio collar (Sirtrack®, Havelock North, New Zealand). Collars were attached around the neck of each pig and secured using the provided fasteners. Collared feral pigs were larger than 15 kg and were an even gender spread: four females and three males. The fitted collars included an ultra high frequency (UHF) radio for tracking and a Global Positioning System (GPS) data-logger; tasking rates were programmed to acquire fixes at 30 min intervals from activation. These fixes are stored on-board the collar and needed to be retrieved from the pigs to download the acquired data points. Blood samples were obtained from the collared pigs from the main vein on the hind leg using a size 19-gauge needle syringe. Blood samples were then processed as above. A single collared male, Male 2, had an additional blood sample taken on recapture for collar retrieval. The radio collars were fitted with the aim to collect six months of movement data per pig; however, four collars were retrieved after these pigs were shot by hunters before the six-month period was complete. The remaining pigs were tracked by UHF after six months and when located were shot. One female could not be located and hence the collar could not be retrieved.

6.2.3 Sampling in the domestic pig population

Blood samples were collected from 86 adult sows from the intensive piggery on the 16th of August and the 14th of October, 2010, at an abattoir. All sows sent to the abattoir by the intensive piggery on these dates as part of routine culling due to age, decreased performance or illness were sampled. Blood samples were collected from the jugular vein

into a small specimen jar immediately post mortem. Serum samples were extracted and divided into three 2 ml eppendorf tubes using disposable pipettes, then frozen at −20 ºC.

6.2.4 Piggery management and disease control history

Information on pig health status and disease and pest control programs implemented on each piggery was obtained through administration of a written questionnaire to each of the piggery managers after sampling of the feral pigs and laboratory tests were performed (Appendix 4). A single page letter outlining results obtained from the feral pigs captured near their piggery was also attached to the four page questionnaire. The questionnaire contained open questions covering pig disease history and pest control management. Questions on disease history focused on information regarding the history of the presence of the pathogens of interest in this study, including how the pathogen presence was confirmed, and the medication used to control illness. Questions pertaining to feral pig control focused on the control measures in place to manage the feral pig population on their property. The general husbandry questions requested information on the herd size and piggery production type. The majority of pig health results were laboratory confirmed, and such were not affected by any potential bias created by knowing the feral pig results prior to questionnaire completion.

6.2.5 Laboratory testing

6.2.5.1 Brucella suis

Serologic testing of antibodies against *Brucella spp.* was done by the Elizabeth MacArthur Agricultural Institute (Menangle, NSW, Australia). For *Brucella suis*, a Rose Bengal test (RBT) was used, followed by confirmation of those that tested positive using a complement fixation test (CFT), following Australian standard diagnostic techniques for animal diseases (Corner 1993). Tests are not specific for *Brucella suis*, as antibodies to *Brucella abortus* and *Brucella melitensis* may also be detected; however, the latter two pathogens are considered to be absent from Australia (WAHID 2010). The RBT is a spot agglutination test. Serum from the feral pig (25 µl) and positive and negative controls were mixed with an antigen (25 µl, BENGA TEST®- Rose Bengal stained acidified buffered Antigen, Synbiotics Corporation) on an 80-well perspex plate (World Health Organisation perspex plate). The serum and antigen were gently mixed together and agglutination scored between 1 and 3, depending on the amount of agglutination present. The RBT is appropriate for rapid screening of samples.

A confirmation CFT was used to increase the specificity of the results. The complement is a constituent of serum and plays an important part in the development of immunity. Antibodies and antigens in serum absorb or fix complement (Corner 1993). In the CFT for *Brucella spp*. the serum to be tested was initially incubated at 60 °C for 45 min to inactivate any complement present in the serum. A diluted CFT antigen was added to 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 dilutions of serum and positive and negative controls. This antigen will bind with any antibodies that may be present in the test serum. This was followed by addition of a dilution of complement (Guinea Pig Serum, Lyophilised). This added complement binds with any antibody-antigen complex that may have formed. Sensitised red blood cells were then added. If the added complement has bound to antibody-antigen complexes, then the red blood cells will not lyse. Once spun, the red blood cells will coalesce at the bottom of the microtitre plate, forming a red spot, indicating a positive result. A negative result will be a uniform pink colour to the well contents, due to lysis of the red blood cells by the free, unbound complement.

6.2.5.2 Leptospira spp.

Testing for *Leptospira spp*. was undertaken at the Queensland Health Forensic and Scientific Services Laboratory (Brisbane, Queensland, Australia), a World Health Organisation, United Nations Food and Agricultural Organization and Office International des Epizooties accredited laboratory. A microscopic agglutination test (MAT) was used for *Leptospira spp*. identification (Faine 1994). The MAT is used most frequently and is the preferred method for diagnosis of *Leptospira spp*. in reference laboratories due to its high degree of sensitivity and specificity (Bajani et al. 2003). The density of cells that agglutinate, or clump together, determines whether a serum is positive or negative. The serum to be tested was diluted 1: 25–1: 6400 in phosphate-buffered saline at pH 7.4 (PBS), in microtitre plates (Millipore, North Ryde, Australia). Live *Leptospira* cell suspensions representing one of a panel of 16 known Australian serovars were then added in the same quantity to each well, including the control cells. Serovars included in the panel of live *Leptospira* included pomona, hardjo, tarassovi, grippotyphosa, celledoni, australis, zanoni, robinsoni, canicola, kremastos, szwajizak, medanensis, bulgarica, arborea and topaz. The serum and live suspensions were gently mixed together and incubated for 90 mins at 30 °C. The level of agglutination in each cell was examined by dark-field microscopy at a magnification of ×100. The endpoint was the weakest dilution of serum that agglutinated at

least 50% of the Leptospires. A MAT titre of at least 1:50 was necessary to determine a sample positive.

6.2.5.3 Lawsonia intracellularis

Serological detection of antibodies against *Lawsonia intracellularis* was done by Ace Laboratory Services (White Hills, Victoria Australia). An enzyme-linked immunosorbent assay (ELISA) antibody test kit was used, following the methods described in the manual distributed with the testing kit (BioScreen ELISA, Svanova, Sweden). The ELISA was used to analyse the samples as a quicker and cheaper alternative to the PCR test. Microplates were provided with the testing kit that were sensitised with *Lawsonia intracellularis* antigens in the bottom of each well. After addition of 100 µl of a 1:10 dilution of each individual sample and positive and negative controls to the wells, antibodies present in the samples would bind with the antigen in the wells. The microplate was gently shaken to ensure mixing and incubated for 1 hr at 37 ºC. Plates were washed three times using a dilution of the wash buffer supplied. An anti-*Lawsonia intracellularis*-horseradish peroxidase conjugate was added to each well, in a quantity of 100 µl, and re-incubated for 1 hr at 37 ºC. This conjugate binds to any free *Lawsonia intracellularis* antigens in the bottom of each well. The microplate was washed again to remove excess conjugate and 100 µl of a buffered peroxidase substrate was added to each well. The plate was shaken and then incubated at room temperature for 10 min. A stop solution in a quantity of 50 µl was then added to each well to halt the reaction. The addition of the buffered peroxidase substrate reveals the enzyme linked to the conjugate as a coloured product. The absence of antibodies for *Lawsonia intracellularis* in a sample will result in a more intense colour reaction. The optical densities of the wells were read at 450 nm in a microplate spectrophotometer. A percentage of inhibition value greater than 30% was considered positive.

6.2.5.4 Mycoplasma hyopneumoniae

Serologic testing of antibodies against *Mycoplasma hyopneumoniae* was done by the Elizabeth MacArthur Agricultural Institute (Menangle, NSW, Australia). An ELISA antibody test kit was used to detect *Mycoplasma hyopneumoniae*, with the procedures detailed in the kit manual (Idexx laboratories, manufactured Westbrook, Maine, USA). The ELISA method is very similar to that used for *Lawsonia intracellularis* antibody detection. The antigen-coated plates will bind any antibodies in the serum samples throughout the

incubation process. After the excess was washed away, a conjugate was then added which binds to the attached antibodies in the wells during incubation. This is the opposite of the *Lawsonia intracellularis* method, which binds to the unattached antigens. The excess, unbound conjugate was washed away and an enzyme substrate was added. The presence of antibodies for *Mycoplasma hyopneumoniae* in a sample results in a more intense colour reaction. A sample to positive ratio of greater than 0.4 indicates a positive result. The sample to positive (S/P) ratio was obtained by using the following formula detailed in the ELISA antibody test kit manual:

$$\text{S/P ratio} = \frac{\text{Sample A(650)} - \text{NC}^{\text{mean}}}{\text{PC}^{\text{mean}} - \text{NC}^{\text{mean}}} \quad \text{.....Equation 6.2}$$

where: Sample A(650) = The light absorbance value of the sample when the wavelength of the spectrophotometer is set to 650nm;

NC^{mean} = The negative control (provided in the kit) mean light absorbance value when the wavelength of the spectrophotometer is set to 650nm;

PC^{mean} = The positive control (provided in the kit) mean light absorbance value when the wavelength of the spectrophotometer is set to 650nm.

A polymerase chain reaction (PCR) was conducted on the 72 lung samples obtained from the feral pigs to compare those animals that may have been infected in the past (determined by the ELISA) to those that are currently infected and shedding the *Mycoplasma hyopneumoniae* bacteria. Lung samples were analysed by the Department of Primary Industries Pig Health Laboratory (Bendigo, Victoria, Australia) for *Mycoplasma hyopneumoniae* DNA using a specific PCR (Mattson et al. 1995). DNA was extracted from the lung samples for both the *Mycoplasma hyopneumoniae* PCR and the *Actinobacillus pleuropneumoniae* PCR using a DNA extraction kit (DNeasy Blood & Tissue, Qiagen DNA) with the procedures detailed in the kit manual. The steps in the DNA extraction can be very simply broken down into lysing of the sample, binding of DNA to the DNeasy membrane, washing and filtering the eluate containing DNA into a clean 1.5 ml microcentrifuge tube.

A 25 µl total reaction volume was used for the PCR, containing 12.5 µl of Qiagen HotStart MasterMix Taq, 7 µl of nuclease-free water (Qiagen), 0.5 µl of both forward and reverse primers (Sigma Aldrich; *Mycoplasma hyopneumoniae* primers final concentration 0.2 µM)

and 2 µl of 10x orange G loading buffer (Monash University). The forward and reverse primers were as follows (Mattson et al.1995):

forward primer: 5'-GAG CCT TCA AGC TTC ACC AAG A-3'

reverse primer: 5'-TGT GTT AGT GAC TTT TGC CAC C-3'

Two and a half µl of extracted DNA sample was then added. For the negative control 2.5 µl of nuclease-free water was added. The PCR cycling involved an initial Taq (*Thermus aquaticus*) polymerase activation step of 5 min at 94 ºC followed by 40 cycles of 45 sec at 93 ºC, 60 sec at 60 ºC, and 2 min at 72 ºC. A final termination step was performed for 10 min at 72 ºC. Ten µl of the PCR product were analysed by gel electrophoreses on a 1% agarose gel (ProBiogen) stained with ethidium bromide (Astral Scientific, Ohio, USA).

6.2.5.5 Actinobacillus pleuropneumoniae

Lung samples were analysed by the Department of Primary Industries Pig Health Laboratory (Bendigo, Victoria, Australia) for *Actinobacillus pleuropneumoniae* using a specific polymerase chain reaction (PCR) (Gram and Ahrens 1998). Spare DNA extracted for the *Mycoplasma hyopneumoniae* PCR was used for the *Actinobacillus pleuropneumoniae* PCR. The PCR process was very similar to that mentioned for the *Mycoplasma hyopneumoniae* resulting in a final reaction volume of 25 µl. Differences between methods were the addition of only 6 µl of nuclease-free water, with more primer used, 1 µl of both forward and reverse primers (*Actinobacillus pleuropneumoniae* primers final concentration 0.2 µM) The forward and reverse primers were as follows (Gram and Ahrens 1998):

forward primer: 5' - AAG GTT GAT ATG TCC GCA CC - 3'

reverse primer: 5' - CAC CGA TTA CGC CTT GCC A - 3'

The PCR cycling parameters also differed with an initial Taq polymerase activation step of 15 min at 95 ºC followed by 30 cycles of 30 sec at 94 ºC, 20 sec at 62 ºC, and 2 min at 72 ºC. A final termination step was the same as for the *Mycoplasma hyopneumoniae* PCR. The DNA was then imaged using the gel electrophoresis step outlined above.

6.2.6 Data analysis

6.2.6.1 Calculation to substantiate freedom from a pathogen and to estimate prevalence

Calculations to substantiate freedom from a pathogen and to estimate true prevalence were conducted as for Chapter 4, Section 4.2.5.1.

The sensitivity and specificity for the laboratory tests used are shown in Table 6.1.

Table 6.1. Specifications of the diagnostic tests used to determine the true prevalence of different pathogens in the feral pig population near two piggeries in Southern Queensland, Australia in 2010 and 2011.

Pathogen	Diagnostic test	Sensitivity	Specificity	Reference
Lawsonia intracellularis	ELISA	72%	93%	Jacobson et al. 2011
Leptospira spp.	MAT	93.8%	97.3%	Bajani et al. 2003
Actinobacillus pleuropneumoniae	PCR	93%	100%	Fittipaldi et al. 2003
Mycoplasma hyopneumoniae	PCR	97.3%	93%	Cai et al. 2007
Mycoplasma hyopneumoniae	ELISA	63%	100%	Erlandson et al. 2005
Brucella spp	RBT and CFT	72.3% [a]	97.7% [a]	Gall and Nielsen 2004

ELISA – Enzyme-linked immunosorbent assay
MAT – Microscopic agglutination test
PCR – Polymerase chain reaction
RBT - Rose Bengal test
CFT - Complement fixation test
[a] Sensitivity and specificity for *Brucella spp.* was a combination of RBT and CFT calculated in series using standard formula (Dohoo et al. 2003). RBT and CFT were assumed to be conditionally independent tests.

6.2.6.2 GPS collar movement analysis

6.2.6.2.1 GPS data: The satellite fixes obtained for each feral pig were stored as GPS location data. These data points were downloaded from the collars using the Sirtrack download interface in comma-separated values (CSV) format. The GPS collars store position data using the Geographic Coordinate System (Lat/Long as decimal degrees) (GCS) in the World Geodetic System (WGS) 84 geographic projection. Data were loaded into the ArcMap 9.2 Geographic Information System (GIS) program (ESRI Inc, Redlands).

Data layers were produced for each pig and examined for spurious data points. All data points taken prior to individual pigs being captured, and after they had been killed to retrieve the collars, were discarded. The coordinates for the boundaries of each of the piggery building locations had not been gathered in the field. The piggeries were located in Google Earth® (Google Inc 2011) on-line satellite imagery program. Using the zoom function in the Google Earth program, the coordinates for the boundaries of the piggeries were obtained from a simulated eye height of 1 km. For the free-range piggery, the boundaries were taken as the extent of the area the pigs use on the piggery, and for the intensive piggeries the boundaries were taken as the perimeter of properties on which pig sheds were located, shown by the grey line encompassing the yellow hatched lines in Figures 6.1 and 6.2. These data were entered into the GIS and data layers formed for each piggery. As Google Earth® displays geographic data using the WGS 84 projection, transformation of data points was not required. Locations of feral pigs and domestic piggeries were overlaid onto SPOT5 2.5 m satellite imagery (SPOT Satellite Imaging Corporation).

6.2.6.2.2 Horizontal dissolution of precision: The GPS collars record a Horizontal Dilution of Precision (HDOP) value with each fix. This HDOP value is calculated based on the position of the satellites in the sky at the time a data point is recorded. The HDOP values are unit-less numbers generally ranging between 1 and 13 that give an indication of the level of spread of satellites the collar communicated with in order to attain that fix location. A small HDOP value indicates that the available satellites were spread widely across the sky, providing highly accurate fixes. A large HDOP value indicates that the satellites were closely grouped in the sky, thus increasing the probability of location error; for example, Recio et al. (2010) found that only 33% of fixes with a HDOP of 9.0 were accurate to within 50 m of the true location. A value of 6.0 was chosen as the cut off point for this study and all fixes with a HDOP value greater than 6.0 were discarded from the analyses to minimise the potential for error. A beacon study is generally undertaken to determine the appropriate cut off value for HDOP values; however, such a study was not undertaken in the current study due to the low number of collars available for use. The cut off value was determined based on the topography of the study area in comparison with other research recently undertaken using Sirtrack collars (Moseby et al. 2009; Hilmer 2010; Buckmaster 2011). Based on the topography in the studies by the above authors, the use of a HDOP cut off in the current study of 6.0 was justified.

6.2.6.2.3 Feral pig proximity to piggeries: Buffers were created around each piggery to a distance of 50, 100, 500 and 5000 m using the buffer tool in ArcMap 9.2. The Hawth's Analysis Tool for ArcGIS (Beyer 2004) plug-in was used to determine the number of data points for each collared pig that fell within each of the buffer zones around each piggery. The 50 and 100 m buffer zones were chosen based on feral pig-domestic pig contact zones determined by Wyckoff et al. (2009), as well as the potential error of the collar. Feral pigs moving within the 100 m buffer were determined to have a high likelihood of contacting domestic pigs. Feral pigs moving within 500 m were in close proximity to the piggery and were also considered to have the possibility of contact with domestic pigs (Wyckoff et al. 2009). The fourth buffer zone of 5000 m was selected based on the tested aerosol dispersal of *Mycoplasma hyopneumoniae*. Dee et al. (2009) detected *Mycoplasma hyopneumoniae* at a maximum tested distance of 4.7 km from the pathogen source. Infected feral pigs in this buffer zone have the potential to transmit *Mycoplasma hyopneumoniae* via the air to domestic pigs.

6.2.6.2.4 Interaction between collared feral pigs: To determine the number of times each collared feral pig was within 50 m of another collared feral pig in an hour, the date and time of each fix for each collared pig were converted to a numeric value in Microsoft Excel, where each day has a value of 1 and each hour has a value of 0.04167 (one hour divided by 24 hours in a day). This converted data were entered into ArcMap 9.2. Each data point for each pig was buffered to a distance of 50 m. The output layers for every pig were overlaid on top of each other using the ArcMap 9.2 intersection tool. This provided an output giving all data points that fell within 50 m of a data point from another collared pig. A structured query language (SQL) interrogation of the attributes of this output was used to then determine which of the identified fixes were taken within one hour of a fix for the other pigs.

6.2.6.2.5 GPS collar fix rate: The GPS collar fix rate for each collar was calculated using the expected number of fixes that would be obtained from the 30 min tasking rate for the number of days the collar was on a live feral pig, compared with the number of successful fixes recorded by the GPS collar.

6.2.6.2.6 Autocorrelated data: Autocorrelation of the GPS data was not considered to have influenced the outcome of these analyses. Regardless of how it is collected, all tracking data are autocorrelated to some degree. However, it is particularly apparent with data collected using GPS collars due to the regularity of the fixes. Autocorrelation of tracking data can introduce confounding effects when analysing home range sizes for the animals being tracked and generally results in a negative bias in home range size calculations (Kernohan et al. 2001; Swihart and Slade 1997). While the data used in these analyses were highly autocorrelated, it was not considered to have introduced bias into either the analyses or interpretation of the results as no analysis of home range size was undertaken.

6.3 Results

Eighty-three feral pigs were tested for pathogen detection, 48% female and 52% male. A total of 6 samples were from weaners (7.2%), 48 were from juveniles (57.8%) and 29 were from adults (34.9%).

6.3.1. Presence of pathogens in the feral pig population.

All pathogens were found to be present in the feral pig population except for *Actinobacillus pleuropneumoniae*, which was statistically determined to be absent, shown in Table 6.2.

Table 6.2. Probability of presence of *Lawsonia intracellularis*, *Leptospira spp*., *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae* and *Brucella suis* in the feral pig populations near two piggeries in Southern Queensland, Australia in 2010 and 2011.

Pathogen	Positive/tested	Probability of presence of pathogen in the feral pig population[a]
Lawsonia intracellularis	76/83	1.000
Leptospira spp.	39/83	1.000
Actinobacillus pleuropneumoniae	0/72	**<0.001**
Mycoplasma hyopneumoniae PCR	23/72	1.000
Mycoplasma hyopneumoniae ELISA	22/83	1.000
Brucella suis	8/83	0.622

[a] Probability that a pathogen is present at the specified minimum expected prevalence (probability ≤0.05, shown in bold font, indicates the population is free from a pathogen at the minimum expected pathogen prevalence). Probability obtained using the hypergeometric formula described by Cameron and Baldock (1998) implemented with the FreeCalc version 2 software (Sergeant 2009b). Parameters used: Population size of 176 individuals in the region of each piggery; Minimum expected prevalence of 10%; test sensitivity and specificity in Table 6.1.

Apparent and true prevalence of the different pathogens in the feral pig population is shown in Table 6.3. Difference between apparent prevalence and true prevalence figures for the five pathogens in the feral pigs arise from accuracy of the laboratory methods used. The exception was *Actinobacillus pleuropneumoniae* with an apparent prevalence and true prevalence both equalling 0%. Five separate serotypes of *Leptospira spp*. were detected: Pomona (36 isolations), Tarassovi (1 isolation), Copenhageni (4 isolations), Robinsoni (1 isolation) and Topaz (7 isolations).

Table 6.3. Apparent prevalence and true prevalence of *Lawsonia intracellularis*, *Leptospira spp*., *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae* and *Brucella suis* in feral pig populations near two different piggeries sampled in 2010 and 2011 in Southern Queensland, Australia.

Pathogen and detection method	Positive /tested	Apparent prevalence %	95% confidence interval	True prevalence %	95% confidence interval
Lawsonia intracellularis ELISA[b]	76/83	91.6	83.6–95.9	100	100–100
Leptospira spp. MAT [b]	39/83	47.0	36.6–57.6	48.6	36.5–60.6
Actinobacillus pleuropneumoniae PCR[c]	0/72	0	0–5.1	0[a]	0–5.3
Mycoplasma hyopneumoniae PCR[c]	23/72	31.9	22.3–43.4	27.6	16.5–40.6
Mycoplasma hyopneumoniae ELISA[b]	22/83	26.5	18.2–36.9	42.1	28.2–59.1
Brucella suis RBT and CFT[b]	8/83	9.6	5.0–17.9	10.5	2.8–22.1

ELISA – Enzyme-linked immunosorbent assay
MAT – Microscopic agglutination test
PCR – Polymerase chain reaction
RBT - Rose Bengal test
CFT - Complement fixation test
Confidence intervals for true prevalence determined using Blakers analysis. Test sensitivity and specificity in Table 6.1.
[a] Statistically confident that the pathogen was determined to be absent from the feral pig population.
[b] In Serum
[c] In lungs

Of the seven feral pigs collared to track movement of feral pigs around piggeries, six had their collars retrieved: three males and three females. All of these feral pigs tested positive for *Lawsonia intracellularis*, while one was positive for *Leptospira pomona* and two were positive for *Mycoplasma hyopneumoniae* (Table 6.4). Male two was sampled a second time upon recapture six months later, and was still positive for *Lawsonia intracellularis* but remained negative for all other pathogens.

Table 6.4. Presence of *Lawsonia intracellularis*, *Leptospira spp*., *Mycoplasma hyopneumoniae* and *Brucella suis* in six feral pigs at time of collar application to track movement near two piggeries in Southern Queensland, Australia in 2010.

Feral pig	*Lawsonia intracellularis*	*Leptospira spp*.	*Mycoplasma hyopneumoniae*	*Brucella suis*
Male 1	+	+		
Male 2	+			
Male 3	+			
Female 1	+		+	
Female 2	+			
Female 3	+		+	

6.3.2. Presence of pathogens in the domestic pig population.

Lawsonia intracellularis, *Leptospira spp*. and *Mycoplasma hyopneumoniae* were all present in the adult sow population sampled from the intensive piggery (Table 6.5).

Table 6.5. Probability of presence of *Lawsonia intracellularis*, *Leptospira spp*. and *Mycoplasma hyopneumoniae* in 86 breeding sows on one intensive piggery in Southern Queensland, Australia in 2010.

Pathogen	Positive/tested	Probability of presence of pathogen in the feral pig population[a]
Lawsonia intracellularis	86/86	1.000
Leptospira spp.	4/86	0.243
Mycoplasma hyopneumoniae ELISA	62/86	1.000

[a] Probability that a pathogen is present at the specified minimum expected prevalence (probability ≤0.05 indicates the population is free from the pathogen at the minimum expected pathogen prevalence). Probability obtained using the hypergeometric formula described by Cameron and Baldock (1998) implemented with the FreeCalc version 2 software (Sergeant 2009b). Parameters used: Population size of 5000 individuals at the piggery; Minimum expected prevalence of 5%; test sensitivity and specificity in Table 6.1.

As with the feral pig samples the apparent prevalence and true prevalence differed for *Leptospira spp*. and *Mycoplasma hyopneumoniae* in the domestic sows due to accuracy of laboratory methods, but were the same for *Lawsonia intracellularis* (100%; Table 6.6). There was one serotype of *Leptospira spp*., Topaz, detected in four of the domestic sows.

Table 6.6. Apparent prevalence and true prevalence of *Lawsonia intracellularis*, *Leptospira spp*. and *Mycoplasma hyopneumoniae* in 86 breeding sows on one intensive piggery in Southern Queensland, Australia in 2010.

