A RISK ASSESSMENT APPROACH TO METICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN PET DOGS

Jane Heller
BSc, BVSc, DipVetClinStud, MVetClinStud, MRCVS

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Department of Animal Production and Public Health,
Institute of Comparative Medicine,
University of Glasgow

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For Ella

ABSTRACT

A risk assessment approach was used to: a) define the risk of acquisition of meticillin-resistant *Staphylococcus aureus* (MRSA) in pet dogs over a defined time period, b) attempt to identify important priority data gaps for future research efforts in this area and c) comment on the usefulness of risk assessment in this regard, in a data sparse area and in a field in which it has not previously been used.

A conceptual model was defined that identified the potential pathways for acquisition of MRSA in a pet dog over any given 24 hours. A qualitative risk assessment, using categorical qualitative estimators and combining estimates using a matrix approach was undertaken. It was found that this approach was unsatisfactory for the specification of this biological model that encompassed a non-modular, non-sequential form, characterised by non-mutually exclusive pathways of acquisition. Modification of the categorical descriptors enabled a relative, rather than absolute assessment of risk and resulted in the conclusion that both veterinary and non-veterinary routes were potentially important for the acquisition of MRSA in pet dogs and that family members and staff were likely to be the most important sources of the organism in the community and veterinary clinic environments respectively. Given the limitations encountered, a quantitative risk assessment was pursued.

Data gaps that were defined within the qualitative risk assessment were addressed through dedicated data-collection studies and an expert opinion elicitation exercise. The studies found a lower veterinary environmental and staff prevalence than had previously been reported, corroborated prior estimates of dog-dog and dog-human interactions and justified the inclusion of dog foods as environmental sources of MRSA rather than independent and important sources in their own right. The expert opinion elicitation exercise used a modified technique to obtain numerous estimates relating to prevalence and transmission of MRSA. However, it was found that experts lacked confidence in estimation of transmission variables in particular, and the resulting distributions for these variables demonstrated divergence between experts and resulted in wide and poorly-informative combined distributions.

These results were utilised, along with published and unpublished data, to parameterise a second order stochastic simulation-based quantitative risk assessment model. The model produced a biologically plausible outcome and allowed the application of sensitivity analyses with the intention of identifying areas of putative importance for future research efforts. The implementation of logistic regression analyses directly to the input/output relationship within the simulation model represented a novel application of a variance-based sensitivity analysis technique in the area of veterinary medicine, and was implemented with and without the consideration of interaction terms. In addition, one-at-a-time (OAT) and Plackett-Burman (P-B) analyses were also completed. The results of the sensitivity analyses were complicated and ambiguous. While family members and the environment were identified as potentially important independent and non-independent sources of MRSA, respectively, it was not possible to discount, or defensibly rank the importance of other sources.

In conclusion, it was found that, despite the application of well researched and previously utilised methods, marked limitations were encountered in the use of risk assessment to address a biologically complex phenomenon that is characterised by sparse data such as this.

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AUTHOR'S DECLARATION

The work presented in this thesis was performed solely by the author except where the assistance of others has been acknowledged. It has not been submitted in any form for another degree or professional qualification.

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LIST OF ABBREVIATIONS

ACME	Arginine catabolic mobile element
AIC	Akaike's Information Criterion
ASM	American Society of Microbiology
BURP	Based upon repeat pattern
BURST	Based upon repeat sequence types
CA-	Community associated
CAC	Codex Alimentarius Commission
CC	Clonal complex
CDC	Centers for Disease Control
CHEF	Clamped homogeneous electric field
DO	Dog owners
EARSS	European antimicrobial resistance surveillance system
FAO	Food and Agriculture Organisation
FAST	Fourier Amplitude Sensitivity Test
HA-	Hospital associated
I/O	Input/Output
IRA	Import Risk Analysis
LR	Logistic regression
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MRA	Microbial Risk Analysis
MRSA	meticillin-resistant *Staphylococcus aureus*
MSSA	Meticillin-sensitive S*taphylococcus aureus*
NAS-NRC	National Academy of Sciences - National Research Council
OAT	One-at-a-time
OIE	World Organisation for Animal Health
P( )	Probability
P-B	Plackett-Burman
PBP	Penicillin binding proteins
PFGE	Pulse field gel electrophoresis
PSM	Phenol-soluble modulins
PVL	Panton-Valentine Leukocidin
QRA	Qantitative Risk Assessment
SBA	Sheep blood agar
SCC*mec*	Staphylococcal Chromasomal Cassette *mec*
SIRN	Scottish Infection Research Network
spa	*Staphylococcus aureus* protein A
ST	Sequence type
TSB	Tryptone soya broth
WHO	World Health Organisation
WTO	World Trade Organisation

CHAPTER 1

REVIEW OF THE LITERATURE

1.1 Introduction

The review of the literature presented below may be conceptually divided in two sections. The first section relates to the methods that are drawn upon within these studies and the second presents the literature surrounding the area of meticillin-resistant *Staphylococcus aureus* (MRSA).

1.2 Risk analysis

Risk analysis can be defined as: 'the quantifying, either qualitatively or quantitatively, of the probability and the potential impact of some risk' (Vose, 2000).

It has been asserted that the origins of risk analysis lie with a group called the Asipu, who served as consultants for risky decision-making in 3200BC (Covello and Mumpower, 1985). While this group almost certainly represent the first risk consultants, it is reported that their data were predominantly of divine origin and as such, the foundation of modern data-driven risk analysis is more recent and likely to have been borne out of environmental health concerns in the 1960s (Covello and Mumpower, 1985; Thompson *et al.*, 2005). The intended outcome of a modern risk analysis is to provide a repeatable and objective assessment of risks that may arise from a particular course of action (MacDiarmid and Pharo, 2003). Risk analysis is common in a number of disciplines, including engineering, toxicology, economics and animal health.

The use of risk analysis as a tool in the animal health field is relatively recent and its genesis may be directly associated with the implementation of the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS agreement) by the World Trade Organisation (WTO) in the 1990s (MacDiarmid and Pharo, 2003; OIE, 2006). The SPS agreement requires the removal of trade barriers unless a risk exists to human, animal or plant health, along with the demonstration of this risk through a dedicated risk analysis (MacDiarmid, 2000). Thus, this trade agreement advocated the use of risk analysis simultaneously to stimulate international trade and protect human and animal health in the importing country (MacDiarmid, 2000). Subsequently, risk analysis techniques have been utilised with two main objectives in the field of animal health (MacDiarmid and Pharo, 2003). Import risk analysis (IRA) is used to assess the disease or pathogen risk associated

with importation of animals, animal products or food (Murray, 2002; OIE, 2003; Peeler and Thrush, 2004; Jones *et al.*, 2005) while microbial risk analysis (MRA) is used to quantify microbial risk relating to food consumption from an animal source (Alimentarius, 1999; Hope *et al.*, 2002; Clough *et al.*, 2006).

1.2.1 Frameworks for risk analysis

Guidelines for undertaking risk analysis in the area of IRA along with other areas of animal health, are published by the World Organisation for Animal Health (OIE) in the form of Terrestrial and Aquatic Animal Health Codes (OIE, 2008b; OIE, 2008a), and MRA guidelines are published by the Codex Alimentarius Commission (CAC), a World Health Organisation/Food and Agriculture Organisation (WHO/FAO) body, as a principles and guidelines document (CAC, 1999). The latter of these frameworks is currently under revision and extension to include Risk Assessment Guidance regarding foodborne antimicrobial resistant microorganisms (FAO/WHO) (CAC, 2009).

While the framework that is used to guide a risk analysis may vary (sections 1.2.1.1 and 1.2.1.2), the holistic process of risk analysis has a common structure, which has been summarised by Vose (2000) to consist of a five step process of;

a) Identifying the risk
b) Undertaking a qualitative description of the risk
c) Quantitative or semi-quantitative analysis of the risk and associated management options
d) Implementation of an approved risk management strategy
e) Communicating the decision and its basis to stakeholders

1.2.1.1 OIE framework for risk analysis

The OIE framework is based on a model developed by Covello and Merkhover (Covello and Merkhofer, 1993). Using this model, the process of risk analysis can be subdivided into the areas of hazard identification, risk assessment, risk management and risk communication (OIE, 2007). Within this framework, the area of risk assessment (less formally defined as the determination of how likely it is for a hazard to occur), is further

separated into the areas of release assessment, exposure assessment, consequence assessment and risk estimation. The interaction between the four main areas of risk analysis as put forward by OIE is illustrated in Figure 1.1.

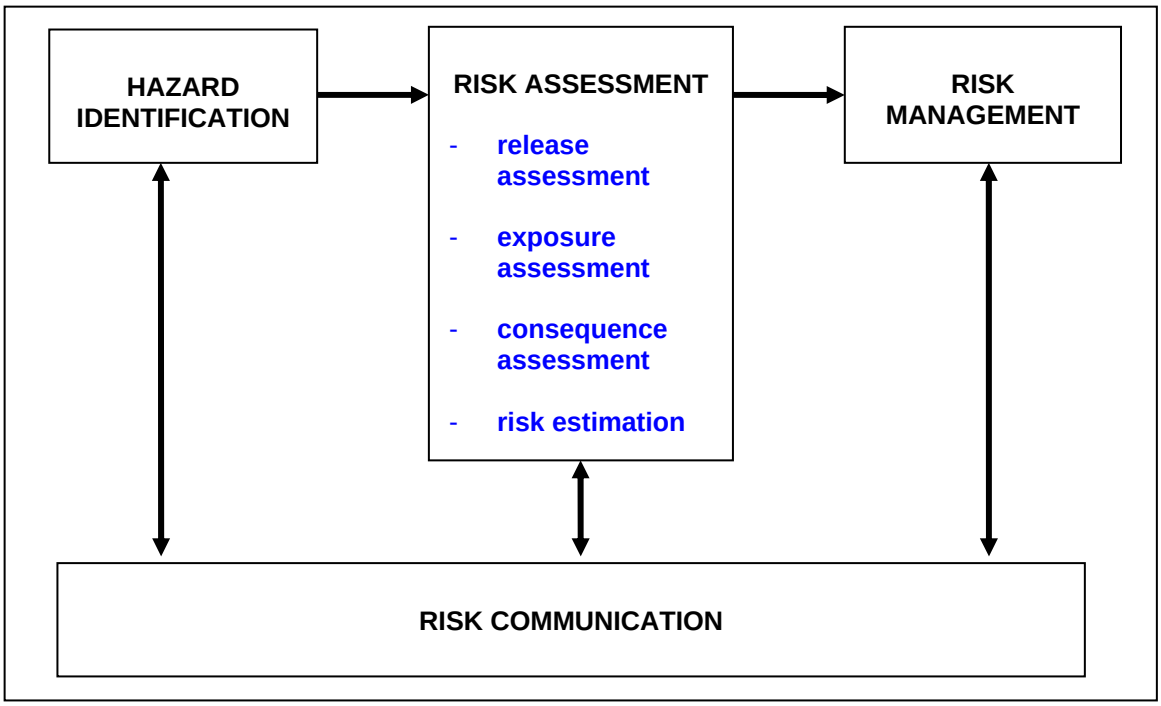

Figure 1.1. The four components of risk analysis as defined by OIE (OIE, 2006).

More specifically, within the OIE framework, the step of hazard identification refers to the identification of one or many hazards (pathogenic agents that could produce adverse consequences) associated with the 'risky' behaviour that is modelled (e.g. importation of a commodity) (OIE, 2006). The most important, and intellectually intensive, component of the risk analysis process presented here is risk assessment, which consists of four inter-related steps that may be assessed either qualitatively or quantitatively. An initial **release assessment** is undertaken to determine the likelihood of an imported commodity being infected or contaminated with a hazard, along with a description of the potential routes of release. The **exposure assessment** describes the pathways necessary for exposure of animals or humans to the hazard(s) identified and estimation of the likelihood of the exposure(s). The **consequence assessment** addresses the consequence of the exposure(s) to the hazard(s) and **risk estimation** refers to the integration of the results from the previous three steps to result in summary measures of the risks associated with the identified hazard(s) (OIE, 2006).

The areas of risk management and risk communication are separate to the risk assessment process. Risk management is ideally undertaken by a dedicated risk manager (who has not been involved with the risk assessment) and involves identification, selection and implementation of measures for risk reduction (National Research Council, 1983; OIE, 2008b), while risk communication refers to the interactive exchange of information on the specified risk among risk assessors, risk managers and other interested parties such as stakeholders (OIE, 2008b).

The OIE framework offers a flexible approach to risk assessment that may be applied to a range of risk questions, not simply restricted to IRA (OIE, 2006).

1.2.1.2 CAC framework for risk analysis

The CAC guidelines for risk analysis follow a related, yet distinct framework to those published by OIE (Figure 1.2) and uses terminology defined by the National Academy of Sciences-National Research Council (NAS-NRC) (National Research Council, 1983).

While the areas of risk management and risk communication can be defined as for the OIE framework (section 1.4.1), the risk assessment component in the CAC framework consists of different steps and definitions. Within the risk assessment area of this framework, the step of **hazard identification** refers to the identification of (known or potential) health effects associated with a particular agent or pathogen. **Hazard characterisation** is the qualitative or quantitative evaluation of the nature of the adverse effects associated with the agents that may be present in food and the **exposure assessment** refers to the qualitative or quantitative evaluation of the degree of intake likely to occur. **Risk characterisation** refers to the integration of the previous three steps to obtain an estimation of the adverse effects that are likely to occur in a given population, including attendant uncertainties (WHO, 2008).

The CAC framework is used extensively in microbial risk assessment to define the maximum amount of hazard advisable for human exposure in food and, while this system is important for use in toxicology and food safety scenarios, it is somewhat less flexible that the OIE approach (OIE, 2006).