Pathogen	Positive /tested	Apparent prevalence %	95% confidence interval	True prevalence %	95% confidence interval
Lawsonia intracellularis ELISA[a]	86/86	100	95.7–100	100	100–100
Leptospira spp. MAT[a]	4/86	4.7	1.8–11.4	2.1	0–9.4
Mycoplasma hyopneumoniae ELISA[a]	62/86	72.1	61.8–80.5	100	98–100

ELISA – Enzyme-linked immunosorbent assay
MAT – Microscopic agglutination test
Confidence intervals for true prevalence determined using Blakers analysis. Test sensitivity and specificity in Table 6.1.
[a] In Serum

6.3.3 Health management and disease control in the domestic pig population.

Health, disease control and biosecurity practices applied at the two piggeries included in this study are presented in Table 6.7. Both piggeries undertook standard biosecurity procedures, such as enclosing feed silos, providing clothing and shoes for piggery visitors, and controlling feral pig populations. In addition, both piggeries had a single fence barrier to prevent entry of feral pigs on to their property, which was reported to be effective against feral pig access by the piggery manager. Limited information on disease status and control measures was provided by the free-range piggery. Both piggeries had had recent outbreaks of *Mycoplasma hyopneumoniae*, of an unknown origin and pigs were currently infected. The free-range piggery did not provide any information on other diseases, other than the use of vaccinations for prevention of Leptospirosis. The intensive/ecoshelter piggery had not had any past infections of *Leptospira spp*., *Brucella suis* or *Actinobacillus pleuropneumoniae*, though it had a history of past infections of *Lawsonia intracellularis*. This piggery vaccinated their breeding herd against *Lawsonia intracellularis* using Enterisol® (a live porcine vaccine) as a result of the 2009 outbreak. They considered the vaccination to be effective when pigs remain seronegative to 6 weeks post-vaccination. The free-range piggery reported vaccinating against Leptospira.

Table 6.7. Health status and pest control for two piggeries in Southern Queensland in 2010.

	Free-range piggery	Intensive/Ecoshelter piggery
Piggery size (breeding sows)	>500 sows	Approximately 5000 sow
Production system	Continuous flow	Breeder continuous flow, all-in all-out by ecoshelter
Food storage	Silos	Silos
Clothing/boot change	Clothing and boots for visitors. Not changed between paddocks.	Clothing and boots for visitors. Not changed between sheds.
Fencing	Yes – Barb wire fence with an electric outrigged wire. Checked daily for holes and erosion.	Yes – single barb wire fence
Feral pig control	Shooting, trapping, and baiting when there is region wide organisation.	Shooting – once per week
Effectiveness of prevention of contact between feral and domestic pigs?	Yes	Yes
***Brucella suis* presence**	Information not provided	No – confirmation details not provided
***Leptospira spp.* presence**	Information not provided	No – confirmation details not provided
***Leptospira spp.* vaccination**	ECOvacLE[a] give to sow pre-farrowing	PLEvac[b] give at weaning
***Lawsonia intracellularis* presence**	Information not provided	Yes, February 2009. Laboratory confirmed via ELISA.
***Lawsonia intracellularis* proportion infected**	Information not provided	2–5% breeders
***Lawsonia intracellularis* medication**	Information not provided	Enterisol live porcine vaccination to pre-weaning pigs post 2009 outbreak
***Mycoplasma hyopneumoniae* presence**	Yes, recent outbreak, pigs currently infected.	Yes, PCR confirmed, recent outbreak, pigs currently infected.
***Mycoplasma hyopneumoniae* medication**	Information not provided	RespiSure®[c] or M+Pac[d]; Chlortetracycline[e], Tilmicosin[f] and Lincomycin[g] used in feed. Commenced immediately post confirmation.
***Actinobacillus pleuropneumoniae* presence**	Information not provided	No – confirmation details not provided

[a] Vaccination for prevention of *Escherichia coli*, *Leptospira pomona* and Erysipelas; [b] Vaccine for prevention of parvovirus, *Leptospira pomona* and erysipelas; [c] vaccination preventing *Mycoplasma hyopneumoniae*, effective for 22 weeks post vaccination; [d] an improved vaccine for *Mycoplasma hyopneumoniae*; [efg] antibiotics.

6.3.4 Feral pig movement

Of the 83 feral pigs sampled for pathogens, seven were collared between June 2010 and December 2010. A collar from one pig was unable to be found. These animals were collared within a 10 km radius of each of the two piggeries in Southern Queensland (Figure 6.1, 6.2 and 6.3). The total number of data points collected on individual animals ranged from 2747 to 5189 GPS fixes, over a range of 64 to 184 days (Table 6.8). The total number of data points per animal ranged from 2079 to 4609 when the HDOP was restricted to ≤ 6 (Table 6.8). The successful GPS fix rate ranged from 52.9–89.4% (mean 72.3%) for the total number of data points obtained for each pig, and 40.3–79.3% (mean 62.8%) for the HDOP ≤ 6 total data points for each pig (Table 6.8).

Table 6.8. The expected number of data points collected per feral per pig and the proportion of actual data points collected per feral pig in Southern Queensland in 2010 and 2011.

	Total days[a]	Expected number of data points[b]	Actual total number of data points[c]	Fix rate[d] (%)	Actual total number of HDOP ≤ 6 data points	Fix rate[e] (%)
Male1	118	5664	3285	58.0	2934	51.8
Male 2	184	8832	4669	52.9	3557	40.3
Male 3	84	4032	2410	59.8	2079	51.6
Female 1	122	5856	5189	88.6	4609	78.7
Female 2	64	3072	2747	89.4	2435	79.3
Female 3	102	4896	4166	85.1	3695	75.5
Mean				72.3		62.8

[a] Number of days over which collars obtained GPS fixes for still living individuals.
[b] Tasking rates were programmed to acquire one fix every 30 minutes.
[c] Total number of GPS fixes collected on individual animals.
[d] Percentage of total GPS fixes (data points) as a proportion of expected fixes (data points).
[e] Percentage of total Horizontal Dilution Of Precision value ≤ 6 (data points) as a proportion of expected fixes (data points).

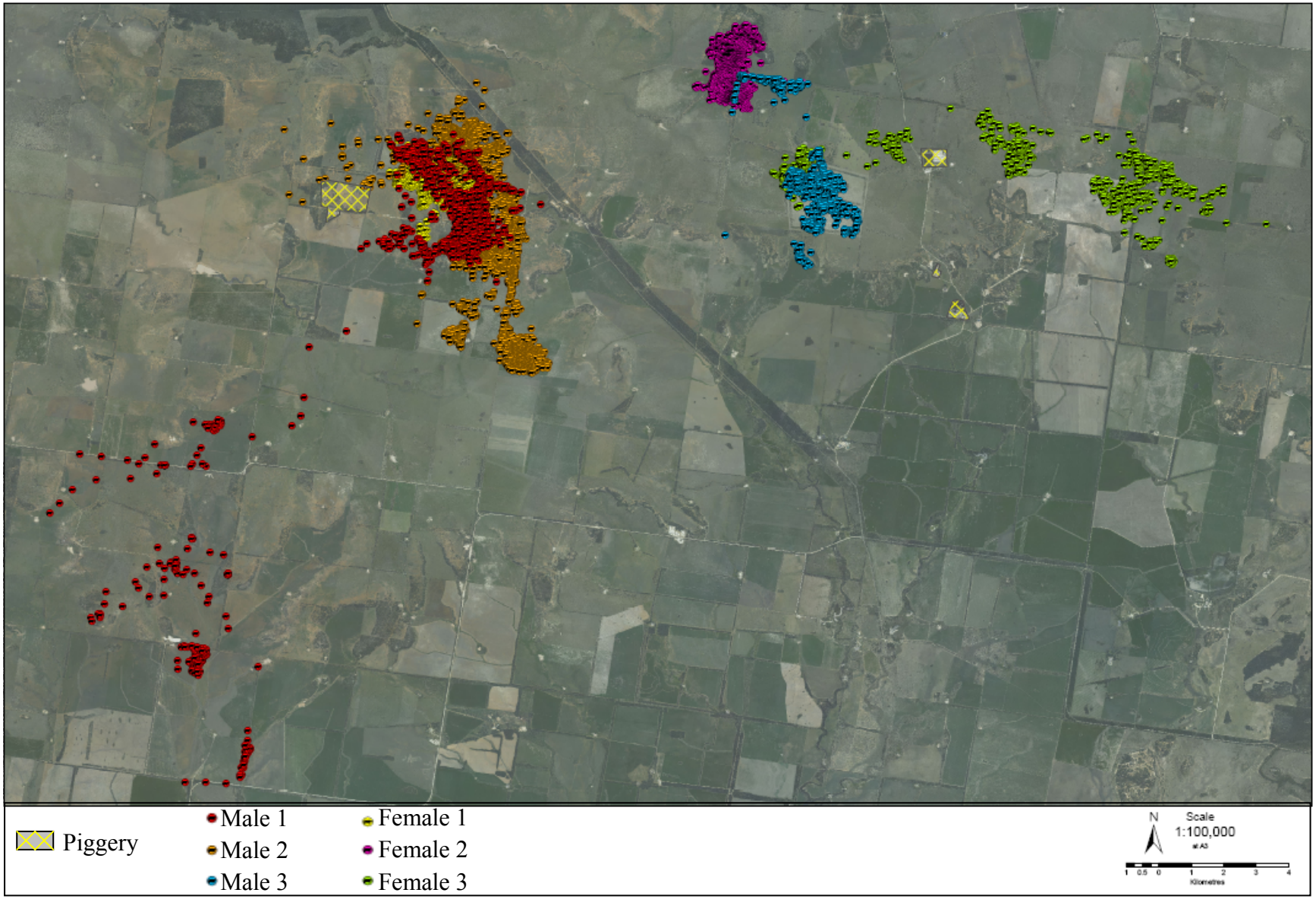

Figure 6.3. Total movement of six feral pigs in the vicinity of two commercial piggeries between June 2010 and December 2010 in Southern Queensland, Australia.

There were 15 data points, corresponding to a single individual, within 500 m of the free-range piggery (Figure 6.4). This corresponded to 0.32% of its total data points (Table 6.9). With the HDOP restricted to ≤ 6, there were 13 data points for this pig within 500 m of the free-range piggery, corresponding to 0.37% of its total data points (Table 6.10). There were 6 data points for the same individual within 100 m of the free-range piggery (Figure 6.4; Table 6.9), and 5 data points with the HDOP restricted to ≤ 6 (Table 6.10). The majority of feral pig data points were within 5 km of either piggery, ranging from 52.3% to 99.7% per individual, with the exception of Female 2 (Table 6.9). These ranges are very similar to the HDOP ≤ 6 data, with 53.6–99.7% of data points within 5 km of the piggeries (Table 6.10).

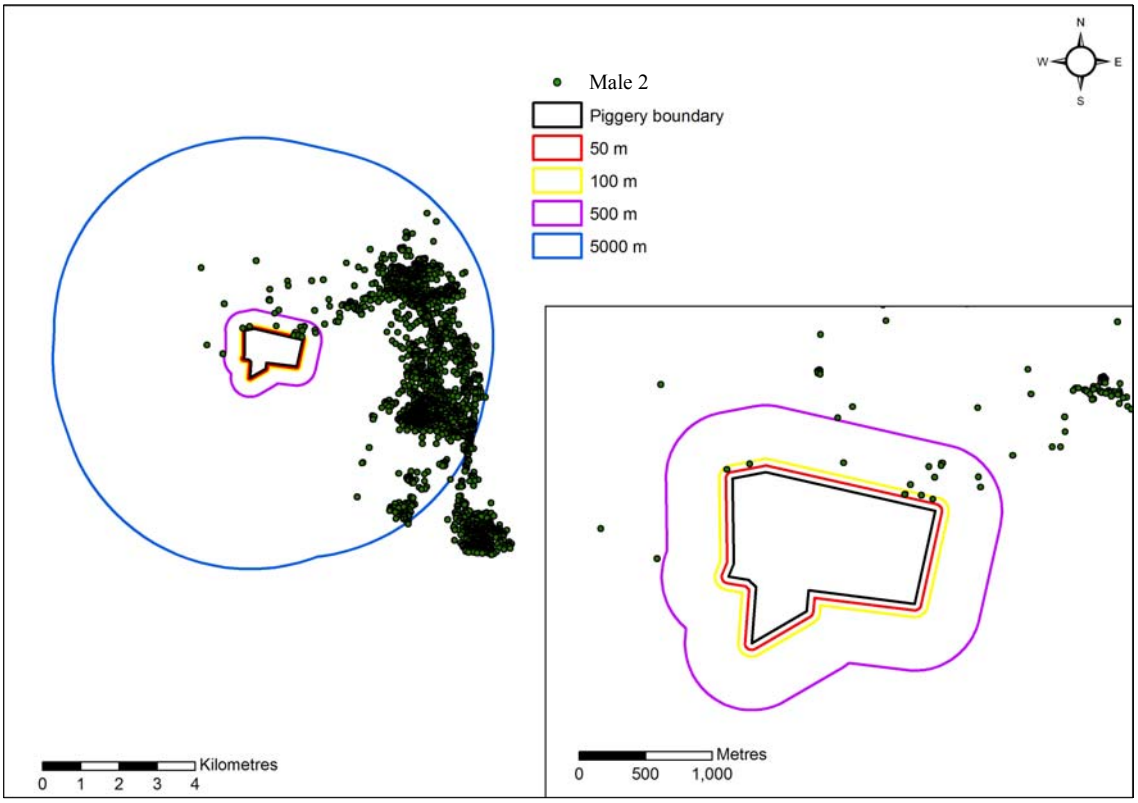

Figure 6.4. Total movement of a single large male feral pig in the vicinity of a commercial free-range piggery between June 2010 and December 2010 in Southern Queensland, Australia.

Table 6.9. Movement of individual collared feral pigs within differing distances of piggeries in Southern Queensland, Australia during 2010–2011.

		Distance from piggery (meters)					
	Sex	<50	<100	<500	<5000	% of locations[a]	Total no. data points[b]
1	Male	0	0	0	3005	91.5	3285
2	Male	0	6	15	3973	85.1	4669
3	Male	0	0	0	1773	62.3	2410
1	Female	0	0	0	5174	99.7	5189
2	Female	0	0	0	3	0.1	2747
3	Female	0	0	0	2180	52.3	4166

[a] Percentage of total data points within 5000 meters of piggery – given distance set on piggery boundary not sheds/shelters where the domestic pigs are housed.
[b] Total number of data points collected on individual animals.

Table 6.10. Movement of individual collared feral pigs within differing distances of piggeries in Southern Queensland, Australia during 2010–2011. The Horizontal Dilution Of Precision (HDOP) value is restricted to ≤ 6 based on Moseby *et al.* (2009).

		Distance from piggery (meters)					
	Sex	<50	<100	<500	<5000	% of locations[a]	Total no. data points[b]
1	Male	0	0	0	2695	91.9	2934
2	Male	0	5	13	3047	85.7	3557
3	Male	0	0	0	1564	75.2	2079
1	Female	0	0	0	4596	99.7	4609
2	Female	0	0	0	2	0.1	2435
3	Female	0	0	0	1943	52.6	3695

[a] Percentage of total data points within 5000 meters of piggery – given distance set on piggery boundary not sheds/shelters where the domestic pigs are housed.
[b] Total number of data points collected on individual animals.

Male 1, Male 2 and Female 1 had the potential for direct contact with each other, as they roamed within 50 m of each other in the same one hour period over multiple occasions (Table 6.11).

Table 6.11. The total number of movement data points that indicate feral pigs were within 50 meters of each other on the same date and in the same hour in Southern Queensland, Australia.

Feral pig individuals	Total number of data points	Total number of HDOP ≤ 6 data points
Male 1 and Male 2	47	36
Male 1 and Female 1	1108	876
Male 2 and Female 1	454	309
Male 1, Male 2 and Female 1	7	5

HDOP = Horizontal Dilution Of Precision value ≤ 6 based on Moseby et al. (2009).
Fix acquired every 30 minutes

Male 1 and Female 1 were in close contact with each other for the greatest proportion of total data points compared to the other feral pigs (Table 6.12). Male 1 was within 50 m of Female 1 for 33.7% of its total recorded data points, and 29.9% of its HDOP ≤ 6 total data points (Table 6.12). Feral pigs collared near the intensive piggery did not have any recorded data points where they roamed to within 50 m of another collared individual.

Table 6.12. The proportion of total data points that feral pigs were within 50 meters of each other on the same date and in the same hour in Southern Queensland, Australia.

	Proportion of total data points (%)	Proportion of total HDOP ≤ 6 data points (%)
Male 1 within 50 m Male 2	1.4	1.2
Male 2 within 50 m Male 1	1.0	1.0
Male 1 within 50 m Female 1	33.7	29.9
Female 1 within 50 m Male 1	21.4	19.0
Male 2 within 50 m Female 1	9.7	8.7
Female 1 within 50 m Male 2	8.7	6.7

HDOP = Horizontal Dilution Of Precision value ≤ 6 based on Moseby et al. (2009).
Fix acquired every 30 minutes.

6.4 Discussion

To calculate the risk of feral pigs spreading pathogens into commercial piggeries, two steps are necessary. First, the presence of pathogens in both feral and domestic pigs must be accurately detected, and secondly, the movements of feral pigs and likelihood of feral and domestic animals coming into close proximity needs to be considered. In the current study, *Lawsonia intracellularis*, *Leptospira spp*., *Mycoplasma hyopneumoniae* and *Brucella suis* were all detected in feral pigs within 10 km of two piggeries in southern Queensland.

The true prevalence of *Lawsonia intracellularis* in feral pigs in this study was higher than that found in Western Australia (18.9%; Phillips et al. 2009). This prevalence was also greater than that found among wild pig populations in the Czech Republic (51.4%, Tomanova et al. 2002; 9.1%, Dezorzova-Tomanova et al. 2006) and Germany (20.6%, Reiner et al. 2011). An ELISA test was used in the current study, which detects antibody presence against a current or past infection with the pathogen. The previously mentioned studies used PCR or a combination of PCR and immunofluorescent antibody test. The PCR results identified the presence of the bacteria in feral pig faeces, and thus determined the proportion of pigs currently shedding the pathogen. Of the feral pigs that have seroconverted against *Lawsonia intracellularis* using the ELISA in the current study, not all would have been shedding the pathogen, and thus been identified as positive via PCR. Brandt et al. (2010) investigated 60 domestic pigs for detection of *Lawsonia intracellularis* using a PCR and the same ELISA used in the current study. Of these 60 pigs, 39 were positive via PCR and 49 positive via ELISA; this is a difference of 16.7%. If we assume a similar proportion of feral pigs in the current study would not have shed the pathogen, the prevalence would still have been much greater in the current study compared to the previous studies, and vastly greater than Phillips et al. (2009), the only other study to detect this pathogen in feral pigs in Australia. Consequently, it can be determined that the pathogen status of feral pigs, for *Lawsonia intracellularis* in particular, differs between different regions of Australia. Thus the risk of pathogen transmission from feral pigs to domestic pigs may differ depending on piggery location, with for example a lower risk for *Lawsonia intracellularis* expected in Western Australia compared to Queensland.

The period of time that *Lawsonia intracellularis* antibodies are present in serum is also longer than the period of time that pathogen DNA is present in the faeces of an infected

animal. Domestic pigs that recovered from proliferative haemorrhagic enteropathy caused by infection with *Lawsonia intracellularis* had serum antibody levels detectable for three months post-infection (Guedes et al. 2002). This time period indicates that the collared feral pig, Male 2, may have been suffering a second infection with *Lawsonia intracellularis* upon recapture six months after initial collar application and serum sample.

Mycoplasma hyopneumoniae was identified for the first time, to the best of the author's knowledge, in Australian feral pigs. This indicates that feral pigs could be a potential pathway of exposure and transmission of this pathogen to naïve domestic pigs. Where there is an infected piggery, such as the two piggeries in the current study, feral pigs may be a minor contributor to the maintenance of *Mycoplasma hyopneumoniae* within a piggery, with the infection cycling between feral and domestic pigs. Cycling of the infection between domestic pigs is likely to be the major contributor to the maintenance of the pathogen. The true prevalence of *Mycoplasma hyopneumoniae* in feral pigs was comparable to levels reported in previous studies in Slovenia (21%, Vengust et al. 2006) and the United States (32%, Baker et al. 2011). The sensitivity of the *Mycoplasma hyopneumoniae* ELISA was lower than the PCR method; however, it was able to detect antibodies that may have remained after an infection, rather than the prevalence of infected individuals (Table 6.1). *Actinobacillus pleuropneumoniae* (APP) was determined to be absent from the feral pig population. This pathogen has not been studied in feral pig populations in Australia previously to the best of the author's knowledge; however, it has been detected in feral pigs in Slovenia (52%, Vengust et al. 2006).

In Australia, *Brucella suis* and *Leptospira spp*. have been detected in previous studies of feral pigs. However, in the current study *Brucella suis* was present at a higher true prevalence than previous Australian studies (Table 6.2; 4.1%, Pavlov 1991; 1.9%, Mason and Fleming 1999). These studies sampled more isolated areas of Australia, including Cape York in Queensland, and were not selected for their vicinity to agricultural premises or urban centres where pathogen transmission risk to commercial pigs is greater. The recommended method for testing in most laboratories in Australia is the RBT, followed by confirmation with the CFT. This method of testing in series increased the overall specificity, filtering out more false positive results than when each test was used individually. However, this method decreases the overall sensitivity, such that some positive samples may have been falsely determined to be negative. The RBT and CFT are

also non-specific for *Brucella suis*, detecting all *Brucella spp*., such as *Brucella abortus* and *Brucella melitensis*, which are presumed to be eradicated from Australia (WAHID 2010). As such the preferred testing method for *Brucella suis* in Australia is reliant upon continued accurate determination of the absence of *Brucella abortus* and *Brucella melitensis* from Australia.

Previous Australian studies of *Leptospira spp*. prevalence in feral pigs present a range of 2% to 51% (Choquenot et al. 1996). The results of this study fall in this range, confirming this finding in feral pigs (Table 6.2). *Leptospira interrogans* serotype Pomona was the most common isolate, which agrees with previous studies (Choquenot et al. 1996), followed by the recently recognised Australian serotype *Leptospira weilii* serotype Topaz. The serotype was detected at a high prevalence in eastern-grey kangaroos (47%, Roberts et al. 2010) and has been isolated from one cow sample (Corney et al. 2008).

Leptospira spp. has previously been detected in feral pigs caught on piggeries in Queensland at a prevalence of 31% (Elder and Ward, 1978). During the time of this study, 1972 to 1976, piggeries were more of a sideline enterprise, and were generally free-range in style, so would have been more accessible to feral pigs than they are presently (Richardson and O'Connor 1978). The prevalence of *Leptospira spp*. in feral pigs has increased since the time of the Elder and Ward (1978) study, despite the incorporation of a vaccination routine on piggeries for *Leptospira interrogans* serotype pomona. This indicates that feral pigs are a stand alone reservoir of *Leptospira* spp. infection, and pose a source of infection for domestic pigs in the absence of protective vaccinations. This again emphasises the particular risk posed by the presence of *Leptospira weilii* Serovar Topaz in the feral pig population at these piggeries.

Lawsonia intracellularis, Leptospira spp. *and Mycoplasma hyopneumoniae* were all detected in the domestic sows on the intensive piggery near to the feral pig population. The true prevalence of *Lawsonia intracellularis* in these breeding sows was the same as that in the feral pigs. The Enterisol® vaccination used for control of *Lawsonia intracellularis* on this piggery does not present as a positive result in the ELISA (Dohoo 2011), indicating sows were infected with the pathogen, suggesting control measures for this pathogen are not effective in breeding sows over the long term. This is additionally supported by research findings by a number of authors (Guedes et al. 2003; Dohoo 2011).

Mycoplasma hyopneumoniae was present at a much higher true prevalence in the domestic sows despite medication compared to the feral pigs. This may be due to the domestic pig's confinement to buildings, allowing for propagation and transmission at a greater level than in feral pigs, as well as the limited duration of the vaccination effectiveness. Due to the infected status of these domestic pigs, and the uncertainty of the infection source, domestic pigs may have transmitted the infection via infected aerosolised particles to the feral pigs, or the feral pigs may have transmitted the infection to the domestic pigs.

Leptospira spp. were present at a lower true prevalence in the domestic pigs than in feral pigs. However, the domestic sows had been vaccinated against *Leptospira interrogans* serotype Pomona. This vaccination is specific to one serotype and does not provide protection against other serotypes (Taylor 2006). The only serotype found in the domestic pigs was *Leptospira weilii* Serovar Topaz. This is the first ever identification of this serotype in pigs, to the author's knowledge. This has important biosecurity implications, as the consequences of this pathogen for herd health and productivity are unknown. The symptoms of infection with this serovar in humans are typical of infection with other *Leptospira spp*. and include fever, headaches, chills and myalgia (Slack et al. 2007). The number of individual isolations of the serotype in the domestic sows was similar to that found in the feral pigs. Due to the identification of this serotype in both wild and domestic pigs, the role of the eastern grey kangaroo in the transmission of this pathogen requires further investigation.

As the domestic pigs sampled from the piggery were specific to one sex and age group, the prevalence of pathogens may be biased, and higher than they actually are in the entire domestic pig population on this piggery. These results may also be biased as only the intensive piggery was sampled for pathogen detection, due to concerns about confidentiality and repercussions of pathogen detection by the other piggery manager. The piggeries were also unwilling to be sampled for detection of *Brucella suis* in the domestic pig herd, as the consequences of a positive sample would be very costly due to the necessary removal of all positive animals within a pig herd and costs of mandatory testing of pigs to confirm pathogen eradication. All positive *Brucella suis* isolates in a commercial piggery must be reported and quarantine procedures ensue. This restriction also impacts the results presented in this chapter.

The presence of these pathogens in both the domestic and feral pig population has implications for the control of the production-limiting pathogens. Management strategies for control or eradication of these pathogens on the piggeries need to consider the risk of re-infection by feral pigs. The feral pigs act as a reservoir for pathogens that may be transmitted in a number of different ways, some not requiring direct contact, such as aerosol transmission for *Mycoplasma hyodysenteriae*.

The results from collared pigs show the majority of movement was within 5 km of the piggeries, with the exception of Female 2. This is a similar result to that found in a study of feral pig movement near piggeries in Texas, US (Wyckoff et al. 2009). None of the collared feral pig data points were within a close enough proximity to piggeries to indicate direct contact with domestic pigs. This prevention of contact with feral pigs is a requirement of the Australian pork industry quality assurance program (APIQ 2010). One individual, a large male boar, moved to within 100 m of the free-range piggery. The natural movement behaviour of the collared feral pigs in the current study may have been disrupted by the eradication of their group members that were trapped with them, as well as through the cull of feral pigs conducted from a helicopter as a part of the QMDC project over a single day in the region of the current study. Displacement of feral pigs from their home ranges has been reportedly associated with continued hunting pressure on the ground or from helicopters (Caley 1993b, Saunders and Bryant 1988). Pigs tend to forage and feed in groups (Kyriazakis and Whittemore 2006), as such, the disruption of the natural structure of feral pig groups in the current study may have caused a shift in the original home range of some of the individuals, affecting their movement in relation to the piggeries, but also providing a possible explanation for the movement and collar retrieval of Male 1 approximately 20 kilometres from initial site of capture. Whether the hunting pressure experienced in the current study was continuous enough to bring about feral pig displacement is unknown.

The collared pigs often overlapped in their movement, and had a number of possible contact events, providing the opportunity for pathogen transmission between feral individuals. The results of the current study were limited by the collaring of only seven feral pig individuals, one of which could not be recaptured. These individuals were captured within a 5 km radius of each of the piggeries, and as such did not allow for collection of movement data from feral pigs that may have been present within the

intermediate region of the two piggeries. However, based on the density of feral pigs in the region (Gentle et al. 2011), it can be assumed there were feral pigs present in the intermediate zone between piggeries, thereby allowing for intermingling and pathogen transmission between feral pig populations. Based on the interaction of feral pigs around the free-range piggery, contacts between these pigs, intermediate pigs and feral pigs around the intensive piggery were likely to occur.

The average GPS fix rate of 72% in this study was similar to that obtained by previous studies (Cain et al. 2005). The feral pig that came into closest proximity to the piggery, Male 2, had the lowest fix success rate at 52.9%, indicating that this pig may have spent more time in close proximity to the piggery than was recorded. There are many factors that affect the fix acquisition, including the location and angle of the antenna on the collar due to the position of the pig's head during fix acquisition, or movement of the collar with pig natural movements (Cain et al. 2005). Topography, vegetation and satellite location can also affect fix acquisition (Cain et al. 2005).

The proximity estimates obtained in the current study demonstrate the threat feral pigs present to domestic pigs. Of the collared pigs, one was positive for *Leptospira interrogans* serotype pomona, five were positive for *Lawsonia intracellularis* and two were positive for *Mycoplasma hyopneumoniae*, based on the apparent prevalence of pathogens. The known prevalence of these pathogens and of the other pathogens in feral pigs in such close proximity to the domestic pigs is a biosecurity risk. Pathogen transmission via infected air provides an additional complication, and risk for pathogen transmission from feral to domestic pigs and visa versa. *Mycoplasma hyopneumoniae* can be transmitted via the air. Dee et al. (2009) detected *Mycoplasma hyopneumoniae* at a maximum tested distance of 4.7 km from the pathogen source. The majority of feral pig movement in the current study was within 5 km, demonstrating the risk associated with feral pigs. Aerosol transmission of exotic highly pathogenic diseases, such as foot-and-mouth disease, has been investigated due to risk of livestock infection from a long-distance source. Spread of foot-and-mouth disease has been measured at 60 to 100 km over land, and is capable of maintaining an infective dose over this distance (Donaldson 1979; Alexanderson et al. 2002). This high density of feral pigs, the major reservoir host of foot-and-mouth disease (Productivity Commission 2002), around piggeries poses great concern for disease control, especially in the event of a foot-and-mouth disease outbreak in Australia.

Aerosol transmission is reliant on climate, temperature and wind conditions, each of which may differ between different pathogens. The optimal conditions for *Mycoplasma hyopneumoniae* aerosol transmission are relatively low humidity levels, less than 30% (Mitscherlich and Marth 1984), and cool temperatures below 27 ºC, which increase the number of airborne particles (Stark 1999). Disease prevention measures such as dust reduction, air filtration, air disinfection and the establishment of disease-free regions are options for mitigation of aerosol transmission that currently are not implemented on piggeries (Stark 1999), likely due to the high infrastructural and running costs and low implementation feasibility in practice.

This study has provided an accurate measure of the pathogen prevalence in a feral pig population in Southern Queensland. Prevalence has been coupled with movement data on feral pigs to estimate time spent in close proximity to piggeries. This increases the risk of transmission of production-limiting pathogens and jeopardises piggery disease management. Only bacterial pathogens were investigated in this study; however, the modes of transmission of all pathogens, apart from vector-borne, have been covered through the selection of pathogens included in this study. Piggery type may have an impact on the level of risk of pathogen transmission. Intensive piggeries have an additional physical barrier between pigs and the outdoors, unlike free-range piggeries. Consequently, the risk of contact between free-range domestic pigs and feral pigs is greater (Wu et al. 2011). It is recommended, where possible, that all piggeries located near a feral pig population double fence their perimeter to create an exclusion zone, with an outrigged electric wire. This minimises the risk of feral pigs making direct contact with domestic pigs through fences, and gaining entry to piggeries by digging. Findings from this study provide accurate information on the potential risks of pathogen transmission from feral pigs to domestic pigs. How likely it is that this transmission could occur is further investigated in Chapter 7.

7 Risk of pathogen transmission from wild animals to domestic pigs in Australia

7.1 Introduction

Pathogen transmission from wild animals to livestock and humans has occurred in the past, with 72% of zoonotic emerging infectious pathogens coming from a wild animal source (Jones et al. 2008). In the event of a new disease outbreak, the index case or source of infection can sometimes be determined; such was the case with fruit bats identified as the source of Nipah virus in pigs in Malaysia, and Menangle virus in pigs in Australia (Chua et al. 2000; Philbey et al. 2008). These cases identify the potential risk of pathogen transmission from wild animal species to domestic pigs. However, in the case of endemic diseases, the source of an outbreak and the level of contribution from wild animal hosts to the introduction and persistence of a pathogen among domestic animals can be difficult to establish. In these instances, determining how much risk wild animals pose to the introduction and maintenance of an infection, and how much effort is needed to mitigate this risk, can be guess work.

The World Organisation for Animal Health (OIE) has determined that, for the purposes of international trade, a country must provide a rigorous and transparent assessment of the risk posed by a product in order to require mitigation or deny importation (OIE 2010). This assessment must follow the OIE risk analysis framework, which provides the scientific principles necessary to determine the probability of an adverse event occurring, and underpins decisions to mitigate unacceptable levels of risk. Risk analysis methods used in animal health were originally applied to evaluate the risk of pathogen or pest introduction via imports to a country (MacDiarmid 1997). In addition, risk analysis methods have been applied to evaluating the risk of exotic disease incursions through other pathways, such as international passenger travel (Gratz et al. 2000; Kilpatrick et al. 2004; Kilpatrick et al. 2006) and migratory birds (Martinez et al. 2011). There have been recent cases when risk analysis has been used to determine the risk of pathogen transmission from wild animals to livestock. Some examples include determining the risk of *Mycobacterium bovis* transmission from badgers to cattle in England (Gallagher et al. 2003), and foot-and-mouth transmission from buffalo to cattle in Zimbabwe (Sutmoller et al. 2000).

Risk can be qualitatively or quantitatively assessed. Both of these methods are accepted under the OIE methodology for risk analysis. In addition, quantitative methods can be deterministic (using point estimates) or stochastic. Stochastic simulation models using probability distributions as input values are used to incorporate uncertainty and variability in quantitative models (OIE 2010).

Quantifying the risk of pathogen transmission from wild animals to pigs has not been previously conducted, to the author's knowledge. This study aims to quantify the risk of exposure to the pathogens *Escherichia coli, Salmonella spp*. and *Campylobacter spp*. from European starlings; *Brachyspira hyodysenteriae*, *Lawsonia intracellularis* and *Salmonella spp*. from rats; and *Mycoplasma hyopneumoniae*, *Leptospira spp*., *Brucella suis* and *Lawsonia intracellularis* from feral pigs to domestic pigs on commercial piggeries in Australia. In conducting this exposure assessment, the most likely pathways of infection as well as those parameters with most influence on the probability of exposure will be identified. Results from these exposure assessments will provide information to determine the most effective mitigation strategies to reduce the risk posed by wild animals for pathogen exposure to pigs on piggeries, and will support decision making for disease management and control in the future.

7.2 Materials and Methods

7.2.1. Risk assessment methodology

This risk assessment follows the World Organisation for Animal Health (OIE) methodology for risk analysis (OIE 2010). As discussed in Chapter 3, Section 3.10, the OIE risk assessment is comprised of four interrelated steps: hazard identification, risk assessment, risk management and risk communication. Chapter 2 of this thesis identified that starlings, rats and feral pigs were commonly observed in and around piggeries. Consequently, these wild animals were targeted for further risk investigation. The subsequent chapters of this thesis identified key pathogens carried by these wild animals that could pose a risk to domestic pigs, which were therefore considered the hazards for this assessment.

Risk assessment is itself comprised of four steps: the release, exposure and consequence assessments, and the risk estimation (OIE 2010). As the wild animals of interest and target pathogens considered in this risk assessment are already present in Australia, a release assessment was not necessary. The exposure assessment describes the potential pathways for infection of domestic pigs with the targeted pathogens from the specified wild animals. These pathways were described in the current study using scenario trees, the common method for representing steps in the process for hazard exposure to a susceptible animal in an import risk assessment (OIE 2010). Consequence assessment investigates the probability of pathogen spread from the index farm and the impacts of this spread. In general terms, the impacts of domestic pig infection with the target pathogens in this study have been previously investigated, and have been considered for the selection of pathogens to be included in the current study. Consequences associated with clinical symptoms and the financial implications of these pathogens have been described in Chapter 3. However, a consequence assessment and subsequent risk estimation for wildlife to pig pathogen transmission was beyond the scope of this study.

The scenario trees for the exposure assessment were implemented in Microsoft Excel (PC/ Windows XP, 2003) and probabilities were calculated using quantitative stochastic simulation modelling with @RISK 5.7 software (Palisade Corporation, USA). Each simulation consisted of 5,000 iterations, using Latin hypercube sampling and a random seeding type. The median, 5% and 95% values were obtained from the simulation output to describe the probability of exposure. Median probabilities of exposure were compared between different pathogens within each animal species. In addition, exposure estimates for the feral pig assessment were compared according to piggery type (indoor vs. free-range) and different animal densities of feral pigs.

The exposure models represent the likelihood of exposure to a pathogen from a wildlife species on an average commercial piggery in Australia at any point in time. The models assume a constant likelihood of exposure year round as seasonality effect on the animal densities and the pathogen prevalence has not been considered.

7.2.2 Population framework and data sources

A variety of data sources were used in this exposure assessment to estimate the quantitative input values required to determine the probability of exposure of domestic pigs to

pathogens from wild animals. Data from Chapters 2, 4, 5 and 6 of this thesis provide the majority of the assessment inputs. Information from literature and expert opinion were also used for a number of parameters that were beyond the scope of those chapters.

7.2.2.1 Postal survey

Chapter 2 reports the results of a postal survey administered to the pork-producing members of Australian Pork Limited. The results of the questionnaire identified the proportion of piggeries experiencing incursions by wild animals, the type of wild animals and animal numbers observed per week by respondents. Information on the control techniques implemented by piggeries to mitigate wild animal entry to piggery housing and control wild animal population size was also obtained. This information was used extensively throughout this exposure assessment.

7.2.2.2 Presence of pathogens in the European starling population in domestic piggeries

Chapter 4 reports the results of a pathogen detection study in starlings captured from piggeries in South Australia. A total of 473 starlings were captured to provide an estimate of the presence of the pathogens *Escherichia coli, Salmonella spp*. and *Campylobacter spp*., as well as an estimation of the prevalence of these pathogens in starling populations.

7.2.2.3 Presence of pathogens in the rat population in domestic piggeries

Chapter 5 reports the results of a pathogen detection study in rodents on two piggeries in Victoria and one piggery in South Australia. A total of 300 rats were obtained to detect the presence of *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli*, *Lawsonia intracellularis* and *Salmonella spp*., and provide an estimate of the prevalence of these pathogens among rats on piggeries that applied different methods of control for *Brachyspira hyodysenteriae* infection in pigs. Control methods include medication, Swiss depopulation and total depopulation, described in Chapter 5. *Brachyspira pilosicoli* has not been included in this exposure assessment as this pathogen was not detected in rats, and has not been detected in rodents in any previous studies, to the author's knowledge.

7.2.2.4 Presence of pathogens in the feral pig population in the area surrounding domestic piggeries

Chapter 6 reports the findings of a pathogen detection study in feral pigs in the vicinity of two piggeries in Southern Queensland. A total of 83 feral pigs were captured and sampled to detect the presence of the pathogens *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Leptospira spp*., *Brucella suis* and *Lawsonia intracellularis*, as well as estimate the prevalence of these pathogens in the feral pig population in this region. Additionally, the movement of feral pigs in the vicinity of these piggeries was assessed by collaring and obtaining GPS data on six feral pigs: three male and three female. As *Actinobacillus pleuropneumoniae* was not detected in the feral pig population, and has not been detected in feral pigs in Australia in any previous studies, this pathogen was not included in this exposure assessment.

7.2.2.5 Literature and expert opinion

Literature and expert opinion were used when suitable data were not available from the current study to estimate input values used in the exposure assessment. For expert opinion, a pig veterinarian, with over 15 years of experience working in Victoria, South Australia and New South Wales; and with previous involvement in research projects with pig producers in Victoria, South Australia, New South Wales, Queensland and Western Australia; was consulted to obtain required information that had not been collected. Consultation was informally conducted by email using open questions.

7.2.3 Pathways and scenario trees

7.2.3.1 European starlings

The scenario tree shown in Figure 7.1 was used to describe the exposure of domestic pigs to the three pathogens studied in starlings. Three pathways of exposure of a domestic pig to a pathogen from a starling were identified: contaminated faeces in the environment, contaminated faeces in food, and contaminated faeces in water. The pathways and nodes of this scenario tree are represented in Figure 7.1, and nodes and branches for each node are described below and summarised in Table 7.1. It was assumed there would be no direct contact between starlings and pigs via consumption of a starling carcass.

7.2.3.1.1 European starlings present around the piggery: Starlings must be present at a piggery for exposure to occur. The proportion of piggeries with starlings present was

identified in Chapter 2. Of the 170 piggeries that reported wild animal observations, 24 recorded specific starling observations and an additional 31 recorded observations of unspecified wild birds, some or all of which may have been starlings. The proportion of piggeries with starlings was incorporated into the model using a Beta distribution with the total starling and wild bird observations, to account for uncertainty around this proportion.

7.2.3.1.2 Number of starlings present: The number of starlings present on piggeries is likely to impact the probability of exposure and subsequent risk of transmission, and thus was considered a risk factor. This node accounted for the differential risk of pigs being exposed, while using a single value for probability of exposure across all piggeries (Martin et al. 2007), and thus is considered a risk node. The number of starlings on piggeries reporting their presence was also obtained from Chapter 2. Of the 24 piggeries that observed starlings, 15 provided estimates of starling numbers. This node had three branches according to the number of starlings reported per week, obtaining piggeries with low, medium and high numbers of birds. Four piggeries observed less than 500 starlings, 8 observed between 500 and 1999 starlings, and 3 observed greater than 2000 starlings a week. A Beta distribution was used for each of these number proportions to account for uncertainty around this estimate. In addition, to incorporate the differential risk associated with the number of starlings around the piggery, an estimate of the risk of exposure in piggeries with high numbers of birds relative to those in the other two piggery categories was required. As no information was available regarding the difference in risk according to the bird number, the relative risk of high and medium number was assumed to be 10 and 5 times higher, respectively, than that in piggeries with a low number of starlings. To incorporate uncertainty around the relative risk, the Pert distributions (5, 10, 15) and (2, 5, 10), were used for a high and medium number of birds, respectively.

7.2.3.1.3 Proportion of piggeries with different starling infection: Results from Chapter 4 indicate that not every piggery had starlings infected with the pathogens studied, or may have a varying level of pathogen presence in starlings. Consequently, the proportion of piggeries with infected starlings was included in the model. For the purposes of this exposure assessment, the sampling events in 2008 and 2009 for piggery B have been treated as separate piggeries due to the different sampling periods and possibly different starling populations, bringing the total number of piggery observations to five. Only one out of the five piggeries selected for capture and sampling of starlings had starlings

infected with *Campylobacter spp*. and *Salmonella spp*. Pathogenic *Escherichia coli* was detected in starlings in all five piggery observations. However, the prevalence of this pathogen varied between piggeries: four piggery observations had a prevalence higher than 3.9% (considered moderate prevalence) and one piggery had starlings infected at a low prevalence (1%). Proportions of piggeries with starlings infected with low or moderate prevalence were incorporated into the model using a Beta distribution.

7.2.3.1.4 Prevalence in starlings: Starlings must be infected with the target pathogen of interest for exposure to occur. Prevalence estimates were obtained from Chapter 4. The true prevalence of *Campylobacter spp*. and *Salmonella spp*. in starlings is shown in Table 4.7 in Chapter 4. True prevalence data of pig-pathogenic *Escherichia coli is* shown in Table 4.8 in Chapter 4. Prevalence was incorporated into the model with a Pert distribution using the lower 95% confidence interval, true prevalence estimate and upper 95% confidence interval for each pathogen. An additional 20% was added to the *Salmonella spp*. prevalence estimates based on Hinton (1988), who identified that cloacal swabs underestimate the proportion of *Salmonella spp*. infected birds by 23% compared to caecal contents. For a pathogen where more than one piggery has a low, moderate or high prevalence, an average of the Pert distributions was obtained. For those piggeries that did not have the pathogen detected in the starling population, the upper 95% confidence interval was not zero, and thus a Pert distribution was calculated for these as well.

7.2.3.1.5 Starling access to pig environment: Whether starlings had access to the pig environment was estimated by information on piggery types and bird control measures described in Chapter 2. Of the 55 pig producers reporting starlings and wild birds, only two used nets that restricted entry of birds to the piggery. Access was assumed to occur in those piggeries without nets. A Beta distribution of the proportion of piggeries without nets according to the results from Chapter 2, [1−(2/55)], was considered to be the maximum value among piggeries in Australia. As such, this value was used as the maximum of a Pert distribution, with minus 5% and 10% for the most likely and minimum values.

7.2.3.1.6 Starling access to pig food and water: Estimation of starling access to food and water, once pig housing was accessed, was based on the types of feed and water distribution systems used in piggeries. As information on the proportion of piggeries using different types of food and water distribution systems in Australia was not available, a pig

veterinarian was consulted. Two different types of feeding systems were identified: ad libitum feeding troughs, and feed distributing conveyor or auger systems. Similarly, two types of water systems were identified: ad libitum water troughs, and enclosed water nipple or bite drinkers.

The troughs and open auger designs allow for starling access. An estimated 75% and 30% of piggeries have feeders and drinkers that allow birds access to pig feed and water, respectively. These proportions were incorporated into the model as the most likely value of a Pert distribution with minus and plus 10% as the minimum and maximum values to account for uncertainty around these estimates.

7.2.3.1.7 Pathogen survival: The survival of pathogens excreted in bird faeces in various substrates was estimated based on literature. However, survival of these pathogens in the same substrates found in piggeries has not been previously studied. As such, a qualitative value was assigned to pathogen survival, which was then translated into a quantitative value using a uniform distribution following the semi-quantitative methodology described in the Guidelines for Import Risk Analysis (DAFF 2004). Standard conversions of qualitative values were as follows: very low probability ranged from 0.001 to 0.05, low probability ranged from 0.05 to 0.3, moderate probability ranged from 0.3 to 0.7 and high probability ranged from 0.7 to 1.0. The same procedure was used for all qualitative estimates throughout the exposure assessment.

Survival of *Escherichia coli* in the pig environment, food and water was determined to be high. This estimation was based on the 66-day survival of *Escherichia coli* in soil seeded with chicken manure and containing 10^3 organisms per gram at room temperature (Mitscherlich and Marth 1984). Although survival of *Escherichia coli* in water was shown to be variable, previous studies reported survival periods of 26 (Cools et al. 2001) and 49 days (Guan and Holley 2003). *Escherichia coli* survive for longer at cooler temperatures.

Survival of *Salmonella spp*. was also estimated to be high. This pathogen was reported to survive for 13 months in dry faeces (Fedorka-Cray et al. 2000). In a study by Temple et al. (1980), the pathogen declined from 10^6 bacteria per gram of soil to 10^4 per gram of soil over an 8-week period, indicating a half life of over 2 months at room temperature. On an organic outdoor Danish piggery, *Salmonella spp*. was detectable for up to 5 weeks in

paddocks after the removal of pigs (Jensen et al. 2006). *Salmonella spp*. also survive for long periods of time in water, as reported by Guan and Holley (2003), who found that the pathogen survived in water for between 45 and 152 days. *Salmonella spp*. survive longer at cooler temperatures.

Survival of *Campylobacter spp*. in different substrates is comparatively lower than survival of the other pathogens. At temperatures between 20 and 30 ºC, survival is less than 2 days in natural water, 10 days in soil and 3 days in cattle manure or slurry (Guan and Holley 2003). As such, survival was estimated to be moderate in the pig environment and low in pig food and water. This pathogen could survive for longer periods of time at lower temperatures, with the longest survival reported at temperatures below 0 ºC.

7.2.3.1.8 Pig contact: Pig contact with the contaminated environment, food and water was also determined by expert opinion. Contact of the pigs with food and water was determined to be certain. Similarly, contact of pigs with bird faeces in their environment, although not certain, was estimated to be very high probability due to the curious nature of pigs. A Pert distribution with a maximum of 1, most likely of 0.95 and minimum of 0.90 was used.

7.2.3.1.9 Infection of pig: The probability of the pig being infected after contact with the pathogen was estimated based on the amount of pathogen shed by birds, as well as the dose required to infect pigs. These parameters were determined from literature. It is assumed that the domestic pigs are totally susceptible.

The probability of pigs being infected with *Escherichia coli* on contact with any of the contaminated materials was estimated to be high. The infective dose of *Escherichia coli* for pigs is low, at approximately 10^4 bacteria (Cornick and Helgerson 2004). Shedding quantity and duration from chickens and other wild birds was used as there was no data available in literature about shedding of these pathogens from starlings. Chickens shed the pathogen at a high level for 35 days, and a medium level for another 35 days. Geese, coots and gulls shed an average quantity of 8.8×10^6, 1×10^7 and 1.2×10^8 colony-forming units per gram of faeces, respectively, though the period of time in which they shed the pathogen is unknown (Meerburg et al. 2011). These levels are sufficient to cause infection in pigs.

Infection of pigs on contact with *Salmonella spp*.-contaminated materials was also deemed to be high probability. Although the infective dose in pigs is much higher than for *Escherichia coli*, with 10^8 bacteria required to reliably achieve infection (Anderson et al. 1998), infected chickens are reported to shed the pathogen at a qualitatively-described high level for up to six weeks in some cases (Shivaprasad et al. 1990).

The probability of pigs becoming infected with *Campylobacter jejuni* was also estimated to be high. Previous work reported an infective dose in pigs of between 10^4 and 10^8 bacteria, which is considered low (Mansfield et al. 2003; Parthasarathy and Mansfield 2009). In addition, gulls, pigeons and wild geese can shed from 10^4 to 10^6 colony-forming units per gram of faeces (Ogden et al. 2009), and shedding can last for approximately 2 months at an average of 3.2×10^6 colony-forming units per gram of faeces in the caeca of chickens (Achen et al. 1998).