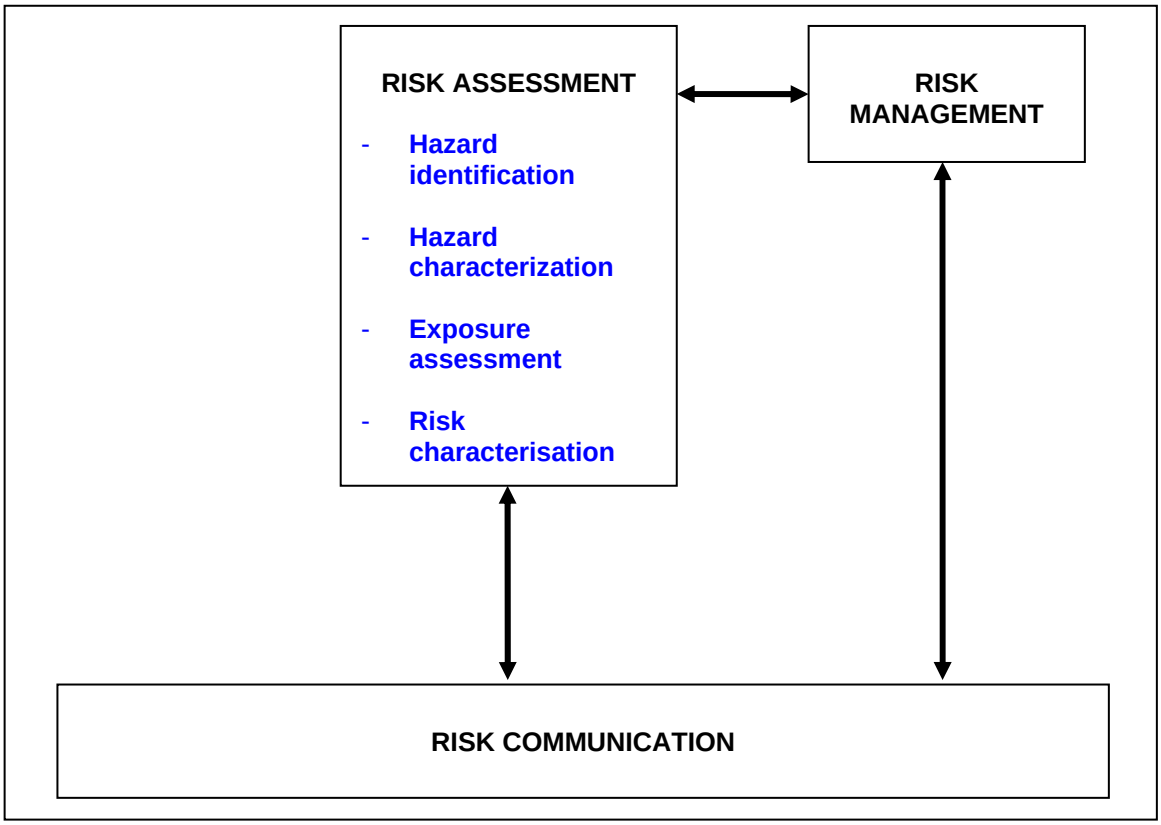

Figure1.2. Relationship between the three components of risk analysis specified by WHO (WHO, 2008).

1.2.2 Risk assessment

The frameworks presented in sections 1.2.1 describe the conceptual nature of the risk assessment process, but the mechanistic process of a risk assessment requires further description. Risk assessments may be undertaken using qualitative, semi-quantitative or quantitative estimates of risk. All techniques are based on an initial conceptual model, whereby the potential pathways that are required for the risk to occur are described, although the process of risk estimation at each step varies with the type of assessment used.

1.2.2.1 Qualitative risk assessment

A qualitative risk assessment is defined as "A risk assessment based on data which, while forming an inadequate basis for numerical risk estimations, nonetheless, when conditioned by prior expert knowledge and identification of attendant uncertainties permits risk ranking or separation into descriptive categories of risk." (CAC, 1999). A qualitative risk

assessment is commonly used as the first step of an overall risk assessment process and has been described as a building block for quantitative assessment (Clough *et al.*, 2006). This technique may also stand alone and is used as the basis of many stakeholder decisions in emerging or data sparse areas and where a rapid analysis or decision-making is required. Although few qualitative risk assessments have been published in the veterinary literature (Moutou *et al.*, 2001; Peeler *et al.*, 2004; Coburn *et al.*, 2005; Clough *et al.*, 2006; Hauser *et al.*, 2007), they are widely utilised by policy makers and examples of those used may be accessed readily on government biosecurity and other websites: http://www.daff.gov.au/ba/ira/final-animal, http://www.scotland.gov.uk/Topics/Agriculture/animal-welfare/Diseases/SpecificDisease/bluetongue/bluetonguevetriskassess, http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_home.htm.

Estimates generated by qualitative assessments are in the form of pre-specified user-defined categories, usually described by terms such as 'low', 'medium' and 'high' (OIE, 2006). The qualitative risk assessments that have been published in the veterinary literature are divided into the areas of food safety (Coburn *et al.*, 2005; Clough *et al.*, 2006; Hauser *et al.*, 2007) and import risk assessment (Moutou *et al.*, 2001; Peeler *et al.*, 2004). The more defensible of these assessments specify conceptual pathways, and follow a matrix approach for the combination of categorical estimates resulting pre-defined outcomes, based on a qualitative interpretation of probabilistic combinations (Moutou *et al.*, 2001; Clough *et al.*, 2006). Combination of risk estimates may also be undertaken using broad subjective conceptual considerations (Bernard and Anderson, 2006; Peeler *et al.*, 2006). Recently, criticism of the use of risk matrices has surfaced, which expounds the possibility of misleading overall risk estimates, lack of fine resolution of input variables and ambiguous input and output measures (Cox *et al.*, 2005; Cox, 2008).

1.2.2.2 Quantitative risk assessment

Quantitative risk assessment (QRA) provides "numerical expressions of risk and indication of the attendant uncertainties" (Codex Alimentarius Commission, 1999). In its most simple form, QRA may be deterministic, consisting of mathematical combinations of point-estimates to result in a single number output (OIE, 2006). However, the probabilistic form of QRA is preferred, in which inputs are in the form of probability distributions and the output represents a distribution of random combinations of samples from each of the

attendant inputs (Vose, 2000; OIE, 2006). Thus, while the use of qualitative risk assessment is often described as the precursor to a quantitative analysis, the benefit of a quantitative assessment is not only to re-define qualitative measures with numerical estimates, but also, in its probabilistic form, to enable consideration of attendant input variability and uncertainties and to quantify overall output uncertainty (OIE, 2006).

The overall quantitative estimation of the probability and magnitude of risk is usually undertaken with the use of mathematical probability theory, and a robust QRA effectively represents a network of inter-related probability distributions (Vose, 2000). Each probability distribution represents the variability and/or uncertainty of the modelled variable, both of which refer to the unpredictability of a system but represent separate and distinct entities, the understanding and modelling of which are central to the accurate implementation of QRA methods. Variability is specific to the system that is modelled and refers to the effect of chance (Vose, 2000). This measure represents the true heterogeneity of the population that is a consequence of the physical system and is not reducible through further study or measurement (Murphy, 1998; Andserson and Hattis, 1999; Vose, 2000; Colyvan, 2008). Conversely, uncertainty refers to the lack of knowledge about a parameter value that can be reduced by further study or measurement (Murphy, 1998; Anderson and Hattis, 1999; Vose, 2000). Furthermore, uncertainty may be categorised as either 'epistemic', which refers to uncertainty about a determinate fact, or 'linguistic' which arises out of language-based vagueness or ambiguity (Colyvan, 2008).

The combination of variability and uncertainty is often referred to as 'overall' or 'total' uncertainty (Vose, 2000), and care must be taken to differentiate this from the previously defined measure of uncertainty. A QRA model may be classified as either first- or second-order, which refers to the inclusion of total uncertainty as a composite measure, or the separation and independent modelling of uncertainty and variability within the risk model (Vose, 2000; Cummins *et al.*, 2008). The distributional output of a quantitative risk assessment can be termed an 'uncertainty analysis' and this refers to the quantification of the overall uncertainty associated with the output of a model as a result of uncertainties (and variability) in the model input (Saltelli, 2000).

Parameterisation of QRA models refers to the population of each step of the model with representative estimates that are to be combined probabilistically. These estimates may be made using previously published data, unpublished data, or expert opinion. In probabilistic QRA modelling, parameter estimates are distributional in nature, and the data obtained,

through any of the aforementioned means, may be used to construct one of numerous discrete or continuous probability distributions. The combination of these distributions to obtain an output risk estimate is undertaken using iterative sampling and combination from each input distribution until output convergence is reached and is referred to as Monte Carlo simulation or analysis (Campolongo *et al.*, 2000b; Vose, 2000)

Sampling from each input distribution may follow Monte Carlo or Latin Hypercube sampling methods (Vose, 2000). Monte Carlo sampling refers to random, or pseudo-random, sampling, whereby a random sample, x, is selected from the input distributions by the substitution of a computer-generated random number, r, from a Uniform(0,1) distribution into the inverse function G(F(x)) of the cumulative distribution function F(x), such that G(r) = x (Vose, 2000). While Monte Carlo sampling represents a truly random technique, this method requires a large number of iterations to ensure that the model will reproduce the desired distribution and may under- or over-sample components of the distribution (Vose, 2000). In contrast, Latin Hypercube sampling provides a stratified sampling technique (without replacement) that requires fewer iterations to enable reproduction of the desired distribution. The method of Latin hypercube sampling involves dividing the range of each input factor into N intervals of equal marginal probability (1/N). Random selection of i) interval and ii) observation within that interval, F(x), is then undertaken and the random sample, x, is computed by x = G(F(x)), for each of N iterations (Campolongo *et al.*, 2000b; Vose, 2000). Both sampling methods are adequate, although the use of Latin hypercube sampling results in greater reliability of sampling of the entire parameter space using fewer iterations (Meinrath *et al.*, 2000; Vose, 2000).

Quantitative risk assessment methods have been used extensively in the fields of import risk assessment (de Vos *et al.*, 2004; Jones *et al.*, 2005; Peeler *et al.*, 2006; Martinez-Lopez *et al.*, 2008) and food safety in the form of microbial risk assessment models (Snary *et al.*, 2004; Bartholomew *et al.*, 2005; Parsons *et al.*, 2005; Vosough Ahmadi *et al.*, 2006; Stacey *et al.*, 2007; Cummins *et al.*, 2008). The methods of QRA are not prescriptive and the mathematical relationships between input variables will vary depending on the system studied. Historically these mathematical relationships have been deterministic, although modern risk assessments rely to a greater degree on simulation modelling, allowing consideration of uncertainty and variability as described above (Cassin *et al.*, 1998). Within the stochastic probabilistic risk assessment process, uncertainty modelling relies on the specification of uncertain input distributions through consideration of the probability distribution most accurately able to represent the uncertainty and variability that requires

modelling (Vose, 2000) and combination of these specified distributions is based on the laws of probability theory, as derived by Cox (Cox, 1946a; Cox, 1946b; Jaynes, 2003). Scenario tree modelling is often utilised in import risk assessment (Nauta, 2002; MacDiarmid and Pharo, 2003; Jones *et al.*, 2005; Peeler *et al.*, 2006; Cummins *et al.*, 2008), whereas modular process modelling with multiplicative combinations or more complex mathematical relationships are more likely to be utilised in microbial risk assessment models (Cassin *et al.*, 1998; Stacey *et al.*, 2007).

1.2.2.3 Sensitivity analysis

Sensitivity analysis may be defined as the evaluation of a model's response to change in inputs (Law and Kelton, 2000; Saltelli, 2000; Ascough *et al.*, 2005). The process of sensitivity analysis is referred to as one of the key steps in construction and utilisation of risk analysis models (Borgonovo, 2006) and is considered imperative to the overall process of risk assessment (Saltelli, 2002). The rationale for undertaking a sensitivity analysis will vary depending on the model to which it is applied, but includes: verification, validation and refinement of the model as it is constructed, evaluation of the robustness of model results for decision-makers, and identification of important uncertainties within model inputs for prioritisation of future data collection and research (Ascough *et al.*, 2005).

One of the classifications of sensitivity analyses defines the methods as either 'screening', 'local' or 'global' (Saltelli, 2000) and allows a structured description of the nature of the analysis that is undertaken and the extent of the inference that may be made from the results obtained. Screening analyses are generally undertaken as an initial step to identify those variables that control most of the output variability in models with large numbers of input parameters (Saltelli, 2000). Local, or nominal range, analyses refer to methods that take into account relatively small perturbations to input variables, are predominantly used for deterministic models and assess the effect of variation of a single model input whilst all other inputs are held constant at their central (nominal) value (Frey and Patil, 2002). In contrast, global sensitivity analyses explore input factors over their full range and shape and also take into account the effects of all other input parameters on the factor being assessed (termed multidimensional averaging) (Saltelli *et al.*, 1999; Saltelli, 2000). Global analyses allow the assessment of interactions and may be extended to include primary and

secondary interaction terms. Furthermore, many global analysis techniques do not require models to satisfy assumptions of linearity or monotonicity (Ascough *et al.*, 2005).

The use of one-at-a-time (OAT) sensitivity analysis refers to the variation of only one input variable at a time, to an upper and lower value in turn, whilst all others are set to a nominal value. While this local method is easily applicable, it requires that the input/output (I/O) function of the model is monotonic, that factors act additively over the range of interest and importantly, does not account for any interaction between inputs, which may not accurately reflect the underlying model (Campolongo *et al.*, 2000a; Vonk Noordegraaf *et al.*, 2003). In order to represent all of the interactions that may be present between input variables (factors) and provide a global analysis, extension of the OAT design to a design whereby each factor is varied with respect to the other is required. However, the number of scenarios required to compute this 'complete factorial' design is n^k, where k represents the number of factors assessed and n represents the number of levels each factor is assessed at (usually set at 2 for upper and lower values). The consideration of this number of scenarios rapidly leads to excessive, and often impossible, requirement of computing time as the number of included factors increases (Campolongo and Saltelli, 2000; Beres and Hawkins, 2001). Implementation of 'fractional factorial' designs are based on the concept that full factorial designs specify numerous 'unimportant' higher order interaction terms that are not detectably different to noise (Campolongo and Saltelli, 2000). The assumption that some of the higher order terms are unimportant allows specification of 2^{k-p} fractional factorial designs, whereby a fraction ($1/2^p$) of the possible 2^k combinations are actually run to result in estimates of main effects and specified interactions (Henderson-Sellers and Henderson-Sellers, 1996; Law and Kelton, 2000). In the specification of fractional factorial designs, confounding occurs between main effects and interaction terms and thus, it is important that the design is specified to avoid confounding between two potentially important factors (Law and Kelton, 2000; Vonk Noordegraaf *et al.*, 2003). The application of a 'foldover', whereby the design matrix is reflected upon itself increases the resolution of the design, resulting in reduced confounding between effects and the addition of orthogonality also increases the accuracy of the estimators (Kleijnen, 1987; Law and Kelton, 2000; Vonk Noordegraaf *et al.*, 2003).