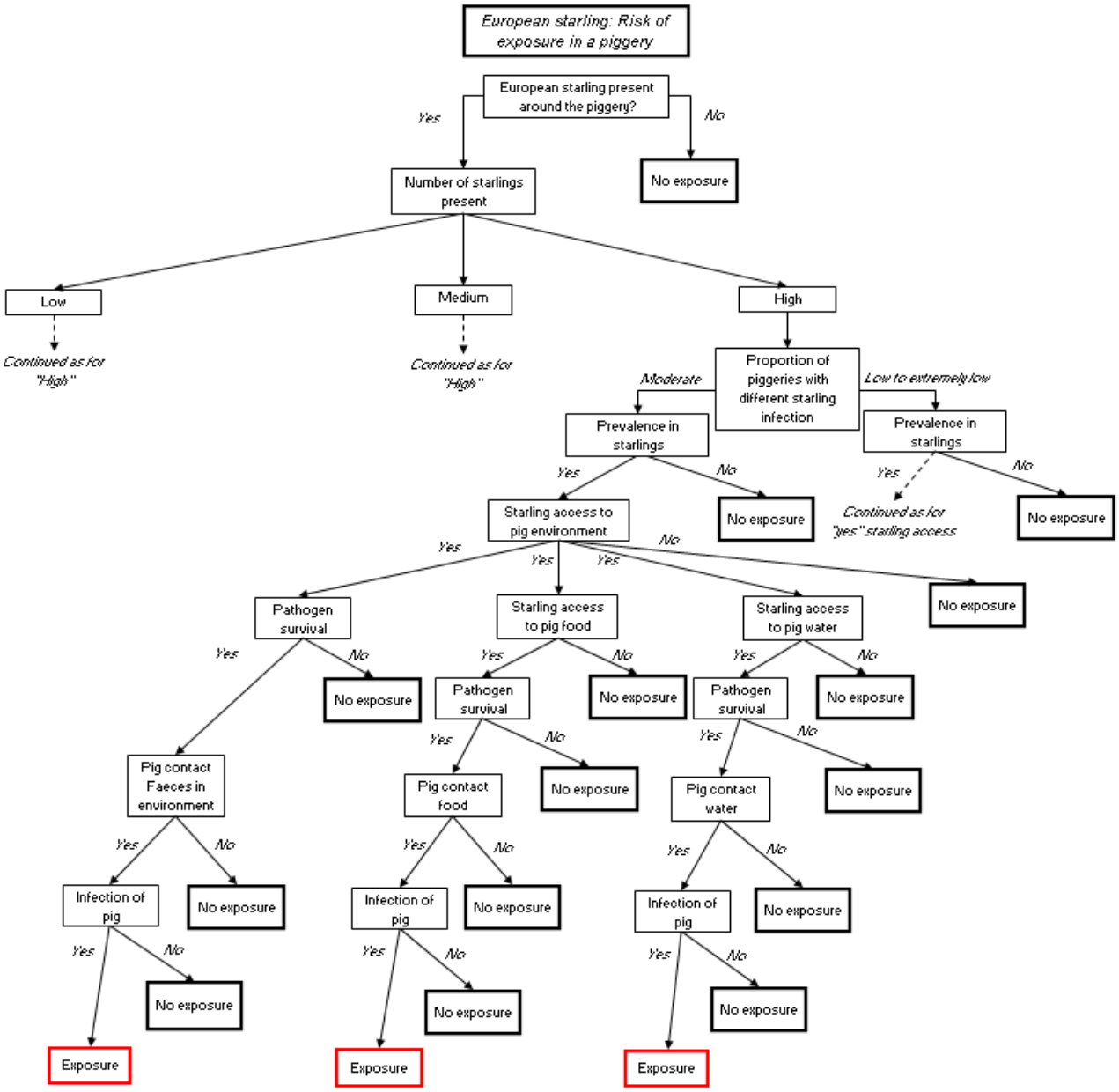

Figure 7.1. Scenario tree representing the exposure assessment evaluating the probability of domestic pigs on piggeries in Australia being exposed to *Escherichia coli*, *Salmonella spp*. and *Campylobacter jejuni* from European starlings.

Table 7.1. Nodes, parameters and input values used for the exposure assessment evaluating the probability of domestic pigs on piggeries in Australia being exposed to *Escherichia coli*, *Salmonella spp*. and *Campylobacter jejuni* from European starlings.

Node	**Branch of node**	**Parameter estimates**	**Input values** [a]	**Data sources**
1. European starlings present around the piggery?	*Yes* *No*	Probability of European starlings being present around the piggery	RiskBeta (56, 116)	Chapter 2
2. Number of European starlings present around the piggery per week	*Low (<500)* *Medium (500–1999)* *High (≥ 2000)*	Proportion of piggeries with low, medium and high number of European starlings observed per week Relative risk of exposure according to the number of European starlings around the piggery per week (*Low being the reference*)	Low: RiskBeta (5, 12) Medium: RiskBeta (9, 8) High: RiskBeta (4, 13) Medium: RiskPert (2, 5, 10) High: RiskPert (5, 10, 15)	Chapter 2 Assumption
3. Proportion of piggeries with different starling infection	*Yes* *No*	Proportion of piggeries with infected starlings. Proportion of piggeries with low and moderate level of infection (for *Escherichia coli,* as all piggeries were infected)	*Salmonella spp*. Moderate: RiskBeta (2, 5) *Campylobacter jejuni* Moderate: RiskBeta (2, 5) *Escherichia coli:* Low: RiskBeta (2, 5) Moderate: RiskBeta (5, 2)	Chapter 4
4. Prevalence in starlings	*Yes* *No*	Prevalence of the pathogen in the European starling population	*Escherichia coli* (two levels of prevalence): Moderate: Average of the output of the following Pert distributions	Chapter 4 and Literature: Hinton 1988.

			1: RiskPert (0.01, 0.04, 0.10) 2: RiskPert (0.04, 0.08, 0.16) 3: RiskPert (0.01, 0.04, 0.10) 4: RiskPert (0.07, 0.12, 0.20) Low: RiskPert (0, 0.010, 0.051) <u>*Salmonella spp*</u>.: Moderate: RiskPert (0.01, 0.04, 0.10) ; + 20% of RiskPert output Low: Average of the output of the following Pert distributions + 20% 1: RiskPert (0, 0, 0.02) 2: RiskPert (0, 0, 0.02) 3: RiskPert (0, 0, 0.02) 4: RiskPert (0, 0, 0.02) <u>*Campylobacter jejuni*</u> Moderate: RiskPert (0.01, 0.03, 0.08) Low: Average of the output of the following Pert distributions 1: RiskPert (0, 0, 0.04) 2: RiskPert(0, 0, 0.04) 3: RiskPert (0, 0, 0.04) 4: RiskPert (0, 0, 0.04)	
5. Starling access to pig environment	*Yes* *No*	Probability that European starlings will have access to pig housing	RiskPert (*maximum −10%*, maximum −5%, *maximum*) *maximum*: 1−Proportion of piggeries with nets to control European Starlings (RiskBeta(3, 54))	Chapter 2 and Expert Opinion
6. Starling access to	*Yes*	Probability that European	<u>*Food:*</u> RiskPert (*most likely* −10%, *most likely*,	Chapter 2 and

pig food/water	*No*	starlings will have access to pig food/water	*most likely* +10%) *Most likely*: 0.75 (75% piggeries with feeders allowing bird access) *Water:* RiskPert (*most likely* −10%, *most likely*, *most likely* +10%) *Most likely*: 0.3 (30% piggeries allow bird water access)	Expert Opinion
7. Pathogen survival	*Yes* *No*	Probability that the pathogen will survive in the environment/food/water	*Escherichia coli*: High: RiskUniform (0.7, 1.0) *Salmonella spp.*: High: RiskUniform (0.7, 1.0) *Campylobacter jejuni*: *Environment*: Moderate: RiskUniform (0.3, 0.7); *Food/water*: Low: RiskUniform (0.05, 0.3)	Literature: Temple et al. 1980; Mitscherlich and Marth 1984; Guan and Holley 2003; Cools et al. 2001; Fedorka-Cray et al. 2000; Jensen et al. 2006
8. Pig contact	*Yes* *No*	Probability pigs will contact the environment/food/water	*Environment*: RiskPert (*maximum*−10%, maximum – 5%, 1) *Food/water*: 1	Expert Opinion
9. Infection of pig	*Yes* *No*	Probability pigs will receive sufficient dose of pathogen and become infected	RiskUniform (0.7, 1.0)	Literature: Anderson et al. 1998; Achen et al. 1998; Cornick and Helgerson 2004; Shivaprasad et al. 1990; Ogden et al. 2009;

				Parthasarathy and Mansfield 2009; Mansfield et al. 2003; Meerburg et al. 2011.

[a] RiskBeta = Beta distribution (*successes + 1, total number – successes + 1*); RiskPert = Pert distribution (*minimum, most likely, maximum*); RiskUniform = Uniform distribution (*minimum, maximum*)

7.2.3.2 Rats

The scenario tree shown in Figure 7.2 was used to describe the exposure of domestic pigs to the three pathogens studied in rats. There were four pathways of exposure of a domestic pig to a pathogen from a rat: contaminated faeces in the environment, contaminated faeces in food, contaminated faeces in water, and ingestion of an infected rat carcass. The pathways and nodes of this scenario tree are represented in Figure 7.2, and nodes and branches for each node are described below and summarised in Table 7.2.

7.2.3.2.1 Rats present around the piggery: This node was calculated as for the European starlings (Section 7.2.3.1.1). Of the 170 piggeries that reported wild animal observations, 66 recorded rodents. This proportion was incorporated using a Beta distribution to account for the fact that this scenario considers rats only (instead of all rodents).

7.2.3.2.2 Number of rats present: This node was calculated as for the European starlings (Section 7.2.3.1.2). Of the 66 piggeries that observed rodents, 28 provided estimates of rodent numbers. This node had two branches, according to the number of rodents reported per week, obtaining piggeries with low and high numbers of rodents. Twenty-two piggeries observed less than 100 rodents, and six observed greater than or equal to 100 rodents a week. A Beta distribution was used for each of these number proportions to account for any uncertainty due to the estimates being for rodents in general, not rats specifically. The differential risk was incorporated as for European starlings, described in Section 7.2.3.1.2, though with only a low and a high category used.

7.2.3.2.3 Proportion of piggeries with different rat infection: Results from Chapter 5 indicate that not every piggery has rats infected with the pathogens studied, or may have a varying level of pathogen presence in rats. As such, the proportion of piggeries with different levels of infection in rats was included in the model. None of the piggeries selected for capture and sampling of rats had rats infected with *Brachyspira hyodysenteriae*, and as a consequence there were not different levels of infection in rats identified in our study. The level of infection in rodents was investigated in literature, see Section 7.2.3.2.4. According to literature a similar level of infection was reported for studies, though these studies were located in different countries and detected the pathogen in rats or mice, as such the literature did not allow for categorisation of piggeries in to different levels of infection. Subsequently for *Brachyspira hyodysenteriae* this node was

not considered. *Lawsonia intracellularis*, detected via real-time qPCR, and *Salmonella spp*. were detected in rats on all three piggeries. Only one piggery had rats infected at a low prevalence (lower than 5%) for *Lawsonia intracellularis*, and only one piggery was infected at a moderate level (greater than 5%) for *Salmonella spp*.. The proportions of piggeries with rats infected and these varying prevalence levels were incorporated into the model using a Beta distribution.

7.2.3.2.4 Prevalence in rats: This node was calculated as for the European starlings (Section 7.2.3.1.4). Pert distributions were included as for European starlings (Section 7.2.3.1.4). Prevalence estimates of the pathogen presence in rats were obtained from Chapter 5. In the case of *Brachyspira hyodysenteriae*, which was not detected in any of the three piggeries undertaking measures to control the disease in domestic pigs, the prevalence of rodent infection was obtained from literature. Hampson et al. (1991) detected 1 infected rat out of a total 44 sampled from a piggery in Australia; Joens and Kinyon (1982) detected 4 infected mice out of a total 157 sampled from piggeries in the United States; and Fellström et al. (2004) detected 3 positive mice out of a total 8 sampled in a piggery in Sweden. The true prevalence of *Lawsonia intracellularis* from the real-time qPCR and *Salmonella spp*. in rats is shown in Table 5.7 and 5.6, respectively, in Chapter 5. Prevalence was incorporated into the model with a Pert distribution using the lower 95% confidence interval, true prevalence estimate and upper 95% confidence interval for each pathogen.

7.2.3.2.5 Rat access to pig environment: This node was calculated as for the European starlings (Section 7.2.3.1.5). There are no strategies available to prevent rodent entry to a piggery that is not entirely enclosed. A Beta distribution was used to incorporate uncertainty around the estimate of the proportion of piggeries that are not completely enclosed, with a maximum figure of 100%. A Pert distribution was used and a most likely value, of 5% less than this maximum figure, and minimum proportion, of 10% less than this maximum figure were included.

7.2.3.2.6 Rat access to pig food and water: This node was calculated as for the European starlings (Section 7.2.3.1.6). According to the expert consulted in this assessment, the majority of piggeries have feeders that would allow rats access to pig feed. The corresponding input value was incorporated into the model using a Pert distribution with a

maximum of 100%, most likely of 95% and minimum of 90%. In contrast, only 30% of piggeries were estimated to be using drinkers that would allow rat access to water, such as water troughs and cups. This proportion was used as the most likely probability of rats accessing pig water in a Pert distribution. The minimum and maximum values of the distribution were estimated as minus 10% and plus 10% of this most likely value (0.3). The rest of the piggeries use nipple or bite drinkers, which are assumed to prevent rat contact with water. Rat access to pig food was estimated to be more likely than that of birds, as rats usually live inside the piggery and can directly access feed in the pig pens.

7.2.3.2.7 Pathogen survival: This node was calculated as for the European starlings (Section 7.2.3.1.7). The survival of pathogens excreted in rat faeces in various substrates was estimated based on literature.

Survival of *Brachyspira hyodysenteriae* in the pig environment, food and in a rat carcass was determined to be high. This estimation was based on the survival of the pathogen in soil and faeces for 112 days at 10 ºC (Boye et al. 2001). Survival in water was estimated to be moderate, with an average survival time of 5 to 6 days with a temperature range between 13 and 32°C (Olson 1995).

Survival of *Lawsonia intracellularis* was also estimated to be high in all substrates. This was based on the survival of the bacteria in sufficient quantities in faeces stored in air at room temperature over a two week period to still cause infection of pigs (Collins 2006). The survival of the pathogen in water has not been investigated, and is an area of data deficiency.

Survival of *Salmonella spp*. was also high in all substrates, and is described in the European starling Section 7.2.3.1.7.

7.2.3.2.8 Pig contact: This node was calculated as for the European starlings (Section 7.2.3.1.8). Contact of pigs with a rat carcass in their environment was estimated to be at a maximum level of 90%. This was based on the curious nature of pigs from expert opinion. A Pert distribution using this maximum, a most likely value 5% lower than this and a minimum value 10% lower than this was used.

7.2.3.2.9 Infection of pig: This node was calculated as for the European starlings (Section 7.2.3.1.9). The probability of pigs being infected with *Brachyspira hyodysenteriae* on contact with any of the contaminated materials was estimated to be high. This was based on the infective dose of *Brachyspira hyodysenteriae* for pigs, being between 10^7 and 10^9 bacteria (Jacobson et al. 2004). Mice shed around 10^7 and 10^8 bacteria per gram of faeces for 180 days (Joens 1980).

The proportion of pigs that would be infected on contact with faeces from a rat infected with *Lawsonia intracellularis* was 29.05%. This was based on a dose rate to cause infection in pigs of 10^5 (Collins et al. 2011). Collins et al. (2011) determined that 29.05% of the wild rodents caught on the three piggeries in the current study were shedding 10^5 bacteria or greater per gram of their faeces.

Infection of pigs on contact with *Salmonella spp*.-contaminated materials from rats was estimated to be moderate, as mouse droppings have been shown to contain up to 10^5 colony forming units per gram of faeces of *Salmonella spp*. (Fedorka-Cray et al. 2000), though pigs require a dose of 10^8 bacteria to reliably achieve infection (Anderson et al. 1998).

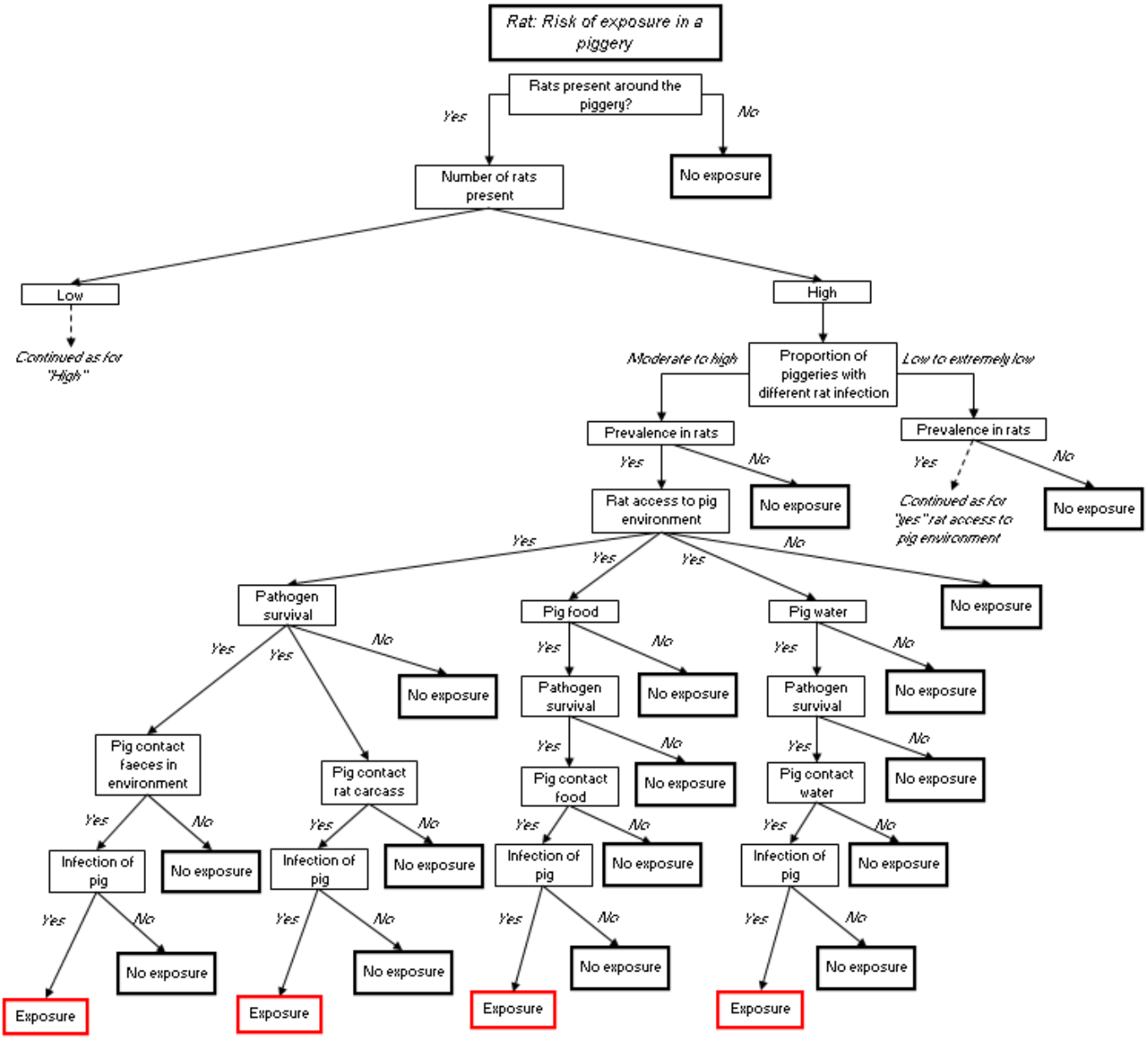

Figure 7.2. Scenario tree representing the exposure assessment evaluating the probability of domestic pigs on piggeries in Australia being exposed to *Brachyspira hyodysenteriae*, *Lawsonia intracellularis* and *Salmonella spp*. from rats.

Table 7.2. Nodes, parameters and input values used for the exposure assessment evaluating the probability of domestic pigs on piggeries in Australia being exposed to *Brachyspira hyodysenteriae*, *Lawsonia intracellularis* and *Salmonella spp*. from rats.

Node	**Branch of node**	**Parameter estimates**	**Input values** [a]	**Data sources**
1. Rat present around the piggery?	*Yes* *No*	Probability of rats being present around the piggery	RiskBeta (67, 105)	Chapter 2
2. Number of rats present around the piggery per week	*Low (< 100)* *High (≥ 100)*	Proportion of piggeries with low and high number of rats observed per week Relative risk of exposure according to the number of rats around the piggery per week (Low being the reference)	Low: RiskBeta (23, 7) High: RiskBeta (7, 23) High: RiskPert (5, 10, 15)	Chapter 2
3. Proportion of piggeries with different rat infection	*Yes* *No*	Proportion of piggeries with infected rats. Proportion of piggeries with low and moderate level of infection for *Salmonella spp*. and proportion of piggeries with low and high for *Lawsonia intracellularis* as all piggeries were infected. Node not relevant for *Brachyspira hyodysenteriae*	*Lawsonia intracellularis*: Low: RiskBeta (2, 3); High: RiskBeta (3, 2) *Salmonella spp.*: Low: RiskBeta (3, 2) Moderate: RiskBeta (2, 3)	Chapter 5

4. Prevalence in rats	*Yes* *No*	Prevalence of the pathogen in the rat population.	*Brachyspira hyodysenteriae* – With control: Average of the output of the following Pert distributions: 1: RiskPert (0.0, 0.0, 0.04) 2: RiskPert (0.0, 0.0, 0.01) 3: RiskPert (0.0, 0.0, 0.01) *Brachyspira hyodysenteriae* – Without control: Average of the output of the following Pert distributions: 1: RiskPert (0.0, 0.01, 0.10) 2: RiskPert (0.0, 0.01, 0.04) 3: RiskPert (0.10, 0.36, 0.71) *Lawsonia intracellularis* (two levels of prevalence): Low: RiskPert (0.0, 0.01, 0.07) High: Average of the output of the following Pert distributions: 1: RiskPert (0.72, 0.84, 0.92) 2: RiskPert (0.61, 0.70, 0.79) *Salmonella spp*. (two levels of prevalence): Moderate: RiskPert (0.016, 0.067, 0.164) Low: Average of the output of the following Pert distributions: 1: RiskPert (0.0, 0.0, 0.04) 2: RiskPert (0.0, 0.0, 0.04)	Chapter 5 (Data for *Brachyspira hyodysenteriae* not shown) Literature: True prevalence calculations from Hampson et al. 1991; Joens and Kinyon 1982; and Fellström et al. 2004

5. Rat access to pig environment	*Yes* *No*	Probability that rats will have access to pig housing	RiskPert (*maximum −10%*, *maximum −5%, maximum*) *maximum*: 1	Chapter 2 and Expert Opinion
6. Rat access to pig food/water	*Yes* *No*	Probability that rats will have access to pig food/water	*Food*: RiskPert (0.9, 0.95, 1.0) *Water*: RiskPert (*most likely* −10%, *most likely*, *most likely* +10%) *most likely*: 0.3	Chapter 2 and Expert Opinion
7. Pathogen survival	*Yes* *No*	Probability that the pathogen will survive in the environment/food/water	*Brachyspira hyodysenteriae*: *Environment/food/rat carcass*: High: RiskUniform (0.7, 1.0) *Water*: Moderate: RiskUniform (0.3, 0.7) *Lawsonia intracellularis*: High: RiskUniform (0.7, 1.0) *Salmonella spp*.: High: RiskUniform (0.7, 1.0)	Literature: Boye et al. 2001; Olson 1995; Collins 2006; Fedorka-Cray et al. 2000; Temple et al. 1980; Jensen et al. 2006; Guan and Holley 2003
8. Pig contact	*Yes* *No*	Probability pigs will contact environment/food/water/rat carcass	*Environment*: RiskPert (*maximum* −10%, maximum –5%, 1) *Food/water*: 1 *Rat carcass*: RiskPert (*maximum* −10%, maximum –5%, 0.9)	Expert Opinion
9. Infection of pig	*Yes* *No*	Probability pigs will receive sufficient dose of pathogen and become infected	*Brachyspira hyodysenteriae*: RiskUniform (0.7, 1.0) *Lawsonia intracellularis*: 0.29	Literature: Joens 1980; Shivaprasad et al. 1990; Anderson et al.

			Salmonella spp.: RiskUniform (0.3, 0.7)	1998; Fedorka-Cray et al. 2000; Collins et al. 2011.

[a] RiskBeta = Beta distribution (*successes + 1, total number – successes + 1*); RiskPert = Pert distribution (*minimum, most likely, maximum*); RiskUniform = Uniform distribution (*minimum, maximum*)

7.2.3.3 Feral pigs

Two different scenario trees, shown in Figure 7.3 and 7.4, were used to describe the exposure of domestic pigs to the four pathogens studied. The first scenario tree (Figure 7.3) describes the two pathways of exposure of a domestic pig to a pathogen, *Mycoplasma hyopneumoniae*, which may be transmitted via direct contact between feral and domestic pigs or via the air. Although it is possible that infection through indirect contact with contaminated secretions may be a route of infection, it is not listed in Taylor (2006), Straw et al. (2006) or Jackson and Cockcroft (2007). Additionally, *Mycoplasma hyopneumoniae* infects pigs via the respiratory system, so inhalation is necessary to start an infection, hence indirect contact was deemed too insignificant to be included in the current study. Direct contact, as well as contact with contaminated feral pig secretions, were the two pathways used for exposure of a domestic pig to the final three pathogens, *Leptospira spp*., *Brucella suis* and *Lawsonia intracellularis* (Figure 7.4). The pathways and nodes of these scenario trees are represented in Figure 7.3 and 7.4, and nodes and branches for each node are described below and summarised in Table 7.3 and 7.4.

7.2.3.3.1 Feral pig present around the piggery: This node was calculated as for the European starlings (Section 7.2.3.1.1). Of the 170 piggeries that reported wild animal observations, 20 recorded feral pigs. To account for uncertainty around this proportion, a Beta distribution of the feral pig observations was used.

7.2.3.3.2 Number of feral pigs present: This node was calculated as for the European starlings (Section 7.2.3.1.2). Of the 20 piggeries that observed feral pigs, 15 provided estimates of feral pig numbers. This node had two branches, according to the number of feral pigs reported per week, obtaining piggeries with low and high numbers of feral pigs. Nine piggeries observed up to one feral pig and six observed more than one feral pig per week. A Beta distribution was used for each of these proportions to account for any uncertainty around the estimates. The differential risk was incorporated as for European starlings, Section 7.2.3.1.2, though with only a low and high category used.

The node for level of infection included in the starling and rat exposure assessment, identifying the proportions of piggeries with a high, moderate or low infection prevalence, was not included in the feral pig exposure assessment. This was due to the fact that only a

single feral pig population around two different piggeries was analysed, so a proportion of infection difference was not ascertained for different piggeries.

7.2.3.3.3 Prevalence in feral pigs: This node was calculated as for the European starlings (Section 7.2.3.1.4). Prevalence estimates of the pathogen presence in feral pigs were obtained from Chapter 6. The true prevalence of *Mycoplasma hyopneumoniae*, *Leptospira spp*., *Brucella spp*. and *Lawsonia intracellularis* in feral pigs is shown in Table 6.3 in Chapter 6. The *Mycoplasma hyopneumoniae* PCR true prevalence and confidence intervals were used, as the PCR indicated current infection and shedding of the pathogen, while the ELISA detected antibodies which may have been as a result of a past infection. Prevalence was incorporated into the model with a Pert distribution using the lower 95% confidence interval, true prevalence estimate and upper 95% confidence interval for each pathogen.

7.2.3.3.4 Feral pig access to piggery property: The probability of feral pigs accessing a piggery property was estimated from information in Chapter 2 and literature. One pig producer among the 20 reporting feral pigs provided comment that feral pigs dig under the perimeter fence to gain access to their property. This probability of 5% was used as the most likely figure of pig access. A Pert distribution was then used with a minimum estimate of 1% and maximum estimate of 10%. The minimum and maximum probability values were estimated through information on piggery sizes in literature, with the small piggeries with less than 10 sows (approximately 70% of pig producers in Australia) assumed to have a higher likelihood of lower biosecurity practices (APL 2008).

7.2.3.3.5 Feral pig direct contact: The probability that feral pigs and domestic pigs were able to have direct contact once the piggery perimeter had been breached was based on expert opinion. On free-range piggeries, contact between feral and domestic pigs was estimated to be very likely. A probability of 90% was used as the most likely value in a Pert distribution, with a minus and plus 10% range as the minimum and maximum values.

The probability of direct contact on an indoor piggery was estimated to be 10%, significantly lower than that for free-range piggeries. This was expected to occur mainly in old indoor piggeries with poor maintenance and damaged infrastructure. High uncertainty was associated with this estimate due to the wide range of characteristics and practices of

indoor piggeries. As such, a minus and plus 50% of the most likely value (0.1) were used as the minimum and maximum figures in a Pert distribution.

7.2.3.3.6 Feral pig proximity to the piggery: This node only relates to *Mycoplasma hyopneumoniae*, as shown in Figure 7.3 and Table 7.3. The proximity of feral pigs to the piggery was based on the data in Table 6.10 in Chapter 6 where data points were restricted to a Horizontal Dilution of Precision of ≤ 6.0. The number of data points for total recorded movement of feral pigs was 19,309. The total number of data points occurring within 100 m, 100 m to 500 m, and 501 m to 5000 m, were 5, 8 and 13839, respectively. Proportions of these proximity measures were incorporated into the model using a Beta distribution.

7.2.3.3.7 Pathogen survival: This node only relates to *Leptospira spp*., *Brucella spp*. and *Lawsonia intracellularis*, as shown in Figure 7.4 and Table 7.4. The survival of pathogens excreted in feral pig secretions was estimated to be high for all three pathogens and was based on literature.

The survival of *Leptospira spp*. in soil and water was estimated to be 45 to 74 days and 7 to 14 days at room temperature, respectively (Faine 1994). Survival of *Brucella spp*. was 120 days in bovine faeces at room temperature, 27 days in soil at room temperature, 81 days in water at 22 ºC, 114 days in water at −4 ºC, and 4 days in cow urine at room temperature (Mitscherlich and Marth 1984). Survival of *Lawsonia intracellularis* was as for rats, shown in Section 7.2.3.2.7.

7.2.3.3.8 Pig contact with secretions: This node only relates to *Leptospira spp*., *Brucella spp*. and *Lawsonia intracellularis*, as shown in Figure 7.4 and Table 7.4. Domestic pig contact with contaminated secretions was estimated by expert opinion to have a most likely value of 0.90 for free-range piggeries, minus and plus 10% for minimum and maximum figures included in the Pert distribution. The most likely figure on indoor piggeries was estimated to be 0.10, minus and plus 20% for minimum and maximum figures included in the Pert distribution.

7.2.3.3.9 Infection of pigs: The probability of pigs being infected upon direct contact or indirect contact with contaminated secretions or air was determined from literature. It is assumed that the domestic pigs are totally susceptible.

The probability of pigs being infected with *Mycoplasma hyopneumoniae* on direct contact with feral pigs was a Beta distribution of 10 out of 12 (Fano et al. 2005). The probability of infection with *Leptospira spp*. and *Brucella spp*. on direct contact was estimated to be high. A Beta distribution of 9 out of 10 pigs became infected with *Leptospira spp*. upon direct contact with an infected individual (Burnstein and Baker 1954). A high estimate was used for *Brucella suis*, as a dose rate of 10^5 is required to cause *Brucella spp*. infection in domestic pigs, while boars eliminate *Brucella spp*. bacteria in their semen in tremendous numbers (Hutchings 1950). A moderate level of infection was estimated for *Lawsonia intracellularis*, as infection through direct contact with this pathogen occurs via the faecal oral route. Faecal ingestion on direct contact was estimated to be less likely, despite the likelihood of feral pigs shedding in sufficient quantities in their faeces to cause infection.

For in-direct contact, the probability of domestic pigs becoming infected with *Leptospira spp*. upon contact with feral pig urine was a Beta distribution of 9 out of 10 (Burnstein and Baker 1954). These figures were used as pigs excrete *Leptospira* bacteria in their urine in enormous numbers, with bacteria excreted in the urine of 32 of 34 pigs in a study by Burnstein and Baker (1954). Additionally, the bacterium survives in cow urine for up to 35 days when stored between 15 and 17 ºC. In the absence of accurate data for *Brucella suis*, an estimate of 8 out of 10 was used due to the similarity in dose and secretions to *Leptospira spp*. For *Lawsonia intracellularis*, four out of five pigs become infected upon contact with infected faeces (Collins 2008). A Beta distribution was used for all of these estimates to account for variability.

The probability of domestic pigs becoming infected with *Mycoplasma hyopneumoniae* through infected air was reliant upon the distance from the infection source and was estimated from literature. Dee et al. (2009) has reported detection of the pathogen 4.7 km from the infection source. However, the quantity of bacteria detected was not investigated. Whether there would be a sufficient quantity to cause infection in pigs at this distance still remains unknown. Cardona et al. (2005) determined an infection rate of 100% with *Mycoplasma hyopneumoniae* at a distance from the source of 150 m. Infection response in pigs located at a greater distance than 150 m from the source of infection has not been studied. In the current study it was assumed that *Mycoplasma hyopneumoniae* was being constantly expired from infected pigs. Due to this high uncertainty, qualitative estimates

were used for airborne infection of this pathogen for feral pigs: less than 100 m from the piggery, defined as high risk; 100 m to 500 m from the piggery, defined as moderate risk; and between 500 m and 5000 m from the piggery, defined as low risk.

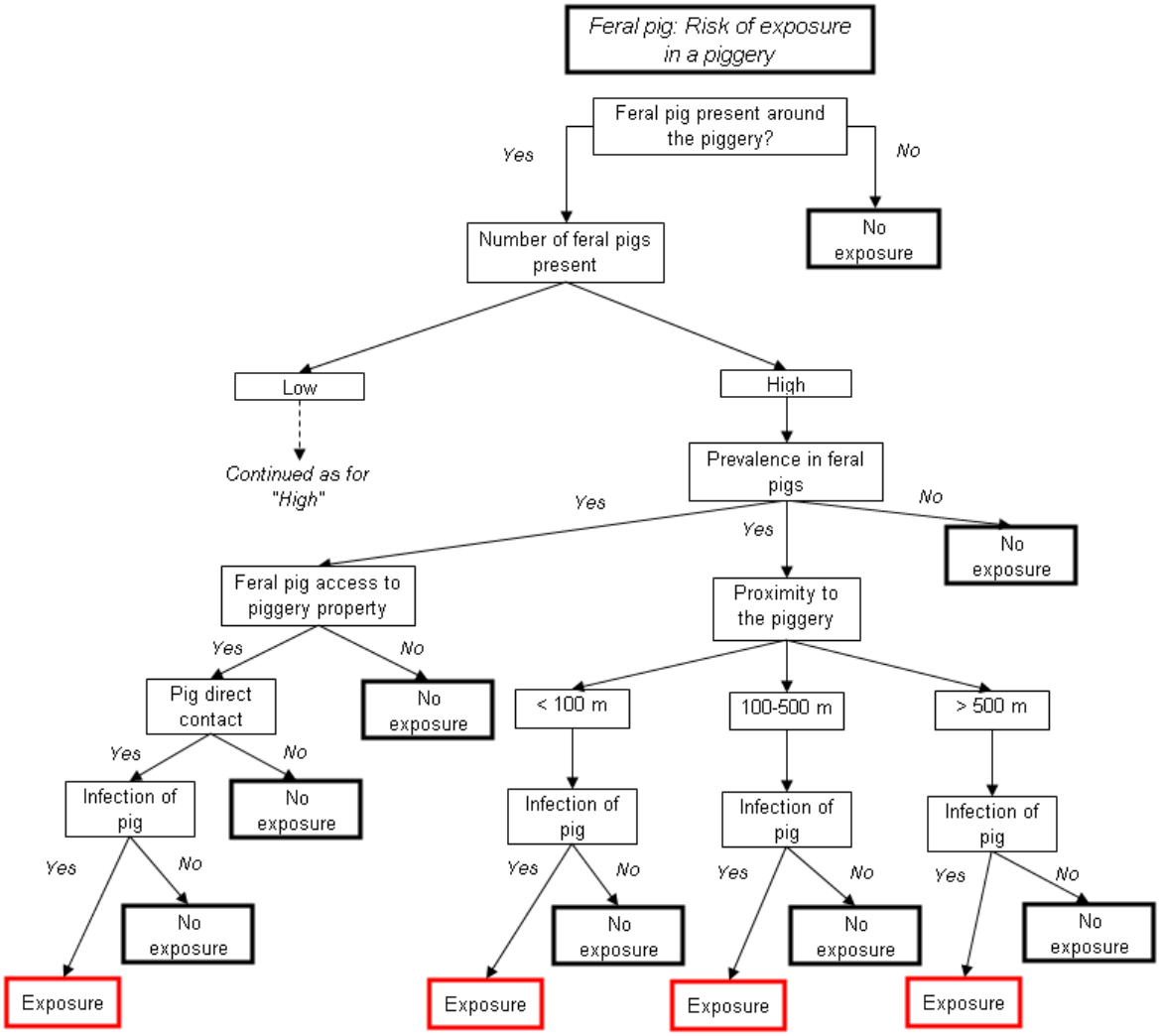

Figure 7.3. Scenario tree representing the exposure assessment evaluating the probability of domestic pigs on piggeries in Australia being exposed to *Mycoplasma hyopneumoniae* from feral pigs.

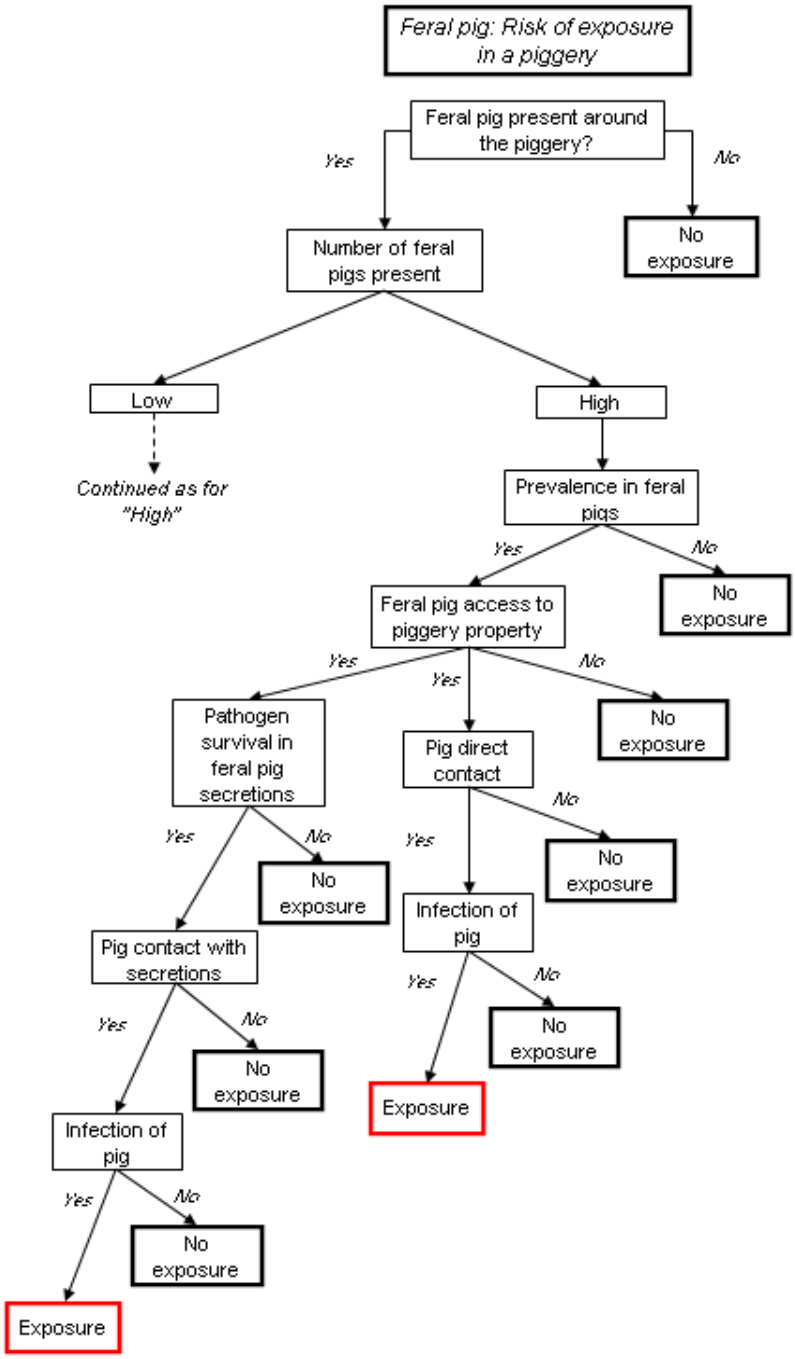

Figure 7.4. Scenario tree representing the exposure assessment evaluating the probability of domestic pigs on piggeries in Australia being exposed to *Leptospira spp*., *Brucella suis* and *Lawsonia intracellularis* from feral pigs.

Table 7.3. Nodes, parameters and input values used for the exposure assessment evaluating the probability of domestic pigs on piggeries in Australia being exposed to *Mycoplasma hyopneumoniae* from feral pigs.

Node	**Branch of node**	**Parameter estimates**	**Input values** [a]	**Data sources**
1. Feral pig present around the piggery?	*Yes* *No*	Probability of feral pigs being present around the piggery	RiskBeta (21,151)	Chapter 2
2. Number of feral pigs present	*Low (≤1)* *High (>1)*	Proportion of piggeries with low and high number of feral pigs observed per week Relative risk of exposure according to the number of feral pigs around the piggery per week (*Low being the reference*)	Low: RiskBeta (10, 7) High: RiskBeta (7, 10) High: RiskPert (5, 10, 15)	Chapter 2
3. Prevalence in feral pigs	*Yes* *No*	Prevalence of the pathogen in the feral pig population.	*Mycoplasma hyopneumoniae* (PCR): RiskPert (0.17, 0.28, 0.41)	Chapter 6
4. Feral pig access to piggery property	*Yes* *No*	Probability that feral pigs will have access to piggery property	RiskPert (0.01, 0.05, 0.1)	Chapter 2 and Literature: APL 2008
5. Feral pig direct contact	*Yes* *No*	Probability that feral pigs will have direct contact with domestic pigs.	*Free-range piggery*: RiskPert (−10% *most likely*, 0.9, +10% *most likely*) *Indoor piggery*: RiskPert (−50% *most likely*, 0.1, +50% *most likely*)	Expert Opinion

6. Feral pig proximity to the piggery	< 100 m 100–500 m > 500 m	Proportion of the reference population of feral pigs' range of movement within 5 km of piggeries. Feral pig movement outside of 5 km are not included as transmission is assumed to not occur.	*< 100 m*: RiskBeta (6, 19305) *100–500 m*: RiskBeta (9, 19302) *> 500 m*: RiskBeta (13840, 5471)	Chapter 6
7. Infection of domestic pig	*Yes* *No*	Probability pigs will receive sufficient dose of pathogen and become infected	*Direct contact*: RiskBeta (11, 3) *Aerosol*: *< 100 m*: RiskUniform (0.7, 1.0) *100–500 m*: RiskUniform (0.3, 0.7) *> 500 m*: RiskUniform (0.05, 0.3)	Literature: Fano et al. 2005; Dee et al. 2009

[a] RiskBeta = Beta distribution (*successes + 1, total number – successes + 1*); RiskPert = Pert distribution (*minimum, most likely, maximum*); RiskUniform = Uniform distribution (*minimum, maximum*)

Table 7.4. Nodes, parameters and input values used for the exposure assessment evaluating the probability of domestic pigs on piggeries in Australia being exposed to *Leptospira spp*., *Brucella suis* and *Lawsonia intracellularis* from feral pigs.

Node	**Branch of node**	**Parameter estimates**	**Input values** [a]	**Data sources**
Nodes 1–2 and 4–5 as for Table 7.3.				
3. Prevalence in feral pigs	*Yes* *No*	Prevalence of the pathogen in the feral pig population.	*Leptospira spp*.: RiskPert (0.37, 0.49, 0.61) *Brucella suis*: RiskPert (0.03, 0.11, 0.22) *Lawsonia intracellularis*: RiskPert (1.0, 1.0, 1.0)	Chapter 6
6. Pathogen survival	*Yes* *No*	Probability that the pathogen will survive in the feral pig secretions in the environment	RiskUniform (0.7, 1.0)	Literature: Faine 1994; Mitscherlich and Marth 1984; Collins 2008
7. Pig contact with secretions	*Yes* *No*	Probability domestic pigs will contact feral pig secretions in the environment.	RiskPert (*minimum*, *most likely*, *maximum*) *Free-range*: RiskPert (−10% *most likely*, 0.9, +10% *most likely*) *Indoor*: RiskPert (−20% *most likely*, 0.1, +20% *most likely*)	Expert Opinion
8. Infection of domestic pig	*Yes* *No*	Probability pigs will receive sufficient dose of pathogen and become infected.	*Direct Contact:* *Leptospira spp*.: RiskUniform (0.7, 1.0) *Brucella suis*: RiskUniform (0.7, 1.0) *Lawsonia intracellularis*: RiskUniform (0.3, 0.7)	Literature: Hutchings 1950; Burnstein and Baker 1954; Collins 2008

			Indirect Contact: *Leptospira spp.*: RiskBeta (10, 2) *Brucella suis*: RiskBeta (9, 3) *Lawsonia intracellularis*: RiskBeta (5, 2)	

[a] RiskBeta = Beta distribution (*successes + 1, total number – successes + 1*); RiskPert = Pert distribution (*minimum, most likely, maximum*); RiskUniform = Uniform distribution (*minimum, maximum*)

7.2.3.4. Probability of exposure

7.2.3.4.1. Adjusted risks: The number of wild animals present around the piggeries was the only risk node for the probability of exposure considered in these models. Relative risks of low, medium and high numbers of wild animals, which have been previously described, were adjusted to maintain their relativity while ensuring that the average risk for all the piggeries (the population) was one (Martin et al. 2007). The following equation has been used:

$$AR_i = \frac{RR_i}{\sum_{i=1}^{I} RR_i \times PrP_i} \quad \text{.....Equation 7.1.}$$

where RR_i is the specified relative risk and AR_i the adjusted relative risk for the ith branch of the node; PrP_i is the proportion of the reference population for each branch; and I is the number of branches in the risk node.

The adjusted risk was then used to calculate the effective probability of exposure for those piggeries with a low, medium and high number of wild animals observed.

7.2.3.4.2. Calculating the scenario tree: For each wild animal species and each pathogen, the multiplication rule was used to calculate the probability of exposure for each pathway and for each piggery category according to the number of specified wild animals present (risk node). Each of these probabilities was then multiplied by the corresponding adjusted risk to account for the differential risk between these piggery categories (Martin et al. 2007). The overall probability of exposure for each pathogen was obtained by adding the probabilities for each of the pathways, given these pathways are independent.

7.2.4 Sensitivity analysis

The sensitivity of the outputs of the model to all of the input parameters was evaluated using the @RISK 5.7 Advanced Sensitivity Analysis (Palisade Corporation, USA). Sensitivity analyses were conducted separately for each pathogen from each wild animal model to identify which input parameters had the greatest influence on the exposure probability outputs. This was evaluated by simulating the outputs for a series of fixed values for each input parameter, while keeping the rest of the input parameters to their base value. Input parameters that were proportions, probabilities or prevalence were allowed to

vary from 0 to 1 in ten steps (0, 0.11, 0.22…) and the simulation was run for 1,000 iterations. The relative risk of having a medium number of animals in the piggery surroundings was fixed to 5, 20 and 50, and for a high number was fixed to 10, 50 and 100. The amount by which the median probability of exposure changed when a parameter input was varied from its base input value was determined by the following equation:

$$\left(\frac{\|\text{Original median} - \text{Changed median}\|}{\text{Original median}}\right) \quad \text{Equation 7.2.}$$

7.3. Results

The risk assessment estimated the probability of starlings, rats and feral pigs exposing domestic pigs on piggeries in Australia to a number of different pathogens.

7.3.1. Risk of pathogen transmission from European starlings to domestic pigs

The median probability of starlings exposing and infecting domestic pigs with *Escherichia coli*, *Salmonella spp*. and *Campylobacter jejuni* was estimated to be 0.03, 0.007 and 0.001, respectively, as shown in Table 7.5. The probability of exposure of domestic pigs to pathogenic *Escherichia coli* from starlings was 4.3 and 30 times higher than that to *Salmonella spp*. and *Campylobacter jejuni*, respectively. Similarly, the probability of exposure to *Salmonella spp*. was 7.0 times higher than that to *Campylobacter jejuni*.

Table 7.5. Predicted median (5 and 95 percentiles) probability of exposure of domestic pigs to different pathogens from European starlings on piggeries in Australia.[a]

Pathogen	Probability (median, 5%–95%)
Escherichia coli	0.03 (0.02–0.04)
Salmonella spp.	0.007 (0.002–0.02)
Campylobacter jejuni	0.001 (0.0004–0.003)

[a] Output distribution of a simulation stochastic model with 5,000 iterations

Results of the sensitivity analysis for the three pathogens are shown in Figures 7.5, 7.6 and 7.7. The greatest influence on the probability of exposing domestic pigs to *Escherichia coli*, *Salmonella spp*. and *Campylobacter jejuni* was the prevalence of infection in starlings, followed by the presence of starlings in the piggery. When the prevalence estimate for *Escherichia coli* was set to 1, the probability of exposure to this pathogen increased 10-fold. Increasing the starling prevalence estimate for *Salmonella spp*. and *Campylobacter jejuni* to 1.0 increased the probability of exposure 19- and 25-fold, respectively. As expected, removing starlings from a piggery would eliminate any risk of pathogen transmission from these animals to domestic pigs. When the probability of starlings being present in the piggery was reduced to 0.11, the probability of exposure to the different pathogens only decreased slightly, by less than 1-fold. However, if the probability of starlings being present was increased to 1, the probability of exposure increased 2- to 3-fold for the three pathogens.

Other influential input parameters on the output of the models were the proportion of piggeries with infected starlings, the proportion of piggeries with a high number of starlings and the pathogen survival in the environment. These varied for each pathogen (Figure 7.5, 7.6, 7.7). The proportion of pigs that develop infection was also influential for *Escherichia coli* and *Salmonella spp*. (Figure 7.5 and 7.6).