The Plackett-Burman (P-B) approach represents a fractional factorial design with further reduced computational requirement, using only k+1 runs to investigate the effects of k factors, providing k+1 is divisible by 4 (Plackett and Burman, 1946; Law and Kelton, 2000). This design is defined as 'context-free' and the type or nature of model that it is

applied to is not prescribed (Plackett and Burman, 1946; Beres and Hawkins, 2001). The addition of a foldover to the P-B design increases the 'resolution' of the design and thus yields main effect estimators not biased by two-factor interactions, although these interactions are confounded with each other (Kleijnen, 1987). The output of factorial designs may be analysed by consideration of main effects and interactions through specified algorithms that estimate the average change in response due to a change in the specified factor, or interaction between factors (Law and Kelton, 2000). Another, more sophisticated option is to fit a metamodel to the I/O relationships defined by the factorial design to enable an overall approximation of the I/O behaviour of the model (Kleijnen and Sargent, 2000; Vonk Noordegraaf *et al.*, 2003; de Vos *et al.*, 2006). Metamodels are often in the form of regression models and linear, tobit and logistic regression (LR) models have been fitted for this purpose within the veterinary literature (Kleijnen and Sargent, 2000; Vonk Noordegraaf *et al.*, 2003; de Vos *et al.*, 2006). While regression analysis has been described as a primary probabilistic technique for sensitivity analysis, its application in this form is sparse (McCarthy *et al.*, 1995).

Numerous alternative techniques are also described for sensitivity analysis, all utilised to enable the estimation of the effect of variation of input variables on the output of the model. These methods include, but are not exclusive to, response surface methods, Fourier Amplitude Sensitivity Test (FAST), extended FAST and the technique of Sobol' (Chan *et al.*, 2000; Frey and Patil, 2002). To the author's knowledge these techniques are yet to be applied to veterinary-based studies.

1.3 Zoonoses and zoonotic disease

Zoonoses can be defined as 'diseases and infections which are naturally transmitted between vertebrate animals and man' (WHO, 1959). Diseases that may be classified as zoonoses are wide-ranging, include those caused by bacterial, viral and protozoal pathogens and may be transmitted by direct contact (including wounds, inhalation and iatrogenic transmission), indirect contact (food, waterborne and environmental), or by vectors such as arthropods (Woolhouse, 2002; Hubalek, 2003). Emerging and re-emerging zoonoses refer to a subset of zoonotic diseases that have an increasing incidence following introduction to a new population, or within an existing population as a result of long-term change in the underlying epidemiology (Woolhouse, 2002).

Zoonotic disease was first described in the 19th Century by Virchow, a physician who also promoted the idea of 'one medicine', referring to the linkage of human and veterinary medicine as a comparative discipline (Brown, 2004; Stewart *et al.*, 2005). While the last century witnessed a dissolution of close interaction between medics and veterinarians (Stewart *et al.*, 2005), recently and subsequent to several instances of high-profile emerging and re-emerging zoonotic diseases, calls have been made to reestablish closer linkage between disciplines, with the aims of enhancing the understanding of zoonotic disease and improvement of prevention and control strategies (Schelling *et al.*, 2005; Stewart *et al.*, 2005; Zinsstag *et al.*, 2005; Kahn, 2006; Hueston *et al.*, 2007).

Global infectious disease emergence is dominated by zoonoses, which account for 60.3% of emerging infectious disease (Jones *et al.*, 2008) and 58% of human infectious pathogens (Woolhouse and Gowtage-Sequeria, 2005). The ability of a pathogen to infect multiple hosts has also been identified as a risk factor for emergence in human and livestock pathogens (Cleaveland *et al.*, 2001). As such, zoonoses are considered a priority area for research and veterinary public health.

1.3.1 Zoonoses in companion animals (in particular pet dogs)

Zoonotic disease is commonly associated with farm animal species and developing countries, and the contribution of companion species to the disease burden in humans is often overlooked or downplayed. This is particularly noted in the developed world, where a combination of factors including increased pet ownership, close living contact between pets and owners and an increasing prevalence of immunocompromised individuals combine to result in a valid threat of companion animal zoonotic disease (Robinson and Pugh, 2002; Poglayen, 2006).

Much has been documented about the potential effects of pet ownership on human health (McNicholas *et al.*, 2005; Knight and Edwards, 2008). Positive effects of pet ownership were first noted by Friedmann and colleagues (Friedmann *et al.*, 1978; Friedmann *et al.*, 1980), who reported increased one-year survival after myocardial infarction in pet owners. These findings were replicated by the same group some 15 years later (Friedmann and Thomas, 1995) and many other benefits of human-companion animal interactions have also

been reported, including blood pressure moderation (Allen, 2003; Virues-Ortega and Buela-Casal, 2006), protection against allergy (Chen *et al.*, 2008) and enhanced physical and psychological health in the elderly (Knight and Edwards, 2008). However, studies undertaken in Australia have disputed the beneficial effect of pet ownership in terms of cardiac, psychological and physical health (Parslow and Jorm, 2003; Parslow *et al.*, 2005). While it is possible that these contradictions reflect a geographical and cultural difference in the role of companion animals between countries, other negative implications of pet ownership have also been reported to include dog or cat bites and their associated infection (Morgan and Palmer, 2007), exposure to allergens and the potential transmission of viral, parasitic, fungal or bacterial zoonotic diseases (Robinson and Pugh, 2002).

Many different zoonotic pathogens have been isolated from dogs and are known to transfer directly between dogs and humans. The most prevalent viral zoonosis that may be transmitted between dogs and humans is rabies, a *Lyssavirus* that, while exotic to the UK, is endemic in other areas of Europe, the Middle East and Africa (Wandeler and Bingham, 2000). Parasitic zoonoses of importance include nematodes such as *Toxocara* (roundworms), *Ancylostoma* (hookworms) and cestodes (tapeworms) such as *Dipylidium* and *Echinococcus*, while important fungal pathogens include dermatophytes, malassezia and aspergillosis (Beran and Steele, 1994; Morris *et al.*, 2005). Bacterial zoonoses number many and are covered in the next section. A non-comprehensive list of the most commonly considered zoonoses that are potentially transmitted by dogs is presented in Table1.2.

1.3.2 Bacterial zoonoses in companion animals (in particular pet dogs)

Many different bacteria may be carried by companion animals, either as commensals or disease-causing organisms, including *Bartonella*, *Pasteurella*, *Campylobacter*, *Staphylococcus*, *Streptococcus* and *Salmonella* species (Chomel and Arzt, 2000). The likelihood of zoonotic transmission of bacterial organisms from animals to humans depends on numerous factors including the number of infected animals, mode of transmission of the agent, behavioral characteristics of the human and existing measures of prevention (Chomel and Arzt, 2000; Robinson and Pugh, 2002; Murphy, 2008). Literature that documents the transfer of bacterial pathogens between companion animals and humans

(excluding dog and cat bite pathogens) is sparse. It has been shown that ownership of puppies and contact with diahorreic animals are risk factors for *Campylobacter spp.* enteritis in children and adults respectively (Saeed *et al.*, 1993; Tenkate and Stafford, 2001), that *Staphylococcus intermedius* may transfer directly to humans from dogs and that human infection with meticillin-resistant MRSA may be obtained from horses (Guardabassi *et al.*, 2004; Weese *et al.*, 2006a). Additionally, while cause and effect cannot be directly established, indistinguishable isolates of MRSA have also been identified in humans and domestic pets (Cefai *et al.*, 1994; Manian, 2003; van Duijkeren *et al.*, 2005; Weese *et al.*, 2006b) and in some cases, infection or colonisation of owners has been unable to be cleared until the pet has also been decontaminated (Cefai *et al.*, 1994; Manian, 2003).

Although transmission of bacterial zoonotic disease from companion animals to humans is known to occur and is cited as a potential negative effect of animal ownership, no randomised controlled trials exist in this area (Hemsworth and Pizer, 2006).

1.4 *Staphylococcus aureus* and MRSA

Staphylococcus describes a genus of bacteria that are identifiable as facultatively anaerobic, catalase positive Gram positive cocci, measure around 1μm in diameter and divide in more than one plane to form grape-like clusters (Wilkinson, 1997; Humphreys, 2002). Staphylococci grow readily on blood agar, nutrient soy agar and brain heart infusion agar as white colonies that are 1-3mm in diameter (Kloos and Lambe, 1991; McCandlish and Taylor, 1998). More than 30 species of staphylococci have been described, of which *S. aureus* represents the most common pathogen (Lindsay, 2008a). *Staphylococcus aureus* exhibits diagnostically distinctive features of the production of an extracellular enzyme, coagulase, thermostable nucleases and the surface-associated protein known as clumping factor (Humphreys, 2002). Although *S. aureus* are found as commensal organisms in approximately 30-50% of healthy humans (Lowy, 1998), infection with these organisms may result in a number of diseases such as pneumonia, skin and soft-tissue infections, bacteraemia, sepsis and toxin-medicated disease such as toxic-shock syndrome (Humphreys, 2002; Lim and Webb, 2005). *Staphylococcus aureus* are the leading cause of nosocomial human infections, and bacteraemia resulting from infection

Table 1.1 Zoonoses potentially transmitted by dogs (adapted from Hemsworth and Pizer (2006)).

Disease	Organism	Category	Transmission	Signs and Symptoms
Ascaridiasis (Roundworm infection)	*Toxocara canis* *Toxascaris leonina*	Parasite	Ingestion of infective eggs in the environment	Dependent on organ damaged during larval migration – visual, neurological or tissue damage.
Campylobacteriosis	*Campylobacter* spp.	Bacteria	Eating or drinking contaminated food or water, unpasteurised milk and direct or indirect contact with faecal material from an infected person or animal	Mild to sever infection of the gastrointestinal. system, watery or bloody diarrhoea, fever, abdominal cramps, nausea and vomiting. A rare complication is Guillain-Barre syndrome.
Cryptosporidiosis	*Cryptosporidium parvum*	Parasite	Faecal-oral route, water	Watery diarrhoea, accompanied by abdominal cramps. Nausea, vomiting, fever, headache and loss of appetite may occur. Rarely, gall bladder inflammation or pneumonia may occur.
Dermatophytosis	*Microsporum canis* *Trichophyton mentagrophytes*	Mycotic	Direct or indirect contact with asymptomatic animals or with skin lesions of infected animals, contaminated bedding	Often mild, self limiting; scaling, redness and occasionally vesicles or fissures.
Giardiasis	*Giardia intestinalis (Giardia lamblia)*	Parasite	Ingestion of contaminated water or food, faecal-oral route	Diarrhoea, fever, severe abdominal cramps.
Hookworm	*Ancylostoma caninum* *Ancylostoma braziliense* *Ancylostoma tubaeform* *Uncinaria stenocephala*	Parasite	Ingestion of infective eggs or contact with contaminated soil	Pruritic skin lesions. Intestinal bleeding and pain.
Rabies	*Lyssavirus*	Virus	Bite wound	Acute encephalitis and death.
Salmonellosis	*Salmonella* spp.	Bacteria	Ingesion of foods contaminated with animal faeces, faecal-oral route	Acute gastroenteritis with sudden onset of abdominal pain, diarrhoea, nausea and fever. May lead to scepticaemia.
Tapeworm	*Dipylidium caninum*	Parasite	Ingestion of infected flea	Proglottids are passed in faeces or are found around anus causing itching.
	Echinococcus granulosus	Parasite	Faecal-oral route	Hydatid cysts on viscera.

with fully susceptible strains of *S. aureus* is associated with a 20-40% mortality rate (Mylotte *et al.*, 1987).

Meticillin-resistant *S. aureus* refers to strains of *S. aureus* that exhibit resistance to penicillins and other β-lactam antibiotics, with or without resistance to other antibiotic classes (Enright, 2008). While *S. aureus* originally exhibited susceptibility to penicillin, resistance rapidly developed through the acquisition and spread of β-lactamase-producing plasmids (Shopsin and Kreiswirth, 2001). Meticillin (originally given the trade name of celbenin), is a synthetic penicillin introduced to overcome this resistance through an ability to be only slowly hydrolysed by staphylococcal β-lactamase (Dyke and Gregory, 1997). However, meticillin-resistant strains of *S. aureus* (through mechanisms discussed below) were isolated within a year of its production (Barber, 1961) and have been globally widespread in human hospitals since the 1980s (Walsh, 2003). Although meticillin has long since been superseded by alternative agents (in particular flucloxacillin in the UK), the acronym MRSA continues to be used (Johnson *et al.*, 2005). Meticillin-resistant *S. aureus* are increasingly recognised as an emerging zoonotic pathogen of considerable importance to public health.

1.4.1 Antimicrobial resistance specific to MRSA

The first antibiotic, penicillin, was discovered as a 'chance observation' (Fleming, 1945) by Scottish physician and bacteriologist Sir Alexander Fleming in 1928. Concentration of this antibiotic into an active form by Sir Howard Florey and Dr Ernst Chain in 1940, and subsequent mass-production of penicillin and other antibiotics for use during the Second World War, heralded the onset of what was thought to be the golden age of treatment of bacterial infectious disease (Cohen, 2000). However, the potential for development of resistance to antimicrobial agents was recognised shortly after the discovery of penicillin (Cohen, 2000). Indeed, the possibility for negligent use of penicillin to result in development of microbial resistance was highlighted by Fleming himself in his Nobel Prize acceptance speech (Fleming, 1945).

Since these early days of antimicrobial development, the number of available antibiotics has increased dramatically, as has the acquisition of resistance to antibiotics in previously susceptible organisms (Neu, 1992). The introduction of these newer drugs constitutes part

of a vicious cycle whereby the continued development of resistance to new antimicrobials requires the continued development of more efficacious antibiotic agents (Cohen, 1992). The inevitable slowing of this cycle, with development of multi-resistant strains of bacteria and reduction of pharmaceutical company interest in antibiotic development, has been acknowledged as an impending crisis since the early 1990s (Soulsby, 2005). Substantial public health implications arise from the possibility of a 'postantimicrobial era' (Cohen, 1992) and calls for the development of alternatives to antimicrobials (Soulsby, 2005), the use of stringent infection control within healthcare settings and stricter regulation of the use of antibiotics in both humans and animals are now widespread (Weber and Courvalin, 2005).

1.4.1.1 Mechanisms of antimicrobial resistance

Antimicrobial resistance may either be intrinsic (occurring without genetic alteration) or acquired (Normark and Normark, 2002; Walsh, 2003). Acquired resistance occurs when susceptible bacteria gain resistance genes either through mutations within the bacterial genome and subsequent vertical transfer or, more commonly, through horizontal transfer, involving the transport of new genetic material into the cell through transformation (uptake of naked DNA), transduction (viral transfer of DNA) or conjugation (plasmid mediated and requiring cell-to-cell contact) (Neu, 1992; Normark and Normark, 2002). The main mechanism of acquisition of antimicrobial resistance appears to be through conjugation, whereby mobile genetic elements such as plasmids and transposons act to transfer resistance between bacteria that are not necessarily of the same species (Walsh, 2003; Lim and Webb, 2005).