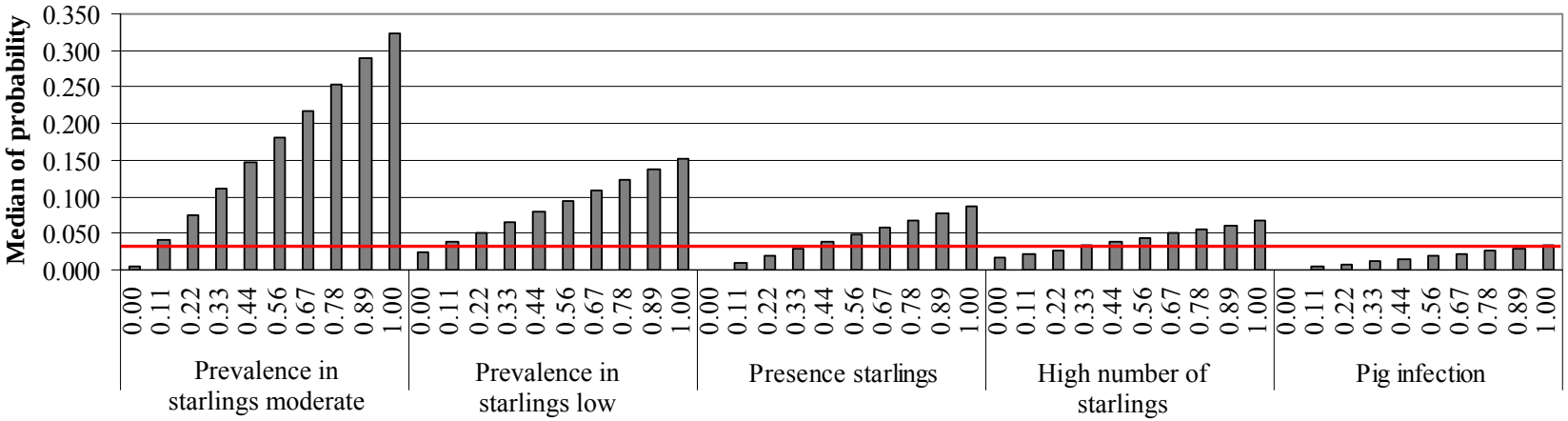

Figure 7.5. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Escherichia coli* from European starlings in piggeries in Australia. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.

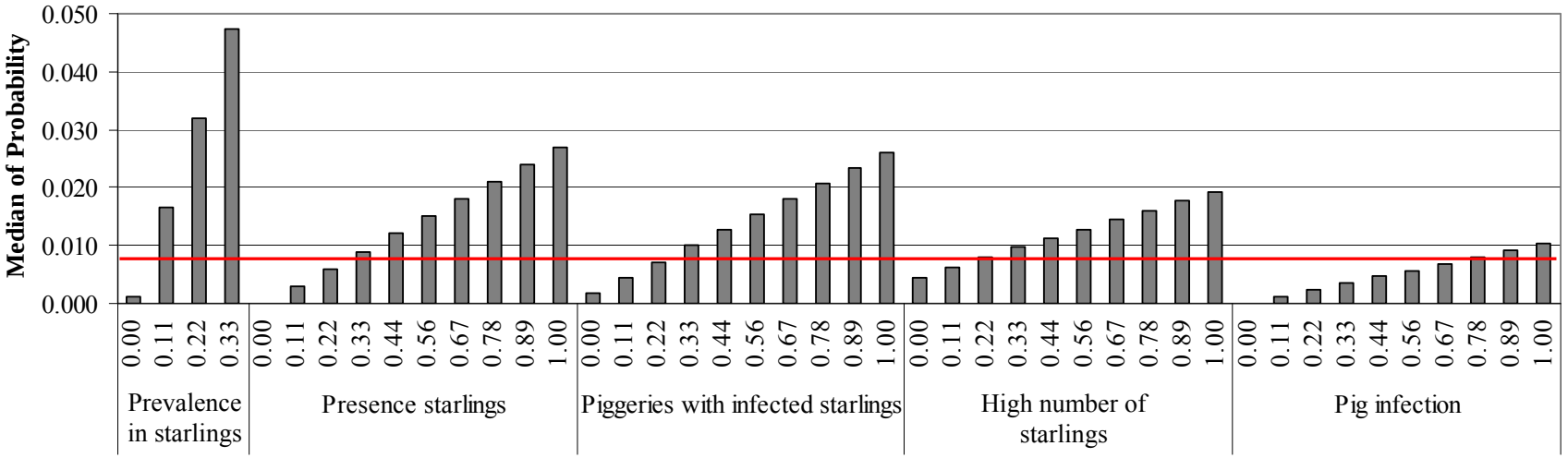

Figure 7.6. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Salmonella spp*. from European starlings in piggeries in Australia. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.
Note: the median of probability estimate increases to 0.139 when the 'Prevalence in starlings' increases to 1.0 (not shown in graph).

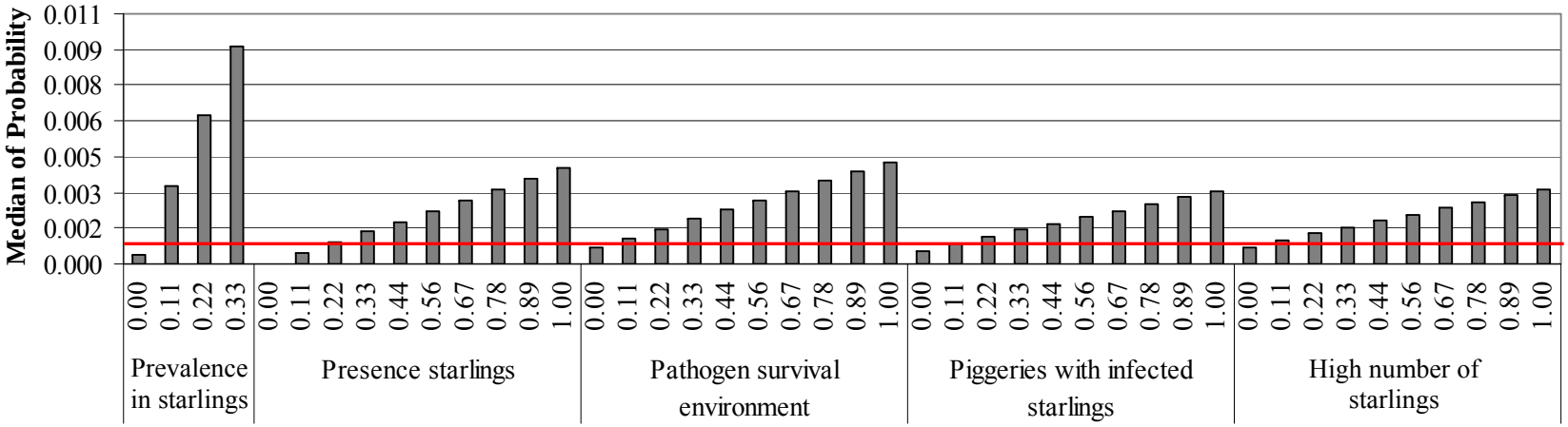

Figure 7.7. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Campylobacter jejuni* from European starlings in piggeries in Australia. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.
Note: the median of probability estimate increases to 0.026 when the 'Prevalence in starlings' increases to 1.0 (not shown in graph).

7.3.2. Risk of pathogen transmission from rats to domestic pigs

The median probability of rats exposing and infecting domestic pigs with *Lawsonia intracellularis* and *Salmonella spp*. was estimated to be 0.13 and 0.01, respectively, as shown in Table 7.6. The probability of rats exposing pigs to *Brachyspira hyodysenteriae* on a piggery that is not using control measures to mitigate infection was 0.10 (Table 7.6). The probability of rats exposing pigs to *Brachyspira hyodysenteriae* was 33.3 times greater on this type of piggery compared to a piggery that had undertaken control measures to mitigate infection in pigs (0.003; Table 7.6).

Table 7.6. Predicted median (5 and 95 percentiles) probability of exposure of domestic pigs to different pathogens from rats on piggeries in Australia.[a]

Pathogen	Probability (median, 5%–95%)
Brachyspira hyodysenteriae in a piggery using control measures to mitigate infection in pigs.	0.003 (0.0007–0.007)
Brachyspira hyodysenteriae in a piggery NOT using control measures to mitigate infection in pigs.	0.10 (0.05–0.19)
Lawsonia intracellularis	0.13 (0.05–0.23)
Salmonella spp.	0.01 (0.004–0.04)

[a] Output distribution of a simulation stochastic model with 5,000 iterations

Results of the sensitivity analysis for the three pathogens are shown in Figures 7.8, 7.9, 7.10 and 7.11. The prevalence of infection in rats was determined to have the most influence on the probability of exposing domestic pigs to *Brachyspira hyodysenteriae* (Figure 7.8 and 7.9) and *Salmonella spp*. (Figure 7.11). When the median prevalence in rats for *Brachyspira hyodysenteriae* was set to 1.0 for a piggery that had undertaken measures to control this infection in pigs (base value of 0.003), the probability of exposure to this pathogen increased 265-fold. Increasing the median prevalence estimate for *Brachyspira hyodysenteriae* to 1.0 on a piggery not controlling this infection in pigs (base value of 0.13) resulted in a 7-fold increase.

The probability of infection in domestic pigs after contacting contaminated material (rat faeces, food, water or rat carcasses), the proportion of piggeries with high number of rats, and the presence of rats had the most influence on the probability of exposing domestic pigs to *Lawsonia intracellularis* (Figure 7.10). A high number of rats and the presence of rats also had an influence on the probability of exposing domestic pigs to *Brachyspira hyodysenteriae* (Figure 7.8 and 7.9) and *Salmonella spp*. (Figure 7.11).

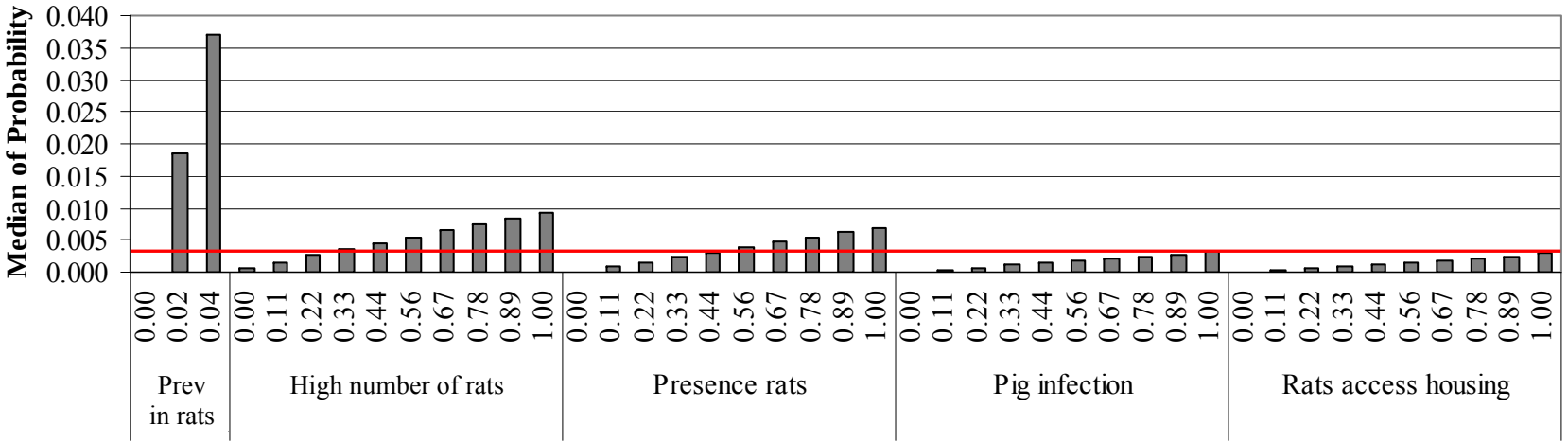

Figure 7.8. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Brachyspira hyodysenteriae* from rats in piggeries in Australia that were using control measures to mitigate infection of pigs with this pathogen (through either medication, Swiss depopulation or total depopulation). Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.
Prev = Prevalence.
Note: the median of probability estimate increases to 0.797 when the 'Prevalence in rats' increases to 1.0 (not shown in graph).

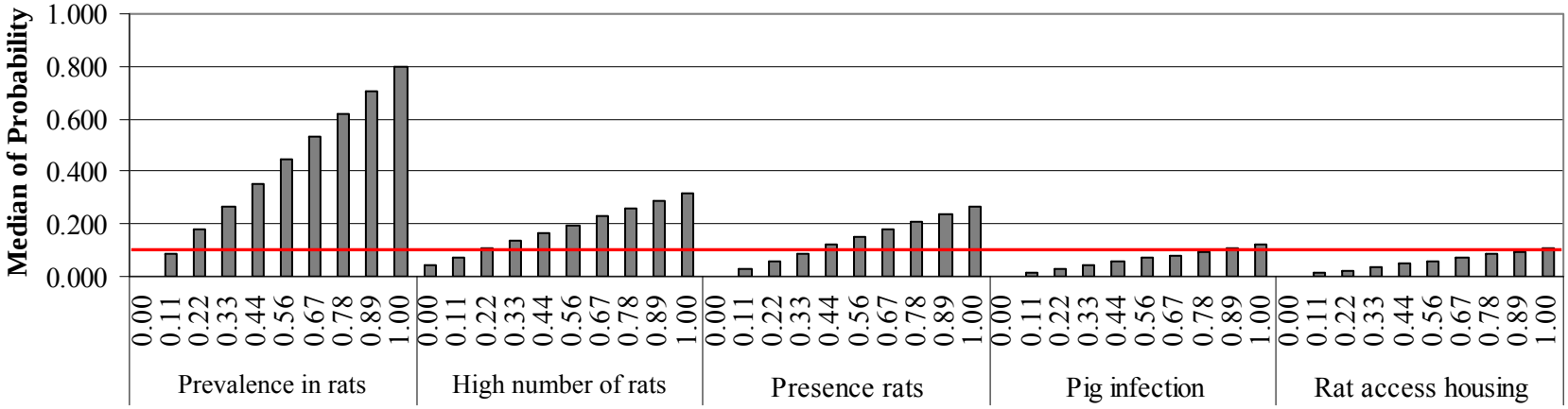

Figure 7.9. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Brachyspira hyodysenteriae* from rats in piggeries in Australia that were not using control measures to mitigate infection of pigs with this pathogen. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.

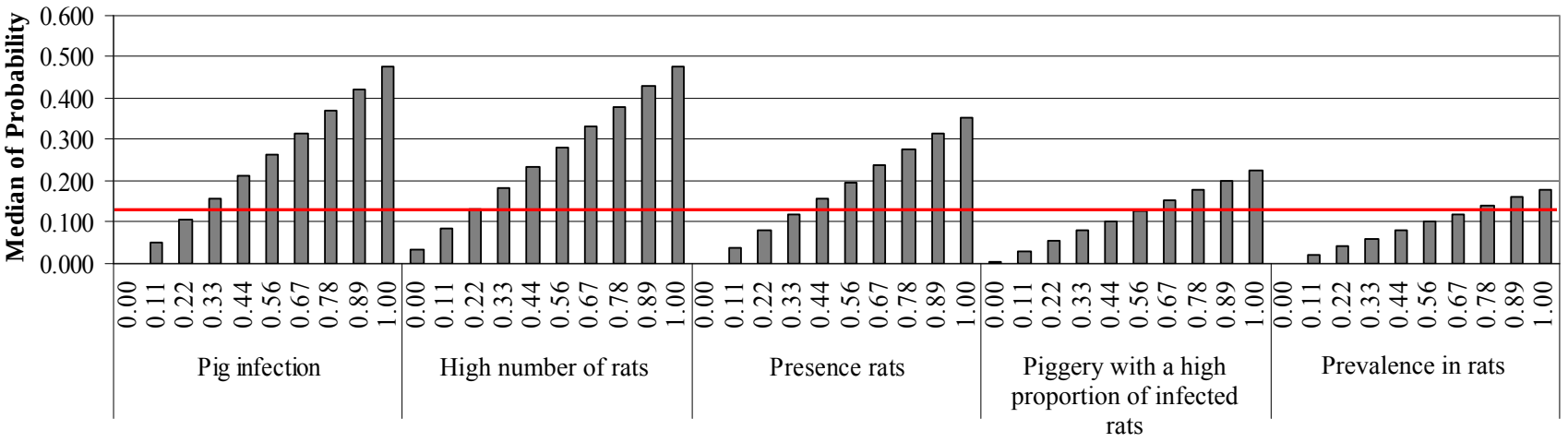

Figure 7.10. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Lawsonia intracellularis* from rats in piggeries in Australia. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.

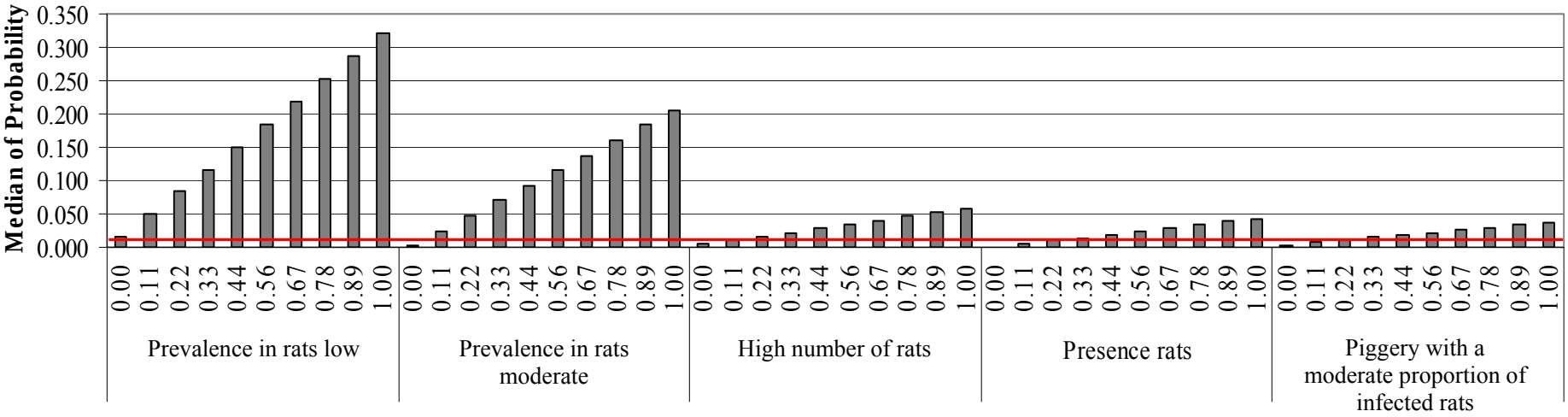

Figure 7.11. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Salmonella spp*. from rats in piggeries in Australia. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.

7.3.3. Risk of pathogen transmission from feral pigs to domestic pigs

The median probability of feral pigs exposing and infecting domestic pigs with *Mycoplasma hyopneumoniae*, *Leptospira spp*., *Brucella suis* and *Lawsonia intracellularis* is shown in Table 7.7. Free-range and indoor piggeries had a similar probability of exposure of domestic pigs to *Mycoplasma hyopneumoniae* from feral pigs. However, the probability of exposure of domestic pigs to *Leptospira spp*., *Brucella suis* and *Lawsonia intracellularis* from feral pigs was 10.0, 10.8 and 8.6 times higher, respectively, on a free-range piggery compared to an indoor piggery. In addition, the probability of exposure of domestic pigs to the different pathogens was higher in piggeries with a high number of feral pigs in their surroundings.

Table 7.7. Predicted median (5 and 95 percentiles) probability of exposure of domestic pigs to different pathogens from a high and low number of feral pigs on free-range and indoor piggeries in Australia.[a]

Pathogen	Piggery Type	Probability (median, 5%–95%)	Feral pig number	Probability (median, 5%–95%)
Mycoplasma hyopneumoniae	Free-range	0.005 (0.002–0.01)	High	0.01 (0.004–0.02)
			Low	0.002 (0.0007–0.005)
	Indoor	0.004 (0.001–0.008)	High	0.008 (0.003–0.02)
			Low	0.002 (0.0005–0.004)
Leptospira spp.	Free-range	0.004 (0.002–0.008)	High	0.009 (0.004–0.02)
			Low	0.0009 (0.0004–0.002)
	Indoor	0.0004 (0.0002–0.0008)	High	0.001 (0.0004–0.002)
			Low	9.8×10^{-5} (7.5×10^{-5}–2.3×10^{-4})
Brucella suis	Free-range	0.001 (0.0004–0.003)	High	0.002 (0.0006–0.004)
			Low	0.0003 (0.0001–0.0008)
	Indoor	9.3×10^{-5} (3.4×10^{-5}–2.1×10^{-4})	High	1.8×10^{-4} (6.2×10^{-5}–5.0×10^{-4})
			Low	3.5×10^{-5} (1.2×10^{-5}–9.4×10^{-5})
Lawsonia intracellularis	Free-range	0.006 (0.003–0.01)	High	0.01 (0.005–0.03)
			Low	0.002 (0.0008–0.005)
	Indoor	0.0007 (0.0003–0.001)	High	0.001 (0.0005–0.003)
			Low	0.0002 (9.4×10^{-5}–0.0006)

[a] Output distribution of a simulation stochastic model with 5,000 iterations

Results of the sensitivity analysis for the four pathogens are shown in Figures 7.12, 7.13, 7.14 and 7.15. The presence of feral pigs in the vicinity of a piggery was determined to have the most influence on the probability of exposing domestic pigs to *Mycoplasma hyopneumoniae*, followed by the close proximity of feral pigs to the piggery. As expected, removing feral pigs from around the piggery reduced the exposure probability to 0.0.

Feral pig access to a piggery was determined to have the most influence on the probability of exposing domestic pigs to *Leptospira spp*., *Brucella suis* and *Lawsonia intracellularis* from feral pigs on both free-range and indoor piggeries. Increasing the median probability of feral pigs accessing a piggery property to 1.0 increased the probability of exposure to *Leptospira spp*., *Brucella suis* and *Lawsonia intracellularis* between 17- and 23-fold.

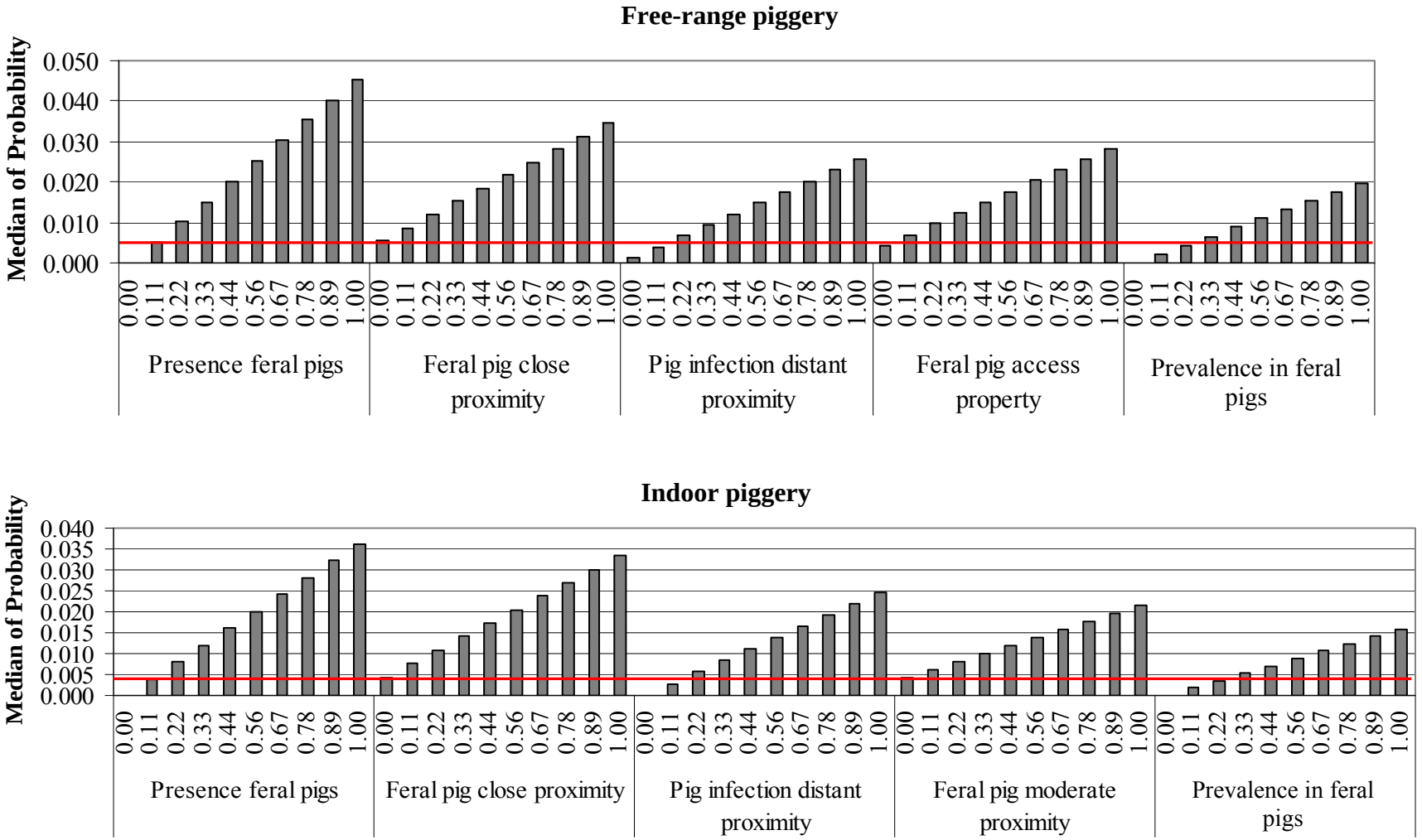

Figure 7.12. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Mycoplasma hyopneumoniae* from feral pigs in piggeries in Australia. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.

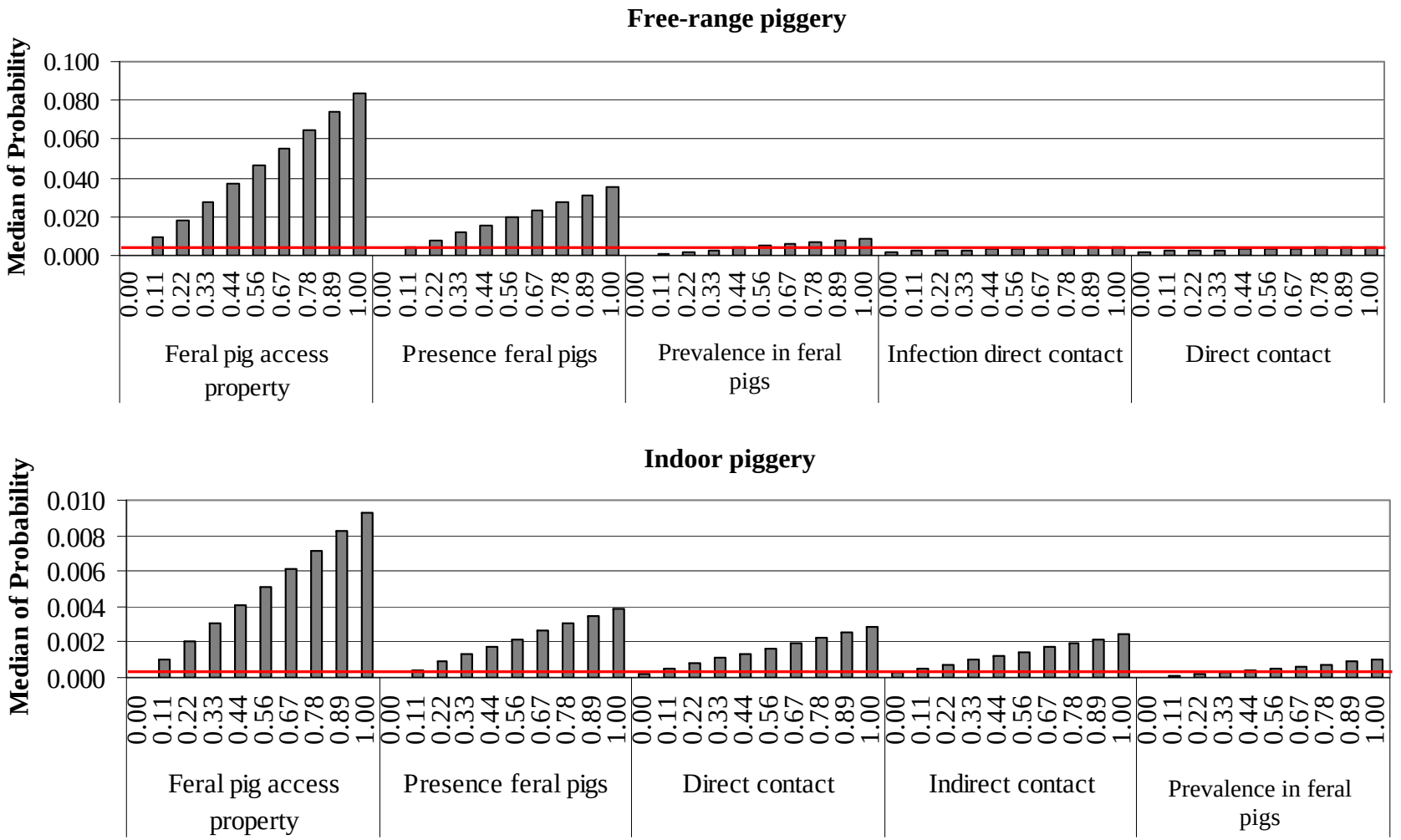

Figure 7.13. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Leptospira spp*. from feral pigs in piggeries in Australia. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.

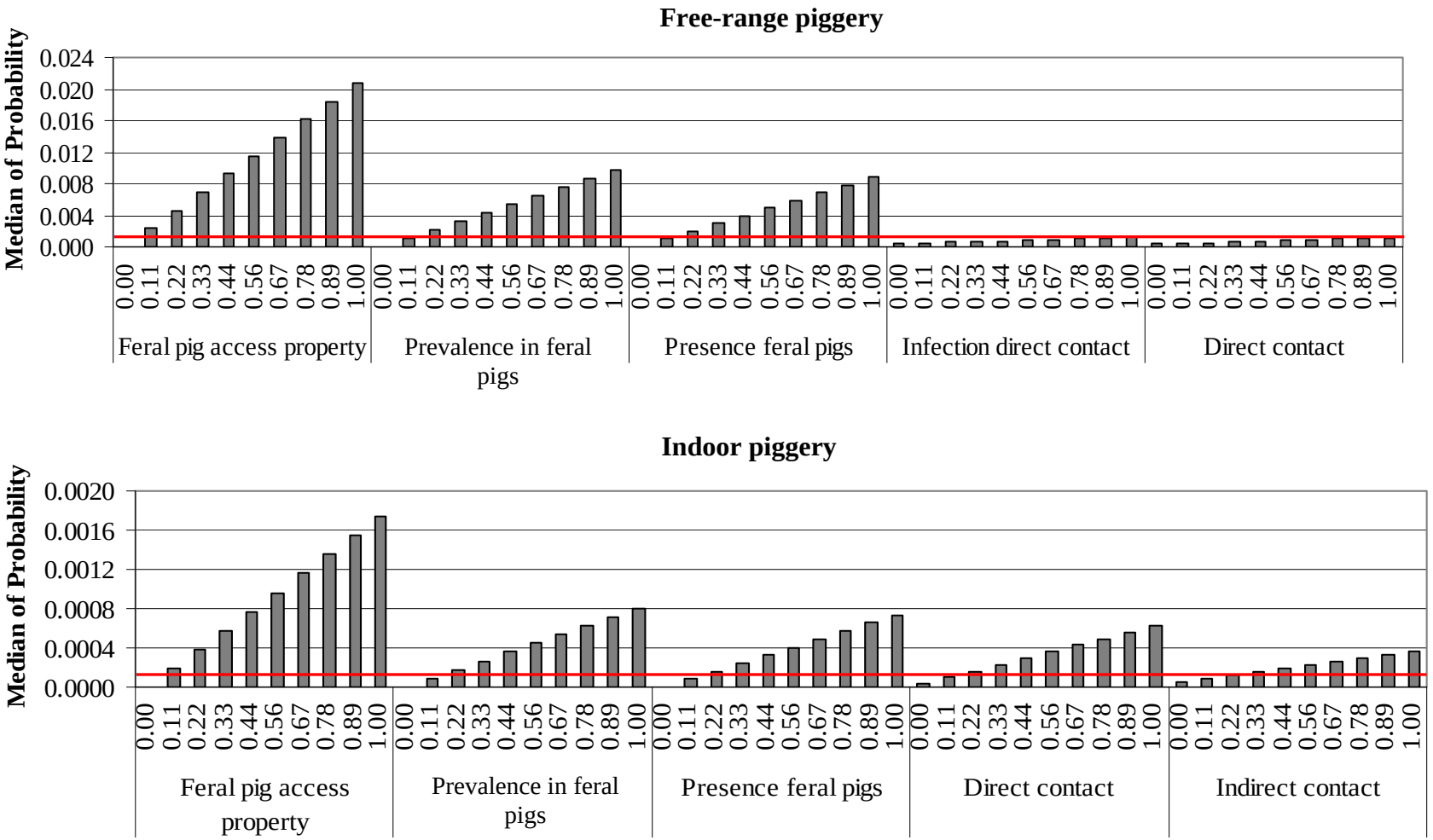

Figure 7.14. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Brucella suis* from feral pigs in piggeries in Australia. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.

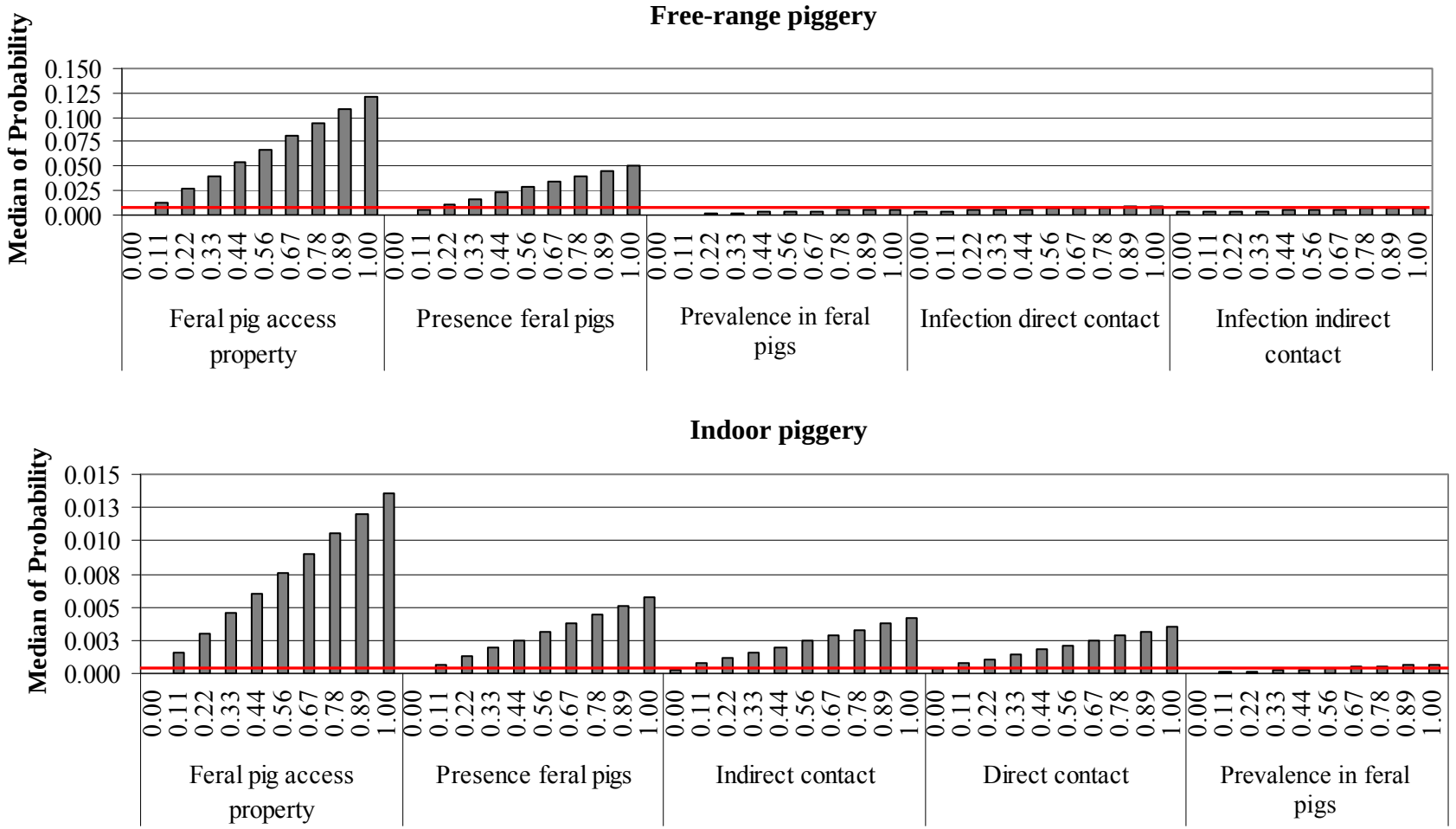

Figure 7.15. Results of the sensitivity analysis representing the influence of different input variables on the median (red horizontal line) probability of exposure of domestic pigs to *Lawsonia intracellularis* from feral pigs in piggeries in Australia. Results were obtained from a simulation of 1,000 iterations using @Risk's Advanced Sensitivity Analysis.

7.4. Discussion

This study quantitatively assesses the probability of exposure of domestic pigs to different pathogens from wild animals present in and around piggeries. The models presented in this study are dynamic, and as such, input parameters can be readily updated when appropriate data becomes available. Most input parameters used in the models have been estimated from extensive data-gathering exercises, reducing the uncertainty around the estimates and supporting the validity of the outputs obtained (Law and Kelton 2000). However, probability distributions have been incorporated in the model to account for natural variation, as occurs for pathogen prevalence in the wild animals, as well as uncertainty around the parameter estimates.

The validity of the models presented was further increased by the modellers understanding of the process undertaken to obtain the data used in the assessment (Banks et al. 2001). In addition, sensitivity analysis can also verify the models' validity (Stärk and Pfeiffer 1999), which is the case in the current study. The sensitivity analysis conducted has identified that the exposure assessment is performing in a logical manner. For example, complete removal of wild animals caused a corresponding reduction in risk to zero; increasing and decreasing the prevalence of pathogens in wild animals caused a corresponding increase and decrease in exposure risk.

For those parameters where data were not available, expert opinion and literature were used to obtain the most accurate estimates. Expert opinion is a recognised information source within the risk analysis discipline (Vose 2008). However, the validity of a risk model can be significantly reduced through the use of expert opinion due to potential bias and errors associated with inaccuracy of estimates (Vose 2008). It is recommended that one or more experts be consulted to provide parameter estimates (Vose 2008). In the current study a single expert with a great deal of experience in the pig industry was consulted. For this reason expert opinion has been used sparingly in this study, with support from literature provided where possible.

Despite the extensive amount of data gathered for the current study, and the use of literature and expert opinion, there are aspects of the exposure assessment where future research could be undertaken to strengthen the estimations of risk. The input parameter of

most importance for future research is pathogen survival. There is a lack of information in literature on the survival of pathogens in the conditions and substrates (including food, water, soil and floor material) present on piggeries. Related to this is the lack of information on the aerosol transmission potential of *Mycoplasma hyopneumoniae*. Pathogen survival information also has an influence on the probability of infection of domestic pigs upon contact with the various substrates. The risk estimate in the current study was provided based on an average commercial piggery; this was limited by the piggeries involved in the pathogen detection studies. Additional piggeries could be assessed to strengthen this. Finally, gathering information via survey of pig producers or observation on-farm about the access of wild animals to pig food, water and environment would also strengthen the study.

Literature was used to determine the survival of all pathogens studied within a number of substrates, although these substrates were not specific to the requirements of this model. Survival of pathogens was most frequently studied in faeces, soil and water sources such as effluent ponds and rivers (Mitscherlich and Marth 1984; Fedorka-Cray et al. 2000; Guan and Holley 2003). However, these substrates were not in piggeries, and thus the conditions for these substrates may differ to the average pig environment, food and drinking water. Additionally, the likelihood of domestic pigs being infected with *Mycoplasma hyopneumoniae* via the air was uncertain. Though literature has reported the detection of contaminated aerosol particles 4.7 km from the infection source (Dee et al. 2005), the quantity was not determined, and thus the infection potential remains unknown. As data available in literature to support these parameter estimates was limited, further research on survival and dose response to *Mycoplasma hyopneumoniae* from distant sources of infection is recommended. Parameter estimates used data from the literature to provide base level inputs, and uncertainty was addressed by providing a range of probabilities.

The exposure assessment assumes a simplified model of piggeries, with the accessibility of piggeries to starlings and rats being equal irrespective of piggery type. This was based on the open nature of most piggeries for ventilation purposes. Nets to restrict entry of birds to piggeries were only used in 3.5% of piggeries responding to the survey in Chapter 2. This was incorporated into the accessibility estimates. The difference in piggery type was only incorporated into the feral pig exposure assessment, due to the difference in accessibility of

domestic pigs to feral pigs and their secretions on free-range piggeries compared to intensive and ecoshelter piggeries.

As the majority of Australian piggery buildings have open sides for ventilation, exposure to aerosols was assumed to be the same regardless of being free-range or indoor. There is a possibility that there will be a difference between the exposure of domestic pigs to aerosols on free-range compared to indoor piggeries in winter, when windows are closed for heating, though this was not considered in the model. More research is needed on the shedding frequency, duration, dose, and distance over which transmission is possible, as well as the effects of weather on transmission of *Mycoplasma hyopneumoniae*. Additionally, the possibility of domestic pigs being exposed to pathogens on free-range piggeries from wild animals, such as feral pigs, through contaminated water run-off sourced from outside of the piggery has not been included as a pathway due to the extensive lack of information on free-range piggeries with creeks, streams or a geographic layout enabling water to run through the piggery. The model could be further developed to tailor to individual piggeries with problems with specific pathogens to determine whether wild animal transmission to domestic pigs is the likely cause.

The highest risk of exposure of domestic pigs to pathogens was from rats, specifically for *Lawsonia intracellularis* (0.13; 0.05–0.23) and *Brachyspira hyodysenteriae* (0.10; 0.05–0.19) on an infected piggery with no control implemented. Potential factors explaining these outcomes are the high prevalence of the pathogen in the rat population, the common presence of rats in piggeries, the absence of rodent control measures to restrict rodent movement, and the rat accessibility to the pig environment, food and water. The sensitivity analysis supported this reasoning, as rat infection, rat presence, density of rats and rat access to pig housing were identified as the most influential factors in the probability of domestic pigs being exposed.

No published studies have investigated the exposure of domestic pigs to endemic pathogens from wild animals, with limited studies conducted on other species. A quantitative release and exposure assessment conducted by Gallagher (2003) identified that badger-to-cattle exposure of *Mycobacterium bovis* did occur, with a mean of 2.5 cattle in a herd size of 74 becoming infected per year in the presence of one badger, or 2.7 cattle in the presence of 7 badgers. This equates to a probability of infection on any given day of

0.00009 and 0.0001, respectively. The probability of exposure of domestic pigs to pathogens from the starlings, rats and feral pigs in the current study was higher. Another study (Sutmoller et al. 2000) in Zimbabwe determined a 0.0002 probability of exposure of cattle to foot-and-mouth virus from antelopes. Most pathways of exposure in the current study were more likely, with only two exposure pathways for feral pigs being of lower probability (*Leptospira spp*. for an indoor piggery with a low number of feral pigs, 0.0001; *Brucella suis* for an indoor piggery, 0.00009)

The sensitivity analysis for all wild animals identified the parameters that had the strongest influence on the risk of exposure. Some of these influential parameters, including the presence of the wild animal at a piggery; a high number of wild animals; access to domestic pig environment; and, in the case of *Mycoplasma hyopneumoniae* from feral pigs, proximity to the piggery; could all be altered through the use of mitigation strategies to reduce the risk of pathogen transmission. Additionally the probability of a domestic pig becoming infected when exposed to these pathways was also identified as an influential parameter, particularly for *Lawsonia intracellularis* from rats. Domestic pig infection can be altered through vaccination protocols. The current study assumes all domestic pigs are susceptible to infection. However, for vaccinations that are effective over the lifetime of a pig, such as vaccinations to protect against *Leptospira pomona*, this probability of infection will decrease. Any future development of equally effective vaccinations for *Lawsonia intracellularis* and *Mycoplasma hyopneumoniae* could also play a large part in reducing the risk of infection. As such, these are areas where efforts and resources could be focused. This will be discussed further in Chapter 8.

The quantitative exposure assessment of the current study is the first assessment to evaluate the risk of pathogen transmission from wild animals to domestic pigs, to the author's knowledge. Many of the parameters incorporated into the models to estimate the probabilities of exposure are based on field data purposively gathered for this study, and when data were not available, literature and expert opinion were used. Results from this study highlight the potential risk of pathogen exposure and transmission from starlings, rats and feral pigs, and those areas where mitigation strategies could be implemented to reduce the identified risk. These findings could support decision-making regarding resource allocation to mitigate these risks.

8 Discussion/Conclusions

Wild animals contribute to emerging, transboundary and endemic infection in livestock and humans. They also contribute to the introduction, reintroduction and maintenance of pathogens. Intensive production systems such as piggeries and cattle feedlots can suffer severe consequences from infection with some pathogens transmitted from wild animals. This is shown by the high mortality rates caused by specific pathogens, and the culling, vaccination and other management practices implemented to control and prevent disease spread. International examples demonstrating the costly impact for piggeries include Nipah virus (Breed et al. 2006), classical swine fever (Fritzemeier et al. 2000), African swine fever (Penrith and Vosloo 2009) and pseudorabies (Hahn et al. 2010). These are cases where transmission has been proven to have occurred from wildlife to livestock. However, with endemic diseases the source of introduction to a piggery may not be known and the extent of wildlife contribution to such local spread is largely unexplored.

A systematic approach to the investigation of this problem would help to identify the level of pathogen-related risk presented by wild animals to Australian piggeries, and facilitate the development of targeted management strategies to mitigate unacceptably high areas of risk. As such, the primary aim of this study was to identify the role wild animals might play in the transmission of pathogens to pigs. The prevalence of specific pathogens in target wild animal species in and around Australian commercial large-scale piggeries was estimated, and the probability of pathogen exposure occurring from these wild animals to domestic pigs was determined. This process supplied a quantitative estimation of the level of pathogen exposure risk.

Prior to this thesis, the extent to which wild animals were present in and around Australian piggeries had not been formally studied. A formal survey among a representative group of commercial pig producers provided a valid basis to direct further research on observed wild animals. Results from the producer survey indicated that birds, cats and rodents were the most common wild animals observed in and around piggeries. This could be related to their ability to adapt to domestic urban environments, travel large distances and live in predator-prey cycles.

Although cats were the most common single animal species observed, this species was not included in the present study as there is substantial information available documenting their role in the transmission of pathogens, such as *Toxoplasma gondii* to domestic pigs (Lehmann et al. 2003). In contrast, scientific literature detailing the role of other observed wild animals in the transmission of endemic pathogens to domestic pigs is lacking. As such, the current study provides information on the infection status of European starlings, rats and feral pigs for specific pathogens and the probability of pathogen exposure to domestic pigs. It was not within the scope of this study to prove transmission of the pathogens.

All three targeted wild animals were carrying pig pathogens. These pathogens could be transmitted through direct and indirect pathways, via contaminated food, water and air. Insect- and fomite-borne transmission were not investigated in this study.

Of the three animals studied, rats were the most likely to expose domestic pigs to pathogens, and *Lawsonia intracellularis* and *Brachyspira hyodysenteriae* were the most likely pathogens to be transmitted. The appropriate level of protection risk allowance for imports into Australia and New Zealand is established at Very Low (Biosecurity Australia 2009), and the probability of exposure obtained in the current study for these two pathogens is higher than this level. It is acknowledged that the appropriate level of protection relates to the outcome of the overall risk estimation, which includes release, exposure and consequences assessments, and thus a direct comparison cannot be made to the probability obtained in this current study. However, as the estimated likelihood of exposure obtained in the current study was not negligible, the application of mitigation strategies should be further investigated to determine their cost-effectiveness.

As the postal survey did not identify rodent association with any particular piggery location, type or size, recommendations for strategically-targeted management and reduction of this risk can be derived from the exposure assessment and sensitivity analysis. Piggeries that were controlling *Brachyspira hyodysenteriae* in domestic pigs through medication, Swiss depopulation or total depopulation of infected piggeries had a 33-fold lower probability of exposure of pigs to the pathogen from rats compared to piggeries that were known to be infected and not controlling the pathogen. The control strategies for

reducing *Brachyspira hyodysenteriae* infection in domestic pigs limit the pathogen transmission cycle by reducing the prevalence in pigs and reducing environmental contamination, causing a corresponding reduction of infection and re-infection in rats, subsequently reducing the likelihood of exposure.

The sensitivity analysis also identified that having a high number of rats in a piggery, irrespective of their infection status, was one of the most influential factors on the probability of exposure. The number of rats present in a piggery can be reduced through more effective rodent control strategies, such as an integrated pest management approach including baiting, trapping and shooting on a region wide basis to prevent reincursion from neighbouring areas (Singleton et al. 2002). The majority of pig producers surveyed were satisfied with the currently applied rodent control strategies. However, effectiveness of these methods could be improved through enhanced knowledge and education. For example, traps and bait stations should be used within 17 m of each other to account for the minimum habitat range of rats (Leung and Clark 2005). Additionally, piggeries should be encouraged to use multiple bait types and toxins. Ideally, rats should not be present in piggeries, and control measures should focus on preventing rat access to pig housing, food and water. However, there are no available techniques to prevent rodent access to piggeries that are not entirely enclosed; therefore, environmental changes to reduce access to feed and water should be implemented. Where possible, removal of open cup and trough water sources and replacement with nipple or bite drinkers; as well as cleaning spilt feed from pens; should be implemented to minimise rodent presence in the piggery and therefore the potential contamination of foodstuffs by pathogens carried by rodents. In addition, those pig producers that mix their own feed should make every attempt to store feed ingredients or feed bags in a manner that reduces the chance of rodents accessing it, such as in silos, bins or plastic food containers.