The presence of resistance genes within bacteria confers a selective survival advantage over sensitive organisms in the face of antimicrobial therapy, and the use of antibiotics in a mixed bacterial population will provide increased selection pressure for resistance (Walsh, 2003). The four mechanisms of resistance identified so far are drug inactivation, target modification, reduced permeability and drug efflux pumps (Lim and Webb, 2005). Selection for resistance may occur slowly, through the continued environmental presence of an antibiotic, or more quickly, through direct antimicrobial prescribing (Walsh, 2003). Although not the only determinant, the development and spread of resistance has been shown to be driven primarily by antimicrobial prescribing (Livermore, 2005).

1.4.1.2 Beta-lactam drugs and resistance

Beta-lactam antimicrobials include penicillins, cephalosporins, carbapenems and monobactams and are defined by the presence of a structural β-lactam ring. These antibiotics function by binding to the bacterial cell wall through native penicillin-binding proteins (PBPs), causing disruption to the peptidoglycan layer of the cell wall and resulting in bacterial lysis (Deurenberg and Stobberingh, 2008). β-lactamase, an enzyme that may be produced by certain bacteria, functions to hydrolyse the β-lactam ring (Figure 1.3), converting the antimicrobial containing this ring into an innocuous form (Dyke and Gregory, 1997).

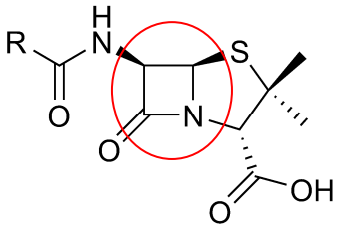

Figure1.3. Penicillin nucleus – circle demarcates β-lactam ring.

1.4.1.3 Molecular resistance mechanisms for MRSA

Meticillin resistance occurs due to the presence of an altered penicillin-binding protein 2a (also denoted as PBP2α and PBP2') which renders the cell wall insensitive to β-lactam antibiotics (Wilke *et al.*, 2005). This PBP is encoded for by the *mec*A gene, which resides on a large mobile genetic element called the staphylococcal chromosomal cassette *mec* (SCC*mec*). At the time of writing, seven different types of SCC*mec* had been defined, with this number rapidly increasing in recent years (SCC*mec* I-VII) (Ito *et al.*, 2001; Ma *et al.*, 2002; Ito *et al.*, 2004; Oliveira *et al.*, 2006; Takano *et al.*, 2008). These genetic elements differ in size and genetic composition, with SCC*mec* types II and III able to confer resistance to multiple classes of antibiotics in addition to β-lactams due to the presence of integrated plasmids and transposons (Deurenberg and Stobberingh, 2008). Additional (non SCC) mobile genetic elements that encode virulence genes and resistance to other antibiotics may also be found within the MRSA genome (Lindsay, 2008b).

1.4.1.4 Evolutionary considerations for MRSA

"Evolution requires genetic change and then selection of the fittest" (Lindsay, 2008b). Genetic change in *S. aureus* may occur by mutation or acquisition of mobile elements.

Two evolutionary theories have been presented for MRSA (Deurenberg and Stobberingh, 2008) and, while it has been postulated that all MRSA clones evolved from a common meticillin-sensitive *S. aureus* (MSSA) ancestor that acquired SCC*mec* on one occasion (Kreiswirth *et al.*, 1993), it is now commonly acknowledged that SCC*mec* was introduced on a number of occasions into different *S. aureus* lineages (Musser and Kapur, 1992). The latter, more common, theory is supported by evolutionary studies using multilocus sequence typing (MLST) combined with SCC*mec* typing and based upon repeat sequence types (BURST) analysis (Enright *et al.*, 2002; Feil *et al.*, 2004). These studies conclude that MRSA has evolved in relatively few independently stable lineages, otherwise known as clonal complexes (CCs), that individual MRSA clones have arisen on multiple occasions from the introduction of SCC*mec* elements into successful MSSA clones and that horizontal transfer of *mec* genes is relatively frequent within *S. aureus* (Enright *et al.*, 2002; Lindsay, 2008b). The implications from these findings are that the *mec* genes are likely to have been introduced into already successful *S. aureus* clones within hospitals that were already well adapted to transmission, resulting a marked potential for adaptability in the face of new antimicrobial treatments (Enright *et al.*, 2002). It is also known that the rate of horizontal transfer of SCC elements between staphylococcal strains is low, although the mechanism of this transfer is yet to be identified (Lindsay, 2008b).

1.4.2 MRSA in humans

Staphylococcus aureus is found as a commensal organism that primarily resides and multiplies in the anterior nares (Williams, 1963) but may also be isolated from areas including the throat, axillae, perineum (Ridley, 1959) and groin and is often shed onto healthy skin (Williams, 1963; Wertheim *et al.*, 2005) (Figure 1.4). Carriage of *S. aureus* is known to occur in 30-50% of healthy humans (Lowy, 1998) and carrier status is associated with a three-fold increase in the risk of clinical infection (Wertheim *et al.*, 2004). A proportion of these *S. aureus* strains are meticillin resistant, resulting in estimates of <1-3% of healthy humans being colonised with MRSA at any given time (Abudu *et al.*, 2001;

Jernigan *et al.*, 2003a; Jernigan *et al.*, 2003b; Kuehnert *et al.*, 2006). Benchmark studies have identified human carriage of any strain of *S. aureus* as either persistent (referring to 10-35% of individuals who predominantly harbour one strain of *S. aureus*), or intermittent (referring to 20-75% of individuals who intermittently carry any strain of *S. aureus*) and between 5 and 50% of the population are referred to as noncarriers as they almost never carry *S. aureus* (Williams, 1963). Persistent carriers are associated with a higher bacterial load of *S. aureus*, resulting in increased dispersal of the organism (Figure 1.4) and association with a higher risk of acquiring bacterial infection (Wertheim *et al.*, 2005).

The clinical importance of MRSA is well established in human medicine. As a hospital acquired pathogen, MRSA may be responsible for outbreaks of infection in health-care settings, but may also be endemic in these institutions. Although the spectrum of disease resulting from MRSA is similar to MSSA, ranging from soft tissue infections to fulminant pneumonia, infection with the resistant organism is associated with a poorer outcome and increased mortality in hospital settings, along with increased consumption of healthcare resources (Whitby *et al.*, 2001; Cosgrove *et al.*, 2003; Kollef and Micek, 2006).

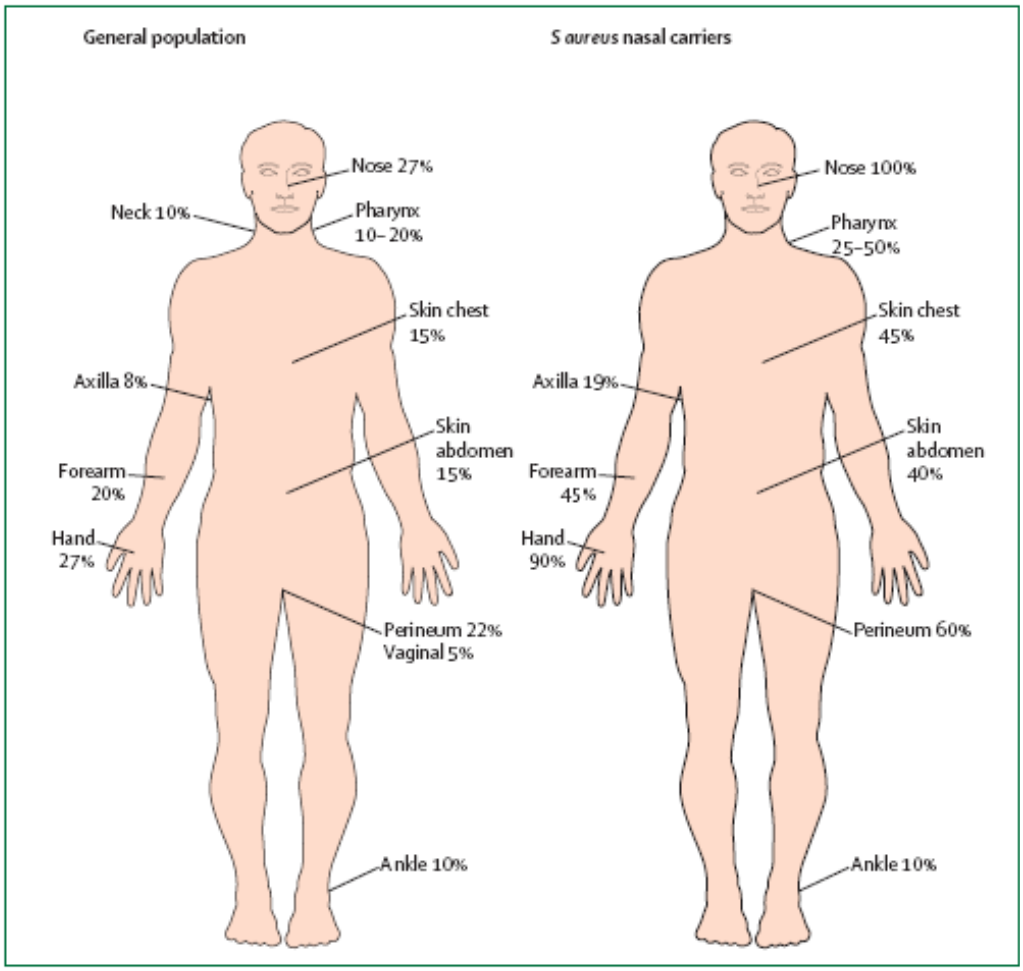

Figure 1.4. *Staphylococcus aureus* carriage rates per body site for the general population and those with persistent nasal carriage of *S. aureus* (after Wertheim *et al.*, 2005).

The prevalence of MRSA within human hospitals is officially assessed by the percentage of blood culture isolates of *S. aureus* that are resistant to meticillin (Johnson *et al.*, 2005). Within the UK, a combination of voluntary reporting dating since 1989, mandatory reporting since 2001, data collection from sentinel laboratories and other surveillance studies, has provided robust information regarding the prevalence of MRSA bacteraemia (Johnson *et al.*, 2005). These data show that an increase in prevalence of MRSA bacteraemia (as a proportion of *S. aureus* bacteraemia) has occurred over recent years, with yearly rates increasing from 2% in 1990/91 to around 40% since 2000 (Johnson *et al.*, 2005; Enright, 2006) (Figure 1.5, 1.6). More recently, the prevalence of MRSA in the UK has been observed to taper (EARSS, 2007; HPS, 2008b). Although these data serve as a guide only (not all cases of MRSA are bacteraemic (Adedeji and Gray, 2005)) and inherent biases in data collection must also be considered, the figures give an insight into recent trends.

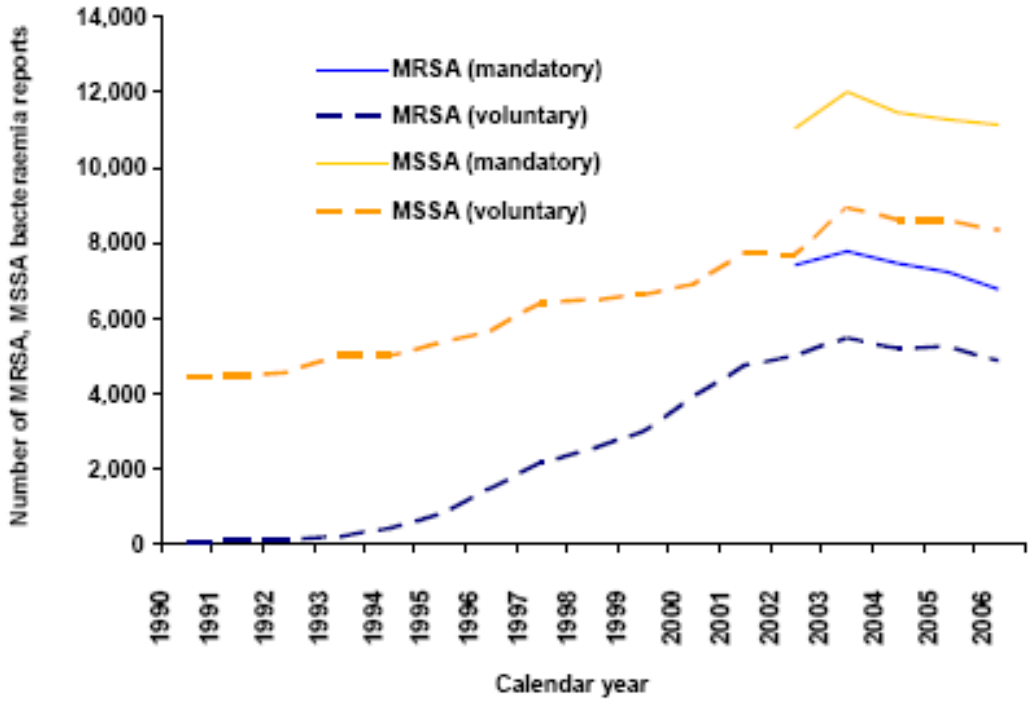

Figure 1.5. Numbers of MRSA and MSSA bacteraemia reports per year in the UK (from http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1196942169446).

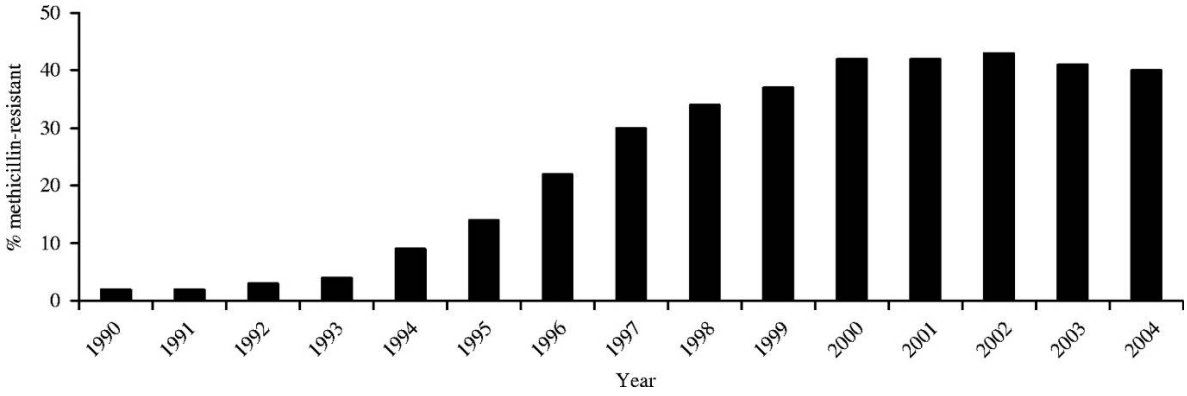

Figure 1.6. Percentage of *Staphylococcus aureus* bacteraemias attributable to MRSA in the UK (after Johnson *et al.*, 2005).

1.4.3 Geographical considerations for MRSA

The prevalence of MRSA varies geographically, and epidemiological characteristics including prevalence, distribution and genotypic variation may alter at a resolution at the level of country, town, institution or ward (Livermore and Pearson, 2007). On a global scale, the distribution of prevalence of MRSA in 2005 has been collated by Grundmann and colleagues (2006) and is presented in Figure 1.7. Similarly, European data have been collated by the European Antimicrobial Resistance Surveillance System (EARSS) (2007) and are presented in Figure 1.8. Brief visualisation of these maps reveals the variation of estimates, both within and between continents. While it is certain that the colour-scheme does not accurately reflect prevalence of MRSA at national borders, the variation in prevalence between countries undoubtedly depends not only on physical geographical boundaries, but other factors such as prevention and control strategies, population densities and is also likely to reflect variation in laboratory methods and reporting (Grundmann *et al.*, 2006; Gould, 2007).

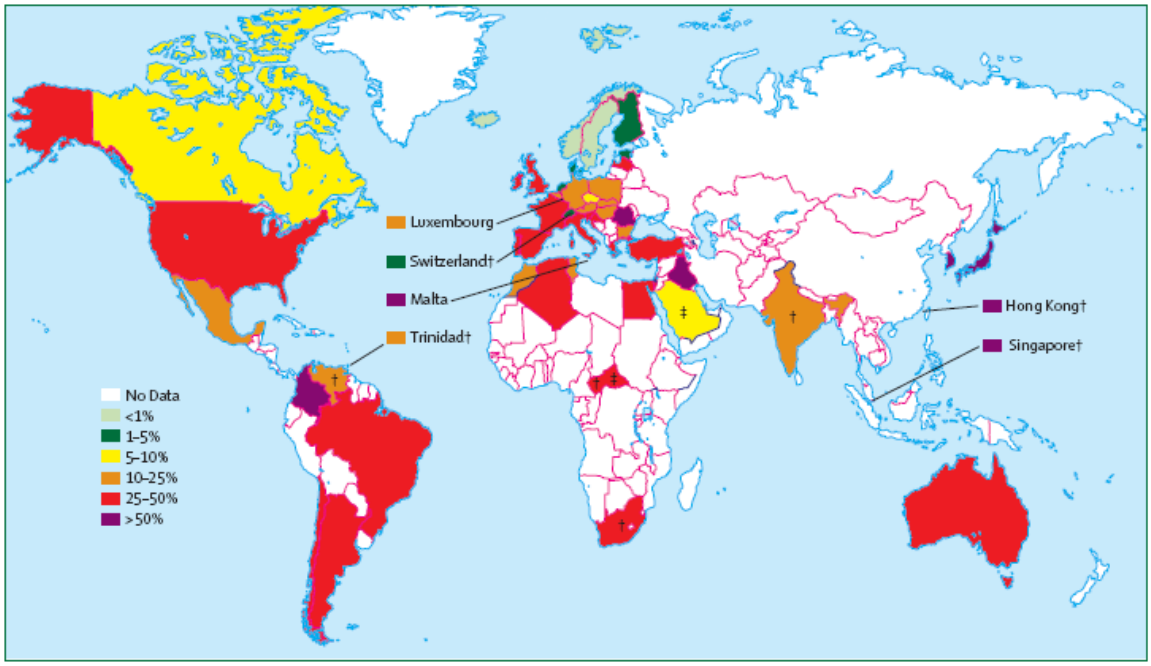

Figure 1.7. Worldwide prevalence of MRSA displayed by country (after Grundmann *et al.*, 2006).

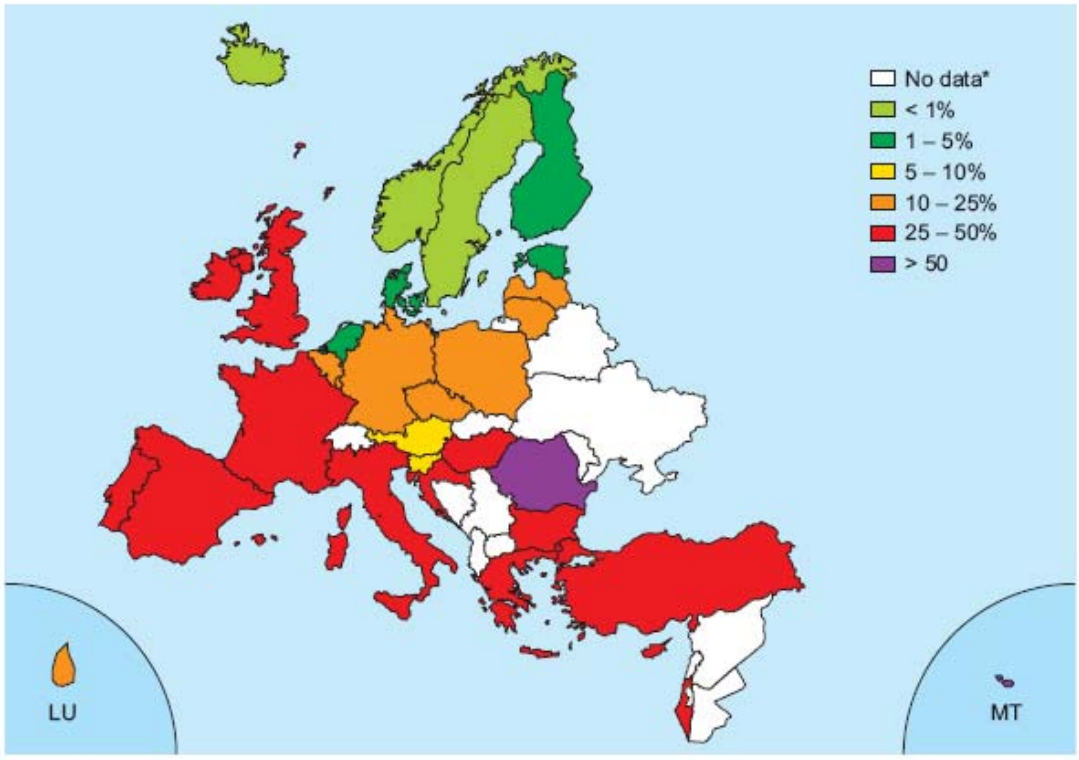

Figure 1.8. Proportion of invasive isolates resistant to MRSA in 2006 (after EARSS, 2007) (* these countries did not report any data, or less than 10 isolates).

1.4.4 Classification of MRSA

Accurate characterisation and classification of MRSA isolates facilitates exchange of information and subsequent monitoring of the spread of disease and antimicrobial resistance (Enright, 2008). This characterisation may be undertaken using epidemiological and/or molecular descriptors.

1.4.4.1 Community-associated and Hospital-associated MRSA

In addition to the organism's prevalence and biological importance within hospital settings, MRSA is also recognised as an emerging pathogen within the community. Since the initial identification of a novel MRSA strain in populations with no prior health-care exposure or other typical risk factors in 1993 (Udo *et al.*, 1993), MRSA has been broadly classified as either hospital or community acquired/associated. Controversy exists over the differentiation between these two categories, and definitions may centre around presence or absence of hospital-associated (HA-) risk factors, appropriate genetic markers and phenotypic profiles (Deurenberg and Stobberingh, 2008).

An epidemiological definition of community associated (CA-) MRSA strains, used by the Centers for Disease Control (CDC), refers to those isolated in an outpatient setting or within 48 hours of hospital admission, in people without: i) a previous history of MRSA infection or colonisation, ii) hospitalisation, admission to a nursing home or dialysis in the last year, or iii) the presence of permanent indwelling devices (Deurenberg and Stobberingh, 2008). However, temporal associations may be misleading due to putative scenarios such as prior carriage of a CA- organism only causing infection after an invasive procedure, or previous exposure to hospitalised individuals within the community and it has recently been shown that defining CA-MRSA by absence of risk factors for healthcare exposure greatly underestimates the burden of CA-MRSA disease (David *et al.*, 2008). Phenotypically, CA-MRSA are more likely to be sensitive to non-β-lactam antibiotics than HA- strains and genotypically are more likely to harbour SCC*mec* type IV, V or VII and the Panton-Valentine Leukocidin (PVL) virulence factor (Grundmann *et al.*, 2006; Deurenberg and Stobberingh, 2008). It is known that the presence of the PVL toxin is associated with the propensity for CA- strains to cause skin and soft tissue infection, along with the more concerning necrotising fasciitis and necrotising pneumonia (Morgan, 2005;

Boyce, 2008). It is also likely that more complicated interactions between multiple genetic factors, including the presence of α-haemolysin, the arginine catabolic mobile element (ACME) and phenol-soluble modulins (PSMs) along with specific variants of PVL are responsible for the heightened virulence of CA- strains, although research continues in this area (Lowy, 2007; Deurenberg and Stobberingh, 2008; Ellington *et al.*, 2008). Clinically, although CA-MRSA strains are often associated with greater antimicrobial susceptibility, the presence of additional virulence factors means that they are more likely to result in, possibly severe, disease in non-compromised individuals (Elston, 2007).

While CA-MRSA and HA-MRSA are often thought of as distinct epidemiological entities, one must consider that there is directional movement of MRSA from hospitals to the community, from the community to hospitals and that there are also independent reservoirs of MRSA within both settings (Kluytmans-Vandenbergh and Kluytmans, 2006). A recent study undertaken in a single hospital in the USA has shown that, while overall prevalence of MRSA blood stream infections did not change over a two year period, community associated genotypes, inferred by phenotypic rules based on an established algorithm using PFGE results and antimicrobial susceptibilities, were twice as prevalent (49% of MRSA infections) in the second year of the study than the first (Popovich *et al.*, 2008). The implications associated with the putative generalisability of the findings from this study are far-reaching, including potentially necessary reassessment of treatment and prevention strategies for endemic hospital strains that display enhanced virulence, along with those with marked resistance (Popovich *et al.*, 2008).

In addition to the classification of MRSA as HA- or CA- is the recent identification of a distinct MRSA clone, typed as ST-398, amongst pigs, pig farmers and veterinarians in The Netherlands (Voss *et al.*, 2005). While this clone does not fit the typical definition of CA- or HA-MRSA, it appears to have originated *de novo* from pigs or cattle and, since its initial identification in 2003, has infiltrated communities and hospital settings in The Netherlands, where currently over 20% of MRSA isolated from humans are attributable to this clone (van Loo *et al.*, 2007b). This strain (ST-398) has also been isolated from pigs in other countries, including Singapore, Canada, Germany, Austria and Denmark (Guardabassi *et al.*, 2007; Sergio *et al.*, 2007; Witte *et al.*, 2007; Khanna *et al.*, 2008; Meemken *et al.*, 2008) and, while ST-398 is not thought to be primarily a human pathogen, it is nevertheless capable of causing infection in humans (Lewis *et al.*, 2008; van Belkum *et al.*, 2008). Furthermore, ST-398 has been shown to transfer readily from animals to humans. Over 12% of delegates at an international pig-health meeting in Denmark were found to

carry ST-398 and over 20% of pig farmers in the Netherlands have been identified as carriers, representing a risk of carriage that is 12 times greater than the general population (Voss *et al.*, 2005; van Loo *et al.*, 2007b; Wulf *et al.*, 2008). Although ST-398 has not yet been isolated from livestock in the UK, the clone has been isolated from three hospitalised humans with no known contact with pigs, farmers or history of travel to The Netherlands or other countries where this strain is present (HPS, 2008a). Recent identification of the PVL toxin within ST-398 in two countries demonstrates the potential for enhanced virulence associated with this infiltrative organism (van Belkum *et al.*, 2008; Yu *et al.*, 2008). The presence of enhanced virulence in such a readily transferable zoonotic organism could result in a marked increase in the prevalence of disease in non-compromised individuals.

1.4.4.2 Molecular Typing of MRSA

A number of different molecular typing methods are available for MRSA. The most important of these are pulse field gel electrophoresis (PFGE), MLST, *S. aureus* protein A (*spa*) typing and SCC*mec* typing (Deurenberg and Stobberingh, 2008). Each of these methods allows assessment and comparison of isolates at varying resolutions, differing in accuracy, discriminatory power and reproducibility (Melles *et al.*, 2007).

The use of the highly discriminatory PFGE, which involves the use of a switching electric field to facilitate separation of large restriction fragments of chromosomal DNA, created by enzymatic cleavage using the enzyme *Sma*I, to produce a restriction pattern or fingerprint on agarose gel (Enright, 2008) (Figure 1.9), is described as the gold standard for investigating outbreaks of *S. aureus* (Deurenberg and Stobberingh, 2008). However, this technique does not allow international comparison of strains and is limited in its reproducibility due to lack of standardised methods between laboratories and sensitivity of the technique to small amounts of genetic change (Enright *et al.*, 2002; Deurenberg and Stobberingh, 2008). MLST examines allelic diversity at seven housekeeping (highly conserved) gene loci by DNA sequencing of 500bp internal fragments and subsequent comparison of sequences with known alleles at each locus to enable generation of an allelic profile (Enright, 2008). In contrast to PFGE, MLST allows robust repeatability that facilitates international strain comparison through the identification of allelic profile designated sequence types (STs) (Enright *et al.*, 2002; Deurenberg and Stobberingh, 2008).

Furthemore, these strain types may be combined in clusters of closely related genotypes, referred to as CCs using BURST algorithms (currently implemented as eBURST), to provide putative evolutionary inference (Figure 1.10) (Enright *et al.*, 2002; Feil *et al.*, 2004; Lindsay and Holden, 2006). While MLST is robust and applicable for the investigation and comparison of long-term global studies and evolutionary relationships, slow accumulation of genetic variation in the housekeeping loci results in a lack of discrimination between epidemiologically unrelated strains (Melles *et al.*, 2007). In contrast, *spa* typing, a single locus sequence typing method based on the determination of the sequence variation of the polymorphic region X (consisting of a variable number of highly diverse 24 nucleotide repeats) of a hyper-variable single (*spa*) locus (Koreen *et al.*, 2004; Deurenberg and Stobberingh, 2008; Enright, 2008) provides intermediate discriminative power and is cheaper and less laborious than MLST (Deurenberg and Stobberingh, 2008). Cluster analysis of spa types as *spa*-clonal complexes (*spa*-CC) is also possible using the based upon repeat pattern (BURP) algorithm, although this grouping method may lack accuracy and discriminatory power in some scenarios (Strommenger *et al.*, 2008). Combination of BURP analysis with additional markers such as SCC*mec* typing has recently been advised to increase the discriminatory power of *spa* typing and BURP analysis (Strommenger *et al.*, 2008). SCC*mec* types (described in 1.4.1.3) may be identified using PCR-based techniques and a combination of SCCmec types and MLST designation results in a robust categorisation that may be compared to PFGE types (Ito *et al.*, 2001; Oliveira and de Lencastre, 2002; Enright, 2008).