Exposure of domestic pigs to pathogens from European starlings was estimated to be lower than the exposure from rats. However, there was a 3% probability of exposure to pathogenic *Escherichia coli*, which was the highest probability among the three pathogens studied in starlings. The infection level of the different pathogens in the starlings themselves had the greatest influence on the exposure likelihood. However, this input parameter could only be modified if a medication strategy was incorporated for wild birds on farms. This strategy has not been considered for pest birds on livestock enterprises

before, but is a potential option, through in-feed and water medication, if it is demonstrated to be a cheaper option than other available approaches to prevent pig exposure. However, the feasibility and usefulness of this strategy must be examined, as it will likely improve the survival of these invasive pest birds, as shown through medication studies for control of blood parasites in wild Blue Tits (*Cyanistes caeruleus*) (Tomás et al. 2007); further increasing their population size and the subsequent impacts would be counter-productive.

The presence and number of starlings on piggeries also had an important influence on the likelihood of pathogen exposure. Both of these input parameters could be altered through effective control strategies. However, the only population control method available to pig producers in Australia is shooting, and this is considered to be ineffective (Fleming 1990; Bomford and Sinclair 2002). Alternative means of controlling starling access to piggeries include netting, which is generally considered ineffective by pig producers due to net damage over time, and use of poison baits for starlings, which are currently not registered for use in Australia. Piggeries from Queensland reported fewer observations of starlings than piggeries in other states of Australia. Control of starlings should be targeted towards regions where this wild animal poses the most significant problem.

The likelihood of pathogen exposure from feral to domestic pigs was found to be very low for some pathogens. The highest probability of domestic pig exposure to feral pig pathogens was found to be for *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis* for pigs in free-range piggeries. As expected, this likelihood was higher in piggeries with a high number of feral pigs in their proximity. Although the probability of exposure was very low, the potential consequences of pathogen exposure and transmission from feral pigs could be vast in many instances (Meuwissen et al. 1999; Productivity Commission 2002). Consequently, mitigation strategies to reduce exposure from feral pigs should be seriously considered. In addition, the role of feral pigs internationally as a carrier and transmitter of exotic diseases, such as foot-and-mouth disease (Productivity Commission 2002), classical swine fever (Fritzemeier et al. 2000) and pseudorabies (Hahn et al. 2010), which have vast consequences on pig and livestock health, should be considered when making decisions about the necessity to mitigate the risk posed by this wild animal.

Free-range piggeries in areas of high density feral pig populations should be the focus for risk mitigation strategies; in particular, those piggeries not applying the recommended

biosecurity practices in relation to the prevention of wild animals contacting domestic pigs are likely to pose the highest risk of exposure (APIQ 2010). To deter feral pig access to piggeries, adequate fencing, such as that provided by a fence with steel posts, net-wire and an electric outrigged wire, is required (Hone and Atkinson 1983). The input parameter describing access of feral pigs to the piggery property had a high influence on the likelihood of exposure; thus, some practical strategies could involve compliance with the biosecurity fencing recommendation, as well as region-wide feral pig population reduction. The latter would reduce by up to 10-fold the likelihood of exposure to the different pathogens. This can be accomplished through integrated baiting with 1080 using a number of targeted delivery mechanisms such as the PIGOUT® bait and the Hog-Hopper™ delivery container (Cowled et al. 2006; Dall 2010), as well as aerial shooting programs, as has been occurring in the area through the Queensland Murray-Darling Committee Blue-print for the Bush project. Undertaking feral pig surveillance through the use of the HogHopper™ and remote cameras can also allow for removal of feral pig populations prior to their establishment.

Proximity of feral pigs to piggeries is also an important factor to consider for the potential aerosol transmission of some pathogens. Having an external property perimeter fence to act as a deterrent by reducing ease of access to a property (Reidy et al. 2008), as well as having a clear zone around piggeries without crops that may attract feral pigs for food, could reduce the exposure risk. These recommendations and practices should not only be considered to reduce endemic disease transmission between feral and domestic pigs, but also to prevent transmission of exotic diseases.

It is important to keep in mind when determining whether to implement a mitigation strategy to reduce risk, that there are inherent problems with predicting risk from imperfect data. The probability of exposure was determined based on an average commercial piggery; individual piggeries may have higher or lower risk levels than those identified in this study. This depends on different factors, such as environmental conditions as well as piggery characteristics. Those piggeries that do not adhere to the Australian pork industry quality assurance program (APIQ✓™), generally small-scale producers, will likely have lower biosecurity standards enabling greater contact with wild animals and a higher risk of exposure to pathogens they may be carrying (Hernandez-Jover et al. 2009; Hernandez-Jover et al. 2011). In addition, estimation of pathogen presence in wild animals was

extrapolated from specific regions in Australia. Alternate regions might have different pathogen prevalence. For example, starlings were only sampled for pathogen detection in South Australia, though the postal survey results indicated starlings were also frequently observed in New South Wales and Victoria. Additionally, piggeries included in this study were only sampled once; for rats this only represented an intensive piggery type, and for starlings it did not include free-range piggeries. Seasonal variability as well as the effect of piggery type on pathogen prevalence has been previously described (Gaulker et al. 2009; Collins and Love 2003). Seasonal variability can also affect wild animal numbers observed on piggeries which were reported as a weekly average by pig producers on the survey in Chapter 2. These differences should be considered before applying any mitigation strategy. Finally all pigs were assumed to be susceptible to infection, with the effects of vaccination and in-feed medication given to pigs on risk of exposure requiring further investigation. In the case of *Leptospira pomona* vaccinations, pigs are protected for life, though the vaccination does not provide protection against other serotypes.

Another limitation of the study was that the wild animal pathogen prevalence used in the exposure assessment was estimated from studies aiming to detect pathogen presence (rather than measure prevalence). Consequently, the confidence intervals around the true prevalence estimate are wider, incorporating greater uncertainty into the exposure assessment probability estimates. Further research involving additional sampling to measure prevalence could reduce this uncertainty. However, the true prevalence was calculated for all pathogens in the three wild animals studied, considering the diagnostic tests used for pathogen detection were imperfect (sensitivity and specificity differ from 100%). This provided more accurate estimates of the point prevalence and allowed for the comparison of the results of the current study with other studies using different diagnostic techniques (Gardner 2004). It is recommended that all pathogen prevalence studies report true prevalence for this reason. Additional to this is the need for laboratories to report the sensitivity and specificity of the diagnostic methods they use. The current study had to make some assumptions about the sensitivity and specificity of some laboratory techniques used from literature due to lack of information directly from the source.

8.1 Future research

Further investigation is necessary to determine the impacts of some of the findings of this thesis. *Leptospira weilii* serovar Topaz was detected in both feral and domestic pigs for the first time. The impacts of infection with this pathogen have not been previously investigated, and as such, the need to control infection with this pathogen in domestic pigs cannot be determined. *Leptospira pomona*, the most common isolate of *Leptospira spp*. in domestic pigs prior to the introduction of vaccination protocols on piggeries, caused a variety of symptoms, the main one being reproductive disorders (Faine 1994; Alston and Broom 1958). If this relatively new isolate, *Leptospira weilii* serovar Topaz, causes similar symptoms, the prevalence of infection in domestic pigs and other livestock needs to be investigated, and a vaccination for control might need to be developed.

The main source of the pathogen, *Leptospira weilii* serovar Topaz, also needs to be investigated. Feral pigs are infected with this serotype at a similar low prevalence to domestic pigs. However, a study in the Warragamba catchment area, near Sydney, identified that almost half of Eastern grey kangaroos were infected with this serotype (Roberts et al. 2010). As such, it is important to determine how widespread this pathogen is in marsupials in Australia, particularly in proximity to livestock. Fencing practices for piggeries are not designed to restrict entry of marsupials due to their climbing or jumping ability. Reported observations of marsupials were higher for piggeries, with greater than 1000 sows ($P<0.05$) in the producer survey, likely due to the rural locations of these larger piggeries. Targeting piggeries with marsupials in their proximity, with particular emphasis on kangaroos on free-range piggeries, and smaller marsupials, such as possums and sugar gliders, on indoor piggeries, is a good focus for future pathogen investigations.

Wild canids and sparrows were also frequently observed by piggery producers and are additional animals that could be investigated for pathogen presence. Wild canids have been identified as the key definitive host of the parasite *Neospora caninum*, which causes illness and abortion in cattle (King et al. 2011), as well as *Echinococcus granulosus*, which is a zoonotic helminth capable of being transmitted between wildlife, domestic animals and humans (Jenkins and Morris 2003). Thus, their role in production-limiting pathogen transmission to domestic pigs should also be investigated. Piggeries with combined indoor and outdoor settings and those with more than 1,000 sows were found to be more likely to observe wild canids. The role of starlings, sparrows and rodents in pathogen transmission could also be further investigated to determine how their movements may contribute to

pathogen transfer between farms. Although a study in the United States has reported starling movement between dairy farms (LeJeune et al. 2008), no information from Australia is available. It is unknown whether starlings are devoted to a single piggery, or alter their feeding location, from day-to-day, or season to season in Australia.

Additional future work is recommended to identify whether there are different levels of wariness towards humans in starling populations on different piggeries and agricultural enterprises. This information will indicate whether control techniques will differ in their effectiveness depending on how cautious a population is. Varying levels of starling wariness was observed in the current study by the varying ease of trapping for the different piggeries.

Transmission and infection of domestic pigs with pathogens from a wild animal source, particularly infection with *Lawsonia intracellularis* and *Brachyspira hyodysenteriae* from rats, is credible, given the probability of exposure arising from this thesis. As such, important future research involves conducting an accurate cost-benefit analysis to determine the cost-effectiveness of mitigation strategies. There are numerous studies that have investigated the cost of infection of some of these pathogens in piggeries, but there is a need to combine this research with a cost analysis of the proposed mitigation strategies outlined in this chapter.

8.2 Industry application

Short of complete enclosure of piggeries and air filtration to prevent exposure, there are a number of practical recommendations for the pork industry to reduce the risk of exposure from wild animals. These recommendations include additional biosecurity practices required for quality assurance programs for piggeries, and improvements to pest animal control. The quality assurance program currently states that pig feed and water should be fresh, palatable and safe (APIQ 2010). APIQ (2010) already recommends storage of pig feed in enclosed silos, bins or bags to prevent access by rodents and birds. However, this recommendation could be expanded to include methods to protect feed and water, once delivered to pigs, to ensure palatability and safety. In indoor piggeries, where possible, feed should be distributed to pigs from enclosed bins or overhead cable-operated auger systems. Feeder designs should take in to account the behavioural requirements of pigs to

feed in groups, while protecting feed as much as possible from birds and rodents. The Penguin Rotational Feeder (Stockyard Industries©, Queensland, Australia) releases limited amounts of feed when it is nudged by the snout of a pig, thus restricting the amount accessible to rodents and birds. An outdoor design restricting wildlife access that may suit some free-range piggeries is an ad-libitum feed hopper, with flaps over feed spaces in the trough which are easily lifted by pigs (Booth©; West Sussex, United Kingdom). In addition to feed delivery, water, where possible, should be delivered in nipple or bite drinkers, not troughs. These practical methods will help reduce the transmission risk from rats and birds, which pose the highest probability of exposure.

Expansion of the quality assurance recommendations in relation to feral pig pest control should indicate the most effective fencing type to deter feral pig entry to piggeries, with emphasis on free-range piggeries. A one metre tall fence, with steel posts, net-wire and an electric outrigged wire described by Hone and Atkinson (1983) is still considered to be the most effective feral pig deterrent over the long term (Seward et al. 2004; Reidy et al. 2008). There should also be an emphasis on a double layer of fencing, such as that provided by pig paddock fencing, as well as a property perimeter fence. The greater the distance between the double layers of fencing, the greater the reduction in the risk of aerosol transmission. This is particularly important for controlling *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*, or any other aerosol-transmitted disease. It is important to reiterate that the risk of non-aerosol pathogen transmission from feral pigs is between 9- and 11-fold greater on free-range piggeries compared to indoor piggeries. Quality assurance recommendations must be strategically targeted towards mitigating risks on this type of piggery.

Pest animal control could also be improved in Australia, particularly in relation to birds. Only one out of the ten pig producers implementing bird control on their piggery were satisfied with its effectiveness. Further the overwhelming majority of producers reporting birds on their piggeries did not use any control technique for reducing bird access to piggery housing. Shooting and the use of nets are the main techniques available for control of large populations. (Fleming 1990). Netting is effective for agricultural crops when applied correctly and maintained, though a high initial investment cost is associated with this method (Bomford and Sinclair 2002). The satisfaction of farmers with its effectiveness

and required maintenance has not been determined (Casal et al. 2007). Clearly an effective and cheap control technique is required for Australian piggeries.

It is recommended that on-farm trials to be undertaken to determine the effectiveness of available netting for piggeries. The extent of damage sustained by rodents and strong beaked birds, such as sparrows, cockatoos and galahs, and the maintenance requirements under these conditions requires formal assessment. Secondly, a lethal population control product needs to be introduced and registered for use in Australia. DRC-1339 is a poison developed and used extensively since 1967 for starling population control in the United States for protection of livestock enterprises, agricultural crops and threatened or endangered species (Lapidge et al. 2006). Its use on cattle feedlots in the United States reduced starling numbers and resulted in a corresponding reduction in *Salmonella spp*. contamination in cattle feed and water (Carlson et al. 2011b). It is expected that a similar positive result would occur on piggeries and feedlots in Australia if it were registered for use. Farm trials on a number of different piggeries and agricultural enterprises is recommended to assess its effectiveness in Australia, to determine the optimal delivery system to encourage starling feeding, to determine whether starlings from different regions will move in to replace eradicated starlings requiring region wide implementation, and to establish its non-target effects on native animals.

In partnership with the United States Department of Agriculture, the extended Invasive Animals Cooperative Research Centre will be investigating water-based poisons for starling and rodent control (Lapidge 2012; Personal communication). Water is a more limiting factor than food for pest animals in piggeries due to the extensive use of nipple and bite drinkers, as well as other intensive agriculture operations, such as feedlots and grain storage areas. The Invasive Animals Cooperative Research Centre also has a suite of feral pig control tools currently available, such as the previously mentioned PIGOUT® and HogHopper™; and those in development and available in the future, including HOGGONE® and HOGGONE® Econobait and nitrite concentrate (Lapidge 2012; Personal communication); that can be used by piggeries to minimise risks of pathogen transmission from feral to domestic pigs. These could also be used for vaccination or medication distribution in the event of an exotic pathogen outbreak in Australia. Although locally effective, it is recommended that any such tools are used as part of a community baiting program due to the large home range of many feral pigs, the long-distance potential of

aerosol transmission of some pathogens and the movement of feral pigs into new areas. All of the previously mentioned mitigation strategies in this chapter will not only reduce the risk of pathogen transmission, but also have a positive affect on other pest animal impacts such as lowering feed costs and lowering building maintenance.

This thesis has identified that there is potential for pathogen transmission from wildlife to pigs in piggeries, given that production-limiting pathogens were detected in wild animals and that the probability of exposure of domestic pigs to pathogens via these animals was not negligible. This finding supports the implementation of mitigation strategies to lower the exposure risk, with particular emphasis on the most likely avenues of infection, as well as the piggery types and geographic locations posing the highest probability of exposure. If the most significant exposure pathways are targeted through wild animal control and enhanced biosecurity practices, the probability of exposure to pathogens would be reduced, subsequently improving pig health and production.

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Appendix

Appendix 1. Questionnaire distributed to the 444 commercial pig producing members of Australian Pork Limited in 2007 in Australia.

help direct research into domestic pig and wildlife interactions in and around piggeries

Page 1 of 1

Domestic pig and wildlife interactions

Survey of commercial producers

*Response is **voluntary** and all information provided by you will remain **private** and **confidential**.*
Wildlife in this survey refers to native Australian wildlife, introduced pest animals and birds and feral animal species.

Name

_____

* Postal address

_____

Post code

_____

* Phone

_____

** Optional. Contact details will be used for verifying results only.*

Region within Australia

_____

Would you like to receive the results of this survey? yes ☐ no ☐

For further information,
Contact Hayley Pearson,
PhD student, Funded by Australian Pork Ltd
ph 0409 233311
email: h.pearson@usyd.edu.au

faxback to: (02) 9351 1693
or *post* to: Domestic pig and wildlife interactions - Survey.
425 Werombi Rd
Camden, NSW, 2570.

The University of Sydney

Australian Pork LIMITED

Invasive Animals Cooperative Research Centre

What piggery type(s) are you involved in (tick one or more boxes)?
☐ Intensive ☐ Free range ☐ Ecoshelter ☐ Other _____
If other – please specify

What size is your piggery (Number of sows)? _____
If you have more than one piggery please state the number of sows at the largest piggery.

Does any wildlife enter your piggery/s or housing facilities (sheds, ecoshelters, paddocks etc?)
☐ Yes ☐ No ☐ Unsure

Does any wildlife visit close (0-5metres) to fence lines that your pigs can access?
☐ Yes ☐ No ☐ Unsure

If you answered Yes or Unsure to either question please complete the below table to the best of your ability. *Please state the average number of individual animals observed in one week. Consider all types of native wildlife and pest/feral animals.*

Species of animal	Average Number/Week	Control method used (e.g. baiting, trapping)	Is the control effective (yes or no)

List specific diseases carried by wildlife that are of importance or concern to you in regard to your piggery/s. (Examples: Toxoplasmosis, Salmonella, Leptospirosis etc)

Disease	Wildlife species/s responsible

Where did you obtain information regarding the diseases carried by wildlife? (e.g. veterinarian, friend, DPI)

_____

What issue related to wildlife in and around piggeries do you consider should be the highest priority for future research and development?

_____

Would you be willing to permit confidential disease sampling of wildlife on your farm?
Yes ☐ No ☐
Other comments (please use over page if more space required)

_____

_____

Appendix 2. Questionnaire distributed to four piggery managers in South Australia involved in confidential pathogen sampling of European starlings on their piggeries

Disease history of piggery

Have you any information (from the period of bird capture in February 2008 –present) on *Salmonella spp.*, *Escherichia coli* and their serotypes and *Campylobacter* spp. on your piggery?

1. **Please, provide information on proportion of animals infected and the serotypes of the pathogens, if available.**
2. **Do you have any health records regarding these infections (serology results, isolations of pathogens, morbidity and mortality records…).**
3. **Are or were any medications being used to control these diseases?**

Escherichia coli:

1. 2008:

   2009:

   Present:

2. Health records:

3. Treatments:

Salmonella spp.:

1. 2008:

   2009:

   Present:

2. Health records:

3. Treatments:

Campylobacter spp.:

1. 2008:

   2009:

   Present:

2. Health records:

3. Treatments:

General information about your piggery

What was the herd size of the piggery?

2008:

2009:

Present:

Which production system was applied?

2008 and 2009

All in/All out:

Continuous flow:

Present

All in/All out:

Continuous flow:

Appendix 2 continued.

How was the food for the pigs stored in the piggery? Is the same storage method still used at present?

_____
_____

Did staff change their boots and/or overalls between different pig sheds? Is this the same method used at present?

_____
_____

Did you undertake any method of control for European starlings? Do you use any methods of control at present?

_____
_____

Specific location information

Are there any other potential food sources for European starlings in the region (region being an approximate measure, ≤ 20 km radius will suffice) that you? Other food sources could include other piggeries, feedlots, poultry farms, feed mills, garbage tips etc.

_____
_____
_____
_____
_____
_____

Appendix 3. Questionnaire distributed to three piggery managers in Victoria and South Australia involved in confidential pathogen sampling of rats on their piggeries.

Disease history of piggery

When was the last confirmed *Brachyspira hyodysenteriae* positive pig and related serotype on your piggery?

On what date did the medication or depopulation of your farm commence and finish for control of swine dysentery (if ongoing, just state ongoing)?

Commencement:
Completion:

On what date were new pigs brought onto the farm if depopulation occurred?

Do you follow a particular medication protocol for treatment of pigs for *Brachyspira hyodysenteriae*? Would you be able to briefly explain or provide a document or reference outlining the protocol you follow, including medications for different age groups of animals?

Did you follow a particular method for you depopulation? Would you be able to briefly explain or provide a document or reference outlining the procedure you followed?

Just prior to the control for *Brachyspira hyodysenteriae* was implemented what proportion of your pigs were infected? If you know any specific age groups this would also be helpful.

Salmonella, *Lawsonia intracellularis* and *Brachyspira pilosicoli*

1. **Do you have any information on the pigs' status regarding Salmonella, *Lawsonia intracellularis* and *Brachyspira pilosicoli* in your piggery over the time of sample collection? Please, provide information on proportion of animals infected and the serotypes of the pathogens, if available.**
2. **Do you have any health records regarding these infections (serology results, isolations of pathogens, morbidity and mortality records…)**
3. **Are or were any medications being used to control these diseases?**

Salmonella spp.:

1. Before:

   After:

2. Health records:

3. Treatments:

Lawsonia intracellularis:

1. Before:

   After:

2. Health records:

3. Treatments:

Brachyspira pilosicoli:

1. Before:

Appendix 3 continued.

After: _____

_____

_____

2. Health records:

_____

_____

3. Treatments:

_____

_____

General information about your piggery

What is the herd size of your piggery?

_____

_____

Which production system is applied in your piggery?

All in/All out:
Continuous flow:

How is the food for the pigs stored in the piggery?

_____

_____

_____

Do you have specific boots and/or overalls to be used only in the piggery?

_____

Does staff in your piggery change their boots and/or overalls between different pig sheds?

_____

Pest control management

Do you have regular measures of control for rodents in your piggery? If yes, please describe these measures and how frequently they are applied.

_____

_____

_____

_____

_____

Are these control measures the same as those conducted prior to the control of swine dysentery on the farm? If the strategies differed, please explain these differences.

_____

_____

_____

_____

_____

General location information

Are there any other potential food sources for rodents in the region (region being an approximate ≤ 5 km radius) that you may be aware of? Other food sources could include other piggeries, feedlots, poultry farms, feed mills, garbage tips etc.

_____

_____

_____

_____

_____

_____

Appendix 4. Questionnaire distributed to two piggery managers in Queensland involved in confidential pathogen sampling of feral pigs near their piggeries.

Disease history of piggery

Brucella suis

Have you ever had, or do you have a confirmed Brucellosis positive pig(s)? Date(s)?

What proportion of animals were/are infected?

Do you have any health records regarding these infections (serology results, isolations of pathogens, morbidity and mortality records…)

Are or were any medications being used to control these diseases?

Leptospira spp.

Have you ever had, or do you have a confirmed Leptospirosis positive pig(s)? Date(s)?

What proportion of animals were/are infected?

Do you have any health records regarding these infections (serology results, isolations of pathogens, morbidity and mortality records…)

Are or were any medications being used to control these diseases?

Lawsonia intracellularis

Have you ever had, or do you have a confirmed *Lawsonia intracellularis* positive pig(s)? Date(s)?

What proportions of animals were infected?

Do you have any health records regarding these infections (serology results, isolations of pathogens, morbidity and mortality records…)

Are or were any medications being used to control these diseases?

Mycoplasma hyopneumoniae

Have you ever had, or do you have a confirmed *Mycoplasma hyopneumoniae* positive pig(s)? Date(s)?

What proportions of animals were infected?

Do you have any health records regarding these infections (serology results, isolations of pathogens, morbidity and mortality records…)

Are or were any medications being used to control these diseases?

Actinobacillus pleuropneumoniae

Appendix 4 continued.

Have you ever had, or do you have a confirmed *Actinobacillus pleuropneumoniae* positive pig? Date(s)?

What proportions of animals were infected?

Do you have any health records regarding these infections (serology results, isolations of pathogens, morbidity and mortality records…)

Are or were any medications being used to control these diseases?

General information about your piggery

What is the herd size of your piggery?

Which production system is applied in your piggery?

All in/All out:
Continuous flow:

How is the food for the pigs stored in the piggery?

Do you have specific boots and/or overalls to be used only in the piggery?

Does staff in your piggery change their boots and/or overalls between different pig sheds?

Pest control management

Do you have a fence or double fence barrier around your piggery? If yes, what type of fence is it, how tall is it, and how far is the distance between the two fences in the event of a double fence barrier?

Do you have regular measures of control for feral pigs in and around your piggery (e.g. are feral pigs being controlled anywhere on your property)? If yes, please describe these measures and how frequently they are applied.

Have you found these control measures effective in preventing physical contact between feral pigs and domestic pigs?

Conference presentations and proceedings

Pearson, H., Lapidge, S., Hernandez-Jover, M., Toribio, J-A. (2011). Are vertebrate pests a disease risk for commercial piggeries? 15th Australasian Vertebrate Pest Conference, Sydney, June, p 138.

Pearson, H., Lapidge, S., Hernandez-Jover, M., Toribio, J-A. (2010). Assessment of the risk of disease transmission from wildlife to domestic pigs in Australia. 5th Annual Conference of the Society for Risk Analysis – Australia and New Zealand, September.

Pearson, H., Lapidge, S., Hernandez-Jover, M., Toribio, J-A. (2008). Survey of domestic pig and wildlife interactions on commercial piggeries in Australia. 14th Australasian Vertebrate Pest Conference, Darwin, June, p 59.