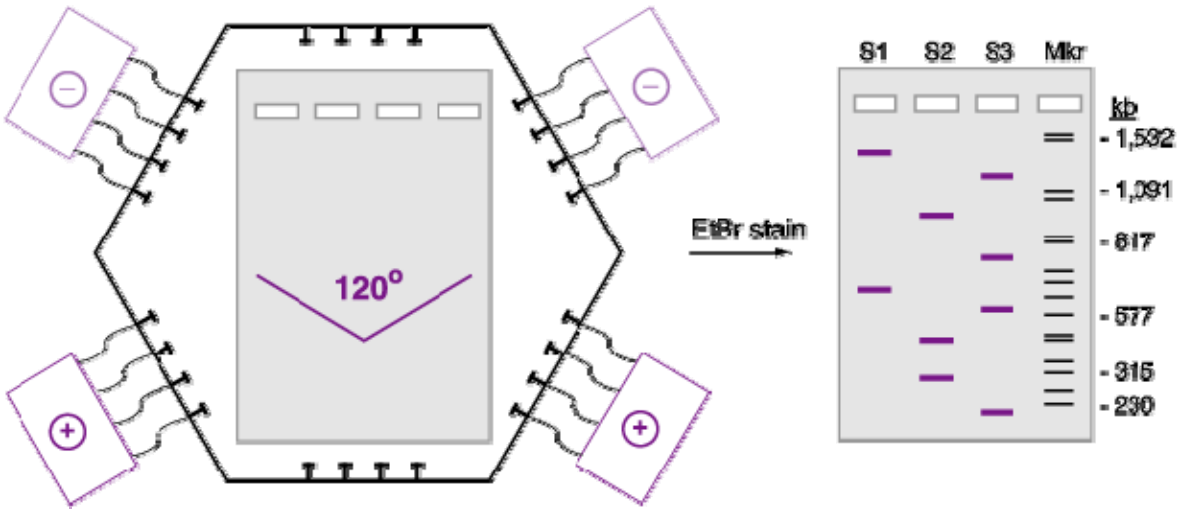

Figure 1.9. Schematic diagram of PFGE process – obtained from http://www.biochem.arizona.edu/classes/bioc471/pages/Lecture8/AMG4.10b.gif.

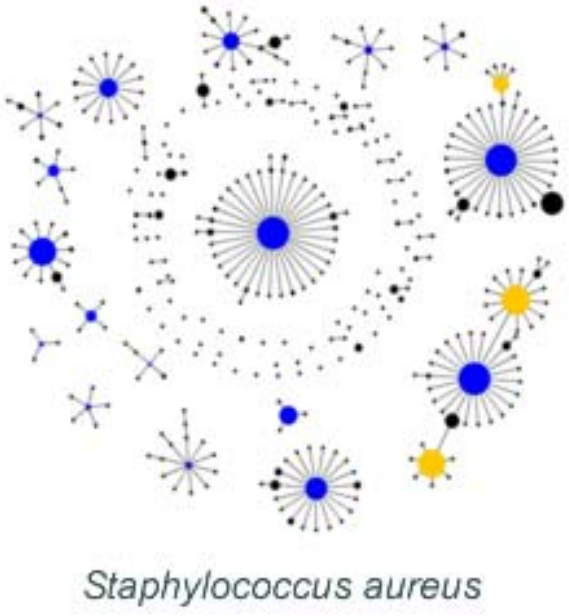

Figure 1.10. Example of eBURST output for *Staphylococcus aureus*. Clusters of linked isolates correspond to clonal complexes (Feil *et al.*, 2004). Each circle represents a genotype (MLST sequence type (ST)) whose prevalence is proportional to its area (Enright, 2008). Primary founders are positioned centrally in the cluster. ST labels removed for clarity – obtained from http://eburst.mlst.net/.

1.4.5 MRSA in animals

Meticillin-resistant *S. aureus* colonisation and infection is a more recently defined phenomenon in the area of veterinary medicine. Reports of MRSA in non-human species have increased in number considerably in the last decade and contamination and infection with MRSA has been documented in wildlife, farm and companion animal species (Lee, 2003; O'Mahony *et al.*, 2005; Rich and Roberts, 2006). The range of clinical signs resulting from MRSA infection in companion animals is similar to those observed in human disease: skin and soft tissue infections predominate and these infections anecdotally appear to be most commonly associated with post-operative wounds, prolonged hospitalisation and/or immunosuppressive therapy (Duquette and Nuttall, 2004; Rich *et al.*, 2005; Leonard *et al.*, 2006; Morris *et al.*, 2006; Griffeth *et al.*, 2008).

The published data that stem from companion animal populations, and with respect to canine species in particular, are largely based on isolation from clinical cases (Tomlin *et al.*, 1999; Morris *et al.*, 2006; Rich and Roberts, 2006) or cross-sectional estimates of prevalence of MRSA colonisation (Baptiste *et al.*, 2005; Loeffler *et al.*, 2005; Rich, 2005;

Abbott *et al.*, 2006; Murphy *et al.*, 2006; Vengust *et al.*, 2006; Bagcigil *et al.*, 2007; Griffeth *et al.*, 2008; Hanselman *et al.*, 2008; Moodley *et al.*, 2008). These data are predominantly obtained from clinical specimens in the first instance, and nasal swabs taken from hospitalised and non-hospitalised dogs and veterinary staff in the latter. Longitudinal information on duration of carriage or likelihood of infection in colonised animals is not available. However, the data that have been presented thus far from small animal veterinary settings do appear to mirror the situation in human populations over the past decades: prevalence appears to be increasing in animal populations and an increased prevalence of MRSA colonisation has also been found in veterinary staff compared to the general population (paralleling a greater prevalence noted in human health care workers) (Cesur and Cokca, 2004; Loeffler *et al.*, 2005; Hanselman *et al.*, 2006a; Moodley *et al.*, 2008).

1.4.6 MRSA as a canine zoonosis

The relationship between MRSA in humans and companion animals is poorly understood. It is known that dogs may act as reservoirs of MRSA for humans, where the term reservoir refers to the potential for direct or indirect transmission, and that the same strain may be found in dogs and humans inhabiting the same household, or veterinary workers in contact with infected dogs (Cefai *et al.*, 1994; Manian, 2003; Leonard *et al.*, 2006). However, in contrast to the stains most commonly recovered in horses (Weese *et al.*, 2005) and pigs (Voss *et al.*, 2005), MRSA found in dogs are indistinguishable from the most common hospital acquired strains found in humans (predominantly EMRSA-15, but also EMRSA-16 in the UK) and as such, it has not been possible to describe the direction of transfer between humans and dogs thus far (Baptiste *et al.*, 2005; Leonard and Markey, 2008). Consequently, while the implication, based on typing data, is that MRSA has emerged in dogs as a result of MRSA in humans (Baptiste *et al.*, 2005; Moodley *et al.*, 2006; Leonard and Markey, 2008), the potential contribution of dogs that are colonised or infected with MRSA to the burden of disease in humans and conversely, the potential contribution of colonised and infected humans to canine disease, has not been directly quantifiable.

1.5 Assessing the risk associated with MRSA in dogs

A number of studies have been published in the human literature that describe transmission models utilised for the assessment of potential outbreak scenarios associated with MRSA in hospitals, the community and other institutions such as gaols (Cooper and Lipsitch, 2004; Cooper *et al.*, 2004; Kajita *et al.*, 2007; McBryde *et al.*, 2007). The mathematical models that have been described are predominantly undertaken to model the transfer of MRSA within contained environments, where information on population admissions, discharges, contact, colonisation and infection rates are generally known. Even in models where the population is relatively large (>8,000 reported cases were modelled in an outbreak of CA-MRSA in a county gaol (Kajita *et al.*, 2007)) these models are able to be populated with known, or well estimated parameters. Most of these models work with the assumption that pathogenic transfer within the community (that is, outside a hospital or other institutional setting) is negligible. Contradicting this assumption are the accounts of transmission of MRSA between family members and persistent re-infection or colonisation with MRSA within the community setting (Kniehl *et al.*, 2005; Huijsdens *et al.*, 2006; Johansson *et al.*, 2007). However, to date, no models have been published that describe or explore the risk of exposure to or transmission of MRSA outwith a contained environment.

1.6 Aims and objectives of the current study

The objective of the current study was to explore the relationship between MRSA in dogs and humans. The aims were to undertake an assessment of the risk of MRSA acquisition in pet dogs in order to a) assess the risk of acquisition of MRSA in dogs over a defined time period; b) identify important priority data gaps for future research efforts in this data-sparse area; c) comment on the usefulness of risk assessment, in a data sparse area and within a field in which it has not previously been used, for the above aims and objectives.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Introduction

The methods adopted throughout these studies centre around the application of a risk assessment to the area of MRSA in pet dogs. This thesis presents a qualitative risk assessment (Chapter 3) and a quantitative risk assessment with attendant uncertainty and sensitivity analyses (Chapters 6 and 7), along with three data collection studies (Chapter 4) and an expert opinion elicitation procedure (Chapter 5) that were required to enhance parameterisation of the quantitative model. The methods used for each of these areas of study are presented below.

2.2 Risk analysis

The risk analysis structure that is followed represents a hybrid of previously described OIE and CAC techniques (Section 1.2.1), designed for use in import and food safety risk analyses respectively (Codex Alimentarius Commission, 1999; OIE, 2006). The structure utilised is discussed fully within Chapter 3, but briefly is composed of two steps: (1) hazard identification and characterisation and (2) risk estimation. Exposure and consequence assessments were undertaken within the step of risk estimation. This structure was adhered to for the purpose of conducting an initial qualitative risk assessment and construction of a conceptual model (Chapter 3). The conceptual model was specified based on the results of hazard identification and characterisation and allowed a structured approach to risk estimation, both for the qualitative (Chapter 3) and quantitative (Chapter 6 and 7) models. Within the qualitative assessment, categorical estimators (negligible, low, moderate and high) were used to define the risk at each point of the conceptual model and a published, repeatable and reproducible matrix was used to combine these categorical estimates (EFSA, 2007). The quantitative risk assessment was specified as a stochastic simulation model that loosely followed the steps set out for a simulation study by Law and Kelton (2000) as displayed in Figure 2.1. The overall estimate of risk obtained from the specified models refers to the risk of acquisition of MRSA in pet dogs in a given 24 hour period. There was no measure of the consequence of acquisition of the organism and as such the outcomes of the qualitative and quantitative models are strictly termed ‘exposure assessments’.

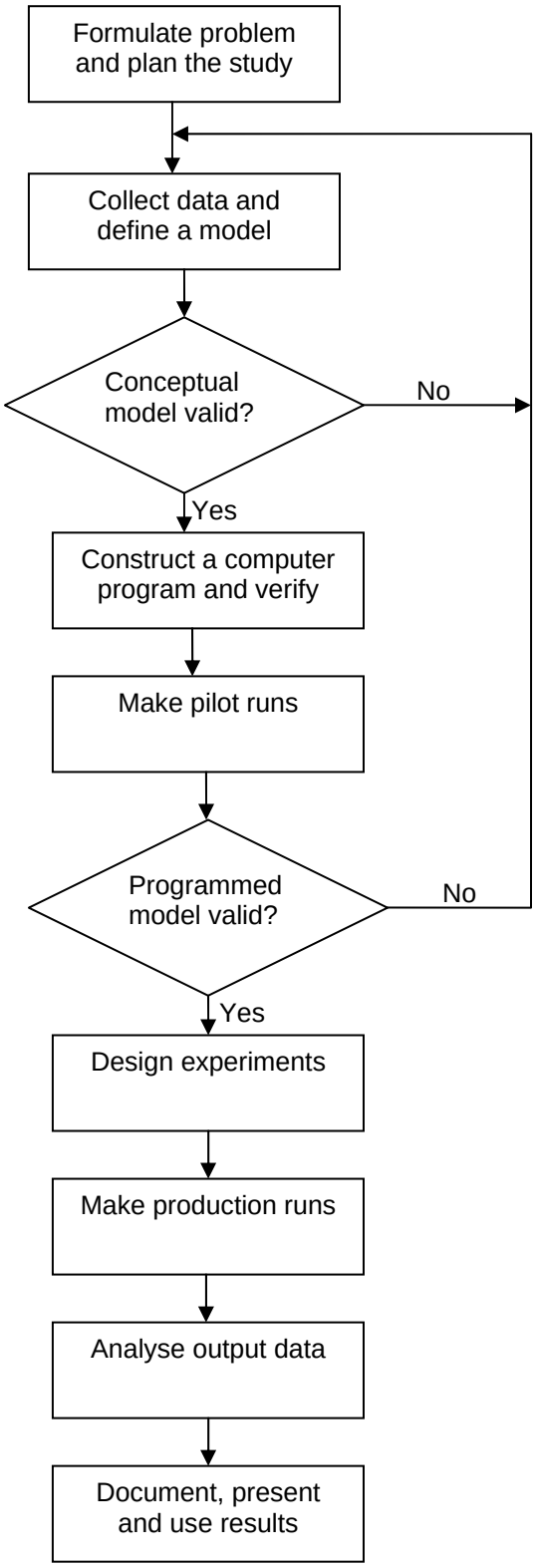

Figure 2.1. Steps in a simulation study, outlined by Law and Kelton (2000).

2.2.1 Simulation modelling

Simulation refers to the imitation of a real-world system through the adherence to a set of assumptions that take the form of mathematical or logical relationships (Law and Kelton, 2000). The simulation model that was specified in this thesis was a stochastic model, incorporating random or probabilistic components and resulting in a random or distributional output. Each of the inputs to the quantitative model was specified as a distribution based on available data and random selection was made from each of these distributions on each model run using Monte Carlo and Latin Hypercube sampling techniques (Vose, 2000) as described in Chapter 1. The model was run for a discrete time period (24 hours) and is described in detail in Chapter 6.

2.2.2 Software

The software predominantly used in these studies was R (R Development Core Team, 2008). R represents a "language and environment for statistical computing" (http://www.r-project.org/) which supports a wide variety of statistical and graphical techniques and consists of a dedicated language and run-time environment (Hornik, 2009). It is an open-source implementation of the S programming language and is supplemented by a large number of 'packages' to undertake complex analyses (Fox and Andersen, 2005). All code was written and edited in the text interface WinEdt® (version 5.5, http://www.winedt.com/) which was incorporated as an R plugin using the RWinEdt package (http://www.r-project.org/). Other R packages that were utilised included epicalc, stats and lhs (http://www.r-project.org/).

The project-specific code that was used to run each of the models and analyses represented in this thesis is discussed in the relevant sections and model-specific programming code is included as appendices where appropriate. All code was written by the author except where specified. Model checking was undertaken for the quantitative risk assessment model (Chapter 6) by running the loops within the model independently and separately assessing the outcome for each step. The model was also checked by two experienced users of R who were familiar with simulation modelling procedures.

A sample of the code used in this thesis is presented as Figure 2.2 as an example of the programming language used. This small portion of code was written to obtain combination expert opinion distributions, the full discussion of which is presented in Chapter 5.

```
qnum <- read.csv("/question.numbers.csv")
qnum <- qnum[,1]

n.experts <- 15
n.sample <- expert.iterations

combination.data <- matrix(nrow=n.sample, ncol=length(qnum))

for(i in 1:length(qnum)){

filename <- paste("/question",qnum[i],".csv",sep="")

data <- read.csv(filename)

opinion <- numeric(n.experts*n.sample)
dim(opinion) <- c(n.experts,n.sample)


for(expert in (1:n.experts)){

if(i>37){
opinion[expert,] <-
rmodbetapert(n.sample,data[expert,2]/100,data[expert,3]/100,data[expert,4]/100,data[expert,5])}
else{
opinion[expert,] <-
rmodbetapert(n.sample,data[expert,2]/1000,data[expert,3]/1000,data[expert,4]/1000,data[expert,5])}
}

combination.data[,i] <- sample(opinion,n.sample,replace=TRUE,
prob=rep(c(0,1,1,1,1,1,1,1,1,1,1,1,1,1,1),n.sample))
}
```

Figure 2.2. Example of R code used within this thesis.

Prior to using R, the quantitative risk assessment model was implemented in @RISK (version 4.5.7, Pallisade Corporation). However, the model was too complex and required too many iterations to be fully implemented in this programme.

In addition, Microsoft Excel 2003 (Microsoft Corporation) and Microsoft Access 2003 (Microsoft Corporation) database programmes were used for data storage and manipualation. All data for input into and output from R were stored within Microsoft Excel spreadsheet format as .csv files.

Minitab (Minitab release 14.1, Minitab Inc. 2003) and StatsDirect (StatsDirect Ltd, 2008) programmes were also used for statistical analyses within the thesis (Chapter 4).

2.2.3 Statistical methods

Sample size calculations that were undertaken in Chapter 4 for the estimation of proportions used the following formula:

$$n = \frac{Z^2_{1-\frac{\alpha}{2}} p(1-p)}{L^2}$$

Where $Z_{1-\frac{\alpha}{2}}$ is the standard normal distribution corresponding to a two-sided confidence level of $1-\frac{\alpha}{2}$, L is the desired precision of the estimate and p is the expected prevalence of interest in the population (Houe *et al.*, 2004).

Chi-square (χ^2) and McNemar's statistics were calculated for the comparison of proportions for un-paired and paired data respectively (Chapter 4) and LR models were specified as part of the sensitivity analyses for the simulation model (Chapter 6 and 7). As discussed in Dohoo *et al.* (2003), using the logistic model and a logit transformation, the relationship between the probability of the outcome (p) and predictor variables ($X = X_j$) can be modelled as follows:

$$\text{Log(odds)} = \ln\left[\frac{p}{1-p}\right] = \beta_0 + \sum \beta_j X_j$$

The probability of the outcome of the logistic model is expressed as:

$$p = \frac{e^{\left(\beta_0 + \sum \beta_j X_j\right)}}{1 + e^{\left(\beta_0 + \sum \beta_j X_j\right)}}$$

and the odds ratio (OR) for the outcome associated with factor X_k can be expressed as e^{β_k}.

The basic assumptions that are required to be met for logistic (or linear) regression are: i) independence of observations and ii) linearity of continuous explanatory variables with the outcome (Dohoo *et al.*, 2003). Further discussion of LR modeling will appear in Chapters 6 and 7 and this statistical technique is described in detail in a number of reference texts, examples of which include Hosmer and Lemeshow (2000) and Dohoo *et al.* (2003).

2.2.4 Sensitivity analysis

In addition to the LR models described above, OAT and fractional factorial Plackett-Burman (P-B) designs were used to implement sensitivity analyses in this thesis (Chapter 7). Literature describing these techniques has been outlined in Chapter 1 and the methods are discussed in full in Chapter 7. Briefly, both of these techniques involve ascribing an upper and lower value to each input variable under consideration and then varying these values one at a time for the OAT analysis, or with respect to a defined pattern that allows consideration of the effect of other variables for the fractional factorial design (Campolongo and Saltelli, 2000).

2.3 Questionnaire design

Questionnaires were used on two occasions in the studies described in this thesis. The first occasion was documented in Chapter 4B and was an orally administered questionnaire designed to assess dog-human and dog-dog interactions and the second, documented in Chapter 5, was an emailed questionnaire used to elicit expert opinion with respect to MRSA in pet dogs. The specific methods of questionnaire construction are covered within the relevant chapters. Briefly, the questionnaire structure, design and administration was based on the Total Design Method, described by Dillman (1991) and its subsequent modification (Yammarino *et al.*, 1991; Eaker *et al.*, 1998), aimed at maximising the response rate while concurrently reducing potential sources of error. The modalities of survey application in these studies (oral and email administration) are less common than mailed (self-administered) although many of the same considerations are required for these modalities. Previously identified techniques for increasing response rate, including repeated contacts (in the form of follow-ups), inclusion of appeals within cover letters, a high level of salience of the topic to the respondent and affiliation of the questionnaire with a University were used where possible (Fox *et al.*, 1988; Yammarino *et al.*, 1991).

2.4 Bacterial isolation and identification

In order to enable parameterisation of the quantitative risk assessment model, two laboratory-based studies were undertaken and are presented in Chapter 4A and 4C.

2.4.1 Environmental and veterinary staff prevalence

Isolation and identification of MRSA from swabs taken of the external nares of veterinary staff members and the environment in the University of Glasgow Small Animal Hospital (Chapter 4A), was undertaken using the following methods.

2.4.1.1 Isolation of MRSA

All swabs were placed directly into Tryptone Soya Broth (TSB, Oxoid Ltd., UK) supplemented with 2.5% NaCl and incubated for 24 hours at 37ºC. A 10µl aliquot of TSB was then streaked onto chromogenic agar (MRSA Select; BioRad, France) and incubated aerobically at 37ºC. The plates were assessed for growth at 24 and 36 hours, in line with manufacturer recommendations. Presumptive MRSA colonies were subcultured onto 5% sheep blood agar. Representative samples from each of these plates were confirmed as *S. aureus* using API ID 32-STAPH (BioMérieux) and *S. aureus* specific latex agglutination kit (Oxoid Ltd, UK).

2.4.1.2 Antimicrobial susceptibility testing

The antimicrobial resistance profile for each MRSA isolate was determined by two methods: (i) the automated Vitek 1 system (BioMérieux, UK) as per manufacturer's instructions to determine the minimum inhibitory concentration (MIC) and (ii) disc diffusion, using diagnostic sensitivity test agar (DSTA, Oxoid Ltd, UK) in accordance with British Society for Antimicrobial Chemotherapy guidelines (Andrews, 2001). The following discs were used; cefuroxime (30ug), chloramphenicol (10ug), ciprofloxacin (1ug), clindamycin (2ug), erythromycin (5ug), fusidic acid (10ug), gentamicin (10ug),

kanamycin (30ug), meticillin (5ug), mupirocin (5ug), neomycin (10ug), oxacillin (1ug), penicillin (1ug), rifampicin (2ug), sulphamethoxazole (25ug), streptomycin (10ug), tetracycline (10ug), tobramycin (10ug), trimethoprim (1.25ug).

2.4.1.3 Genotypic characterisation of MRSA

PCR was performed to identify *mec*A and PVL genes, and to identify SCC*mec* types of the MRSA isolates. *Mec*A and PVL detection were performed as described below (Bignardi *et al.*, 1996; Lina *et al.*, 1999) with an internal *S. aureus* specific *nuc* control included (Brakstad *et al.*, 1992).

DNA extraction was carried out by the suspension of approximately 5 bacterial colonies in NET buffer (10mM NaCl, 1mM EDTA, 10mM Tris (Sigma, UK)) containing 100U achromopeptidase (Kobayashi *et al.*, 1994; Leonard *et al.*, 1995), and incubation of the suspension at 50ºC for 15 minutes. Two microlitres of DNA template was then added to PCR Master Mix (12.5µl ReddyMix (0.625 units Thermoprime Plus DNA Polymerase, 75mM Tris-HCl (pH 8.8 at room temperature), 20mM $(NH_4)_2SO_4$, 1.5mM $MgCl_2$, 200mM of each dNTP and 0.01% (v/v) Tween 20)) (Abgene,UK) containing 1µl of each of the appropriate primers (MWG Biotech, UK) at the correct concentration (Table 2.1). Tissue culture grade water (Sigma, UK) was added to give a final reaction volume of 25µl. PCR amplification was carried out with a mastercycler® ep thermal cycler (eppendorf, Hamburg, Germany) and using the following cycle: 95ºC for 5 minutes; 30 cycles of 95ºC for 30 seconds, 55ºC for 30 seconds, 72ºC for 1 minute; and a final extension at 72ºC for 5 minutes. Six µl of PCR product was loaded into 1.5% agarose (Mast, UK) gel and electrophoresis performed in 0.5 x TBE buffer at 180V for 90 min using a power-pac 300 pwer pack (BioRad, France). Gels were stained with 1µg/ml ethidium bromide (Sigma, UK) for 20 min, examined under UV light and photographed.

SCC*mec* typing was performed according to the Oliveira method (Oliveira and de Lencastre, 2002). Details of extraction, master mix preparation and electrophoresis are as described above. The following cycle was used: 94ºC for 4 minutes; 30 cycles of 94ºC for 30 seconds, 53ºC for 30 seconds, 72ºC for 1 minute; and a final extension at 72ºC for 4 minutes. Primers and product sizes are detailed in Table 2.1.

Table 2.1. Primers and product sizes used for PCR for *nuc*, *mec*A and PVL gene identification and SCC*mec* typing.

Primer	Sequence (5'-3')	Product size (kb)	Concentration (µM)
*mec*A-1	CTCAGGTACTGCTATCCACC	449bp	1
*mec*A-2	CACTTGGTATATCTTCACC		1
nuc-1	GCGATTGATGGTGATACGGTT	280bp	1
nuc-2	AGCCAAGCCTTGACGAACTAAAGC		1
pvl-1	ATCATTAGGTAAAATGTCTGGACATGATCCA		1
pvl-2	GCATCAASTGTATTGGATAGCAAAAGC (S=G/C 1:1 ratio)	433bp	1
CIF2 F2	TTCGAGTTGCTGATGAAGAAAGG	495bp	0.4
CIF2 R2	ATTTACCACAAGGACTACCAGC		0.4
KDP F1	AATCATCTGCCATTGGTGATGC	284bp	0.2
KDP R1	CGAATGAAGTGAAAGAAAGTGG		0.2
MEC1 P2	ATCAAGACTTGCATTCAGGC	209bp	0.4
MEC1 P3	GCGGTTTCAATTCACTTGTC		0.4
DCS F2	CATCCTATGATAGCTTGGTC	342bp	0.8
DCS R1	CTAAATCATAGCCATGACCG		0.8
RIF4 F3	GTGATTGTTCGAGATATGTGG	243bp	0.2
RIF4 R9	CGCTTTATCTGTATCTATCGC		0.2
RIF5 F10	TTCTTAAGTACACGCTGAATCG	414bp	0.4
RIF5 R13	GTCACAGTAATTCCATCAATGC		0.4
IS431 P4	CAGGTCTCTTCAGATCTACG		0.8
PUB110 R1	GAGCCATAAACACCAATAGCC	381bp	0.4
IS431 P4	CAGGTCTCTTCAGATCTACG		0.8
PT181 R1	GAAGAATGGGGAAAGCTTCAC	303bp	0.4
MECA P4	TCCAGATTACAACTTCACCAGG	162bp	0.8
MECA P7	CCACTTCATATCTTGTAACG		0.8

2.4.1.4 Pulsed-field gel electrophoresis (PFGE)

Pulsed-field gel electrophoresis typing of *Sma*I (Invitrogen, UK) digested DNA was performed by a modification of a previously described method (Bannerman *et al.*, 1995). Briefly, *S. aureus* colonies from overnight cultures were incorporated into agarose plugs. After bacterial lysis, genomic DNA was digested using *Sma*I (Invitrogen, UK). PFGE was performed by clamped homogeneous electric field (CHEF) electrophoresis with a CHEF-mapper system (Bio-Rad Laboratories, California, USA). The fragments were separated with a linear ramped pulse time of 6.8–63.8 s over a period of 23 h at 14°C. Gels were analysed visually and the epidemiological relationship between isolates was assessed using the criteria described by Tenover and colleagues (Tenover *et al.*, 1995).

2.4.1.5 *Spa* typing

Amplification of the *spa* repeat region was performed using primers spa-1113f (5'-AAGACGATCCTTCGGTGAGC-3') and spa-1514r (5'-CAGCAGTAGTGCCGTTTGCTT-3') as previously described (Harmsen *et al.*, 2003). PCR products were sequenced using BigDye v1.1 (Applied Biosystems, Foster City, USA) and *spa* types were determined using the Ridom StaphType 1.4.1 software (Harmsen *et al.*, 2003). The BURP (based upon repeat patterns) algorithm was used to cluster resulting *spa* types into different groups. *Spa* types with less than five repeats were excluded from the analysis.

2.4.2 Meat and dried pig's ear treat prevalence study

2.4.2.1 Isolation of MRSA and MSSA

As in the previously reported study (2.4.1.1), swabs were initially placed directly into Tryptone Soya Broth (TSB, Oxoid Ltd., UK) supplemented with 2.5% NaCl. However, at this NaCl concentration, overgrowth of enterococci was observed, so the NaCl concentration was increased to 6.5% and all samples were re-incubated in the modified enrichment broth. Broths were incubated at 37.1ºC for 48 hours and after enrichment, 25µL was transferred to each of a 5% sheep blood agar (SBA) and MRSA-Select agar

(MRSA Select; BioRad, France) plate via pipette. The innoculant was then spread over the plate using a sterile loop and these samples were incubated at 37ºC for 24 hours (SBA) and 48 hours (MRSA-Select). The MRSA-Select plates were assessed at 24 and 48 hours of incubation.

Catalase production was assessed by mixing a loop of cells from the agar culture with a drop of 30% hydrogen peroxide on a glass microscope slide. The immediate appearance of bubbles was indicative of the presence of catalase. The presence of DNase was assessed using DNase agar plates (Oxoid Ltd, UK) and following the manufacturer instructions. In short, isolates were inoculated onto the DNase agar, incubated at 37ºC for 24 hours. After incubation, the plates were flooded with 10% HCl and those with zones of clearance around the colonies were classed as positive.

2.4.2.2 Further identification

Presumptive MRSA and MSSA colonies were confirmed as such using standard PCR techniques to identify *mecA* and *nuc* as described above (Section 2.4.1.3). Antimicrobial susceptibility testing was carried out using the Vitek 1 system only and PFGE typing as described above (2.4.1.2 and 2.4.1.4) on all samples that were *mecA* and *nuc* positive.

CHAPTER 3

QUALITATIVE RISK ASSESSMENT OF THE RISK OF ACQUISITION OF MRSA IN PET DOGS

3.1 Introduction

The relationship between MRSA in humans and companion animals is poorly defined. It is known that dogs may act as reservoirs of MRSA for humans and that the same strain is often found in dogs and humans inhabiting the same household, or veterinary workers in contact with infected dogs (Cefai *et al.*, 1994; Manian, 2003; Leonard *et al.*, 2006; Weese *et al.*, 2006b). However, in contrast to the strains most commonly recovered in horses (Weese *et al.*, 2005) and pigs (Voss *et al.*, 2005), MRSA found in dogs are indistinguishable from the most common hospital acquired strains isolated from humans (predominantly EMRSA-15, but also EMRSA-16 in the UK) and as such, it has not been possible to describe the direction of transfer between humans and dogs thus far (Baptiste *et al.*, 2005; Rich *et al.*, 2005; Weese *et al.*, 2006b; Leonard and Markey, 2007). While it is commonly acknowledged that dogs are likely to be the recipients of MRSA from humans and act as a secondary reservoir for potential reinfection or colonisation, rather than providing their own host-adapted source (Duquette and Nuttall, 2004; Rich *et al.*, 2005), no conclusive evidence exists to support this. Consequently, the potential contribution of dogs that are colonised or infected with MRSA to the burden of disease in humans and conversely, the potential contribution of colonised or infected humans to canine carriage or disease, has not been directly quantifiable. In order to begin to quantify these contributions, an initial assessment of the risk of acquisition of MRSA in dogs will facilitate the identification of important sources of the pathogen and the overall risk of acquisition of MRSA.

Qualitative risk assessment is often used as the first step in assessing the risk from a putative hazard. This chapter presents a qualitative assessment that aims to define the risk of acquisition of MRSA in a dog over any given 24 hour period.

3.2 Materials and Methods

Given the lack of alignment of the topic of this risk assessment with either of the OIE or CAC risk analysis frameworks (sections 2.1.1, 2.1.2), the qualitative study presented here was undertaken following a combination of the aforementioned structures (Codex Alimentarius Commission, 1999; OIE, 2003). The combined framework that was used is represented by the two main areas of **hazard identification and characterisation** and **risk**

estimation. **Exposure** and **consequence assessments** were undertaken within the step of risk estimation.

3.2.1 Hazard identification and characterisation

In the current assessment, the hazard is defined as MRSA. A generalisation of 'MRSA positive' or 'MRSA negative' is used consistently within this thesis for human, animal and environmental status and refers to a source from which MRSA can be cultured directly and that has the ability to contaminate, colonise or infect others.

The term 'MRSA positive' makes no effort to distinguish between carriage, colonisation and infection with this organism, and these states are considered as a spectrum within the definition of the hazard. It is acknowledged that colonisation and carriage states differ by the presence or absence of attachment of the bacteria to target areas (cells or extracellular matrices) respectively (Projan and Novick, 1997). However, reference in this work to 'carriage' does not preclude 'colonisation', as the resolution of the data are not sufficient to differentiate these states in much, if not all, of the literature. Furthermore, as defined by Williams (1963), carriage of *S. aureus* (and consequently MRSA) may be considered to be persistent or intermittent. Persistent carriers predominantly harbour a single strain of *S. aureus*, whereas intermittent carriers may carry any strain on an intermittent basis (Williams, 1963). Again, while this information represents important epidemiological distinctions, the resolution of the data presented herein is not sufficient to differentiate between these states and as such they are considered in combination. Similarly, while it is acknowledged that epidemiological differences exist, no attempt has been made to differentiate between different species or subtypes of MRSA within this qualitative assessment and the term MRSA encompasses CA-MRSA, HA-MRSA and all potential phenotypic and genotypic combinations.

Finally, marked geographical disparity exists in prevalence estimates for MRSA in humans and animals and variation may occur at national, community, institutional and individual levels (Livermore and Pearson, 2007). This is taken into account where possible in the current study.

3.2.2 Risk grading

In all steps of the risk assessment, the pre-defined qualitative risk categories of negligible, low, moderate and high were used. These categories were defined separately for the assessment of absolute risk (Table 3.1) and risk relative to a (qualitative) median approximation (Table 3.2).

Table 3.1. Definitions of the qualitative descriptors used for the qualitative risk assessment of MRSA acquisition in pet dogs (Moutou *et al.*, 2001).

Term	Definition
Negligible	When the probability of exposure or transmission is sufficiently low to be ignored, or if the event is possible only in exceptional circumstances
Low	When exposure or transmission may occur in some cases
Moderate	When exposure or transmission may occur in all cases
High	When exposure or transmission is likely to occur

Table 3.2. Definitions of the relative qualitative descriptors used for the qualitative risk assessment of MRSA acquisition in pet dogs.

Term	Definition
Negligible	When the probability of exposure or transmission is sufficiently low to be ignored, or if the event is possible only in exceptional circumstances
Low	When exposure or transmission is less than the qualitatively assessed median (central) level
Moderate	When exposure or transmission is around the qualitatively assessed median (central) level
High	When exposure or transmission is higher than the qualitatively assessed median (central) level

3.2.3 Risk estimation

A conceptual model, representing a chain of potential events that may befall a single animal in any given 24 hour time period to result in becoming 'MRSA positive', was defined as the first step of risk estimation.

Individual exposure assessments were undertaken to define the risk of exposure to MRSA through each potential route of acquisition outlined in the conceptual model. A systematic qualitative review of the available appropriate literature was used to consider all influential factors for exposure of an individual dog to MRSA during a 24 hour period for each route. Following the exposure assessment, a consequence assessment was undertaken, also by consideration of available literature. The consequence was defined as the likelihood of transmission of the organism over a 24 hour period, given that an individual dog is exposed to a MRSA positive individual, animal or environment. Relevant literature was sought that addressed the dependence of the consequence on transmission dynamics of the organism, host defence mechanisms and interactions between the at-risk dog and source of the hazard. An overall qualitative grading of risk of exposure and subsequent risk of transfer of MRSA (consequence) were derived using both absolute and relative categorical rankings (Tables 3.1, 3.2).

The resulting rankings (exposure and consequence) were then combined for an overall estimation of risk using a matrix approach, modified from an approach used by EFSA (2007), whereby the probability of exposure was combined multiplicatively with the probability of transmission for each putative route of MRSA acquisition (Table 3.3). This approach takes into account the reduction in probability that occurs with multiplicative combination, unlike other previously utilised methods (Sein, 1998; Moutou *et al.*, 2001). This combination was undertaken separately for absolute and relative risk estimates (Tables 3.1 and 3.2). As noted by Clough and colleagues (Clough *et al.*, 2006), the assignment of each risk value is subjective and no attempt was made to interpret qualitative outcomes numerically at any stage.

Table 3.3. Matrix combination of qualitative categories (adapted from EFSA (2007)).

Result of the assessment of parameter 2	Result of the assessment of parameter 1			
	Negligible	**Low**	**Moderate**	**High**
Negligible	Negligible	Negligible	Negligible	Negligible
Low	Negligible	Negligible - Low	Low	Low
Moderate	Negligible	Low	Low	Moderate
High	Negligible	Low	Moderate	Moderate

3.3 Results

A conceptual model, describing the potential routes or series of events that may occur leading to MRSA acquisition in a single dog over a 24 hour time period, was specified and is presented as Figure 3.1. Seven non-sequential and non-mutually exclusive routes for acquisition of MRSA in a dog were defined. Each pathway (A to G) represents a combination of the risk of exposure to a particular source of the pathogen and the risk of transmission of the organism to the at-risk animal. This combination corresponds to an estimate of overall risk.

It was assumed that exposure to MRSA could occur through direct contact with MRSA positive humans, dogs, other animals or through an environmental source. The environment was considered to be dependent on MRSA positive humans and dogs and alternative (non-canine) animal sources. Exposure pathways were stratified into exposure through attendance at a veterinary hospital and those exposed through 'the community' in a given 24 hour period. For the purpose of this thesis, 'the community' refers to any location outwith a veterinary hospital. Similarly, the term 'owner' refers to the primary carer of the dog in any given 24 hours (this may change from day to day), while the term 'secondary person' refers to a non-owner and may be another member of the household, or a non-household member.

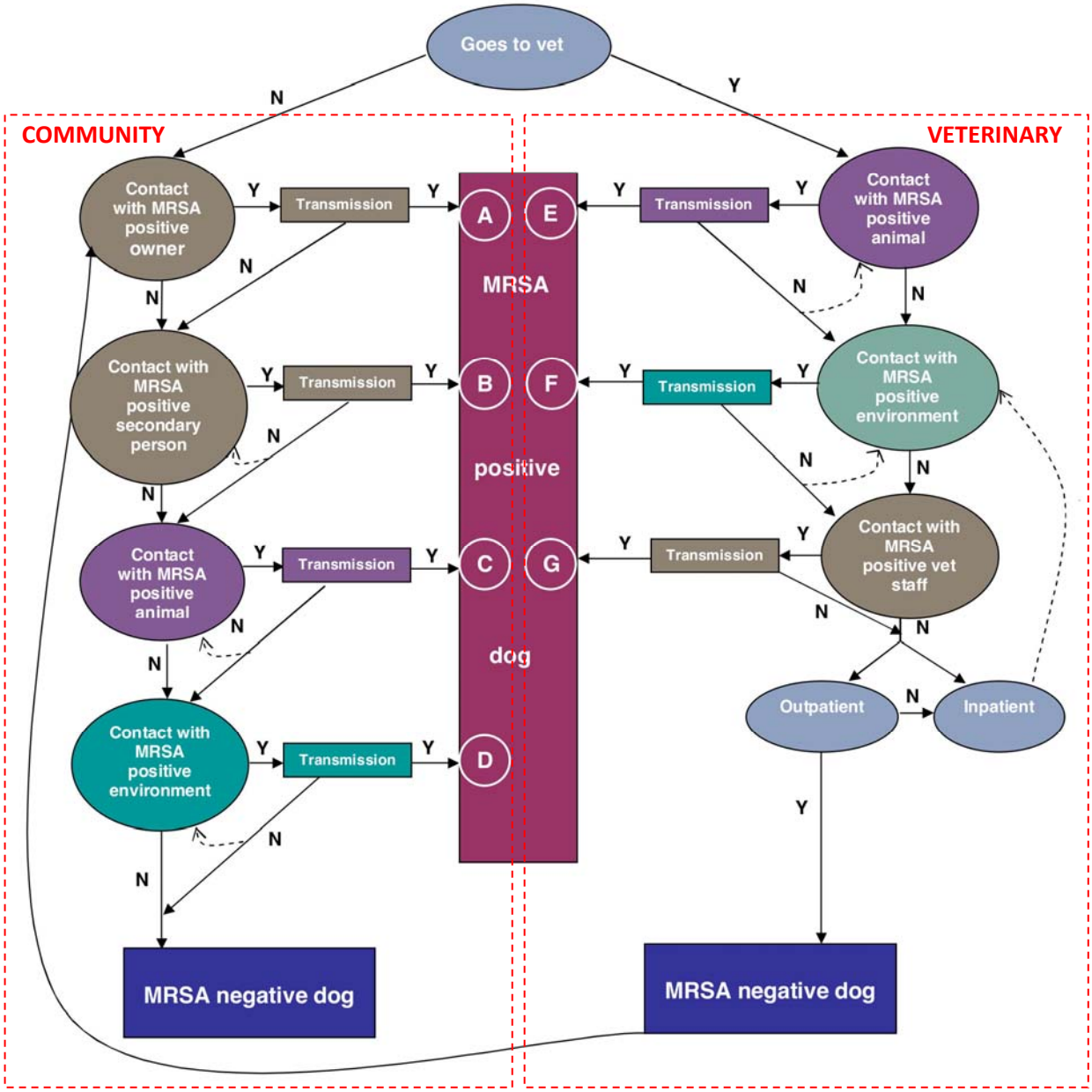

Figure 3.1. Conceptual pathways for exposure assessment of MRSA acquisition in a dog for any given 24 hour period
Y – yes, N – no, A-G – pathways resulting in MRSA acquisition. Pathways A to D and E to G are interchangeable

For an individual dog in any given 24 hour period, consideration of the potential routes of acquisition of MRSA will depend on whether they have attended a veterinary hospital or not. Animals that attend a veterinary hospital within the period of consideration also have the potential for exposure to MRSA through the community pathways if they are treated on an outpatient basis.

3.3.1 Exposure assessment

The risk of exposure to MRSA through any of the described routes is defined following consideration of previously published data.

3.3.1.1 Veterinary clinic attendance

A recent targeted questionnaire study found that 84% of dogs about which owners were questioned had attended a veterinary clinic at least once within the previous year, 62% had been vaccinated and 4% were presented for vomiting and diarrhoea (Westgarth *et al.*, 2008). Another large questionnaire study has reported a vaccination rate of 98% for dogs that attended a veterinary clinic in the last year, with the vast majority of these dogs being vaccinated within the year prior to questioning (Edwards *et al.*, 2004). This study also reported signs of ill health in over 18% of the dogs during a 2 week period, while another study estimated 16% of animals (dogs and cats) had at least one 'disease' (encompassing a broad range of chronic and acute conditions) at a single point in time (Freeman *et al.*, 2006). The proportion of ill animals that are presented for veterinary attention is not reported and, given that only 12% of ill humans will seek medical care (Dwight *et al.*, 2005), it is likely that owner-reported illness is a vast overestimation of veterinary clinic attendance due to illness. These data are further limited in their applicability for exposure assessment because detailed information on frequency and length of attendance and admission per disease and per animal at veterinary clinics are also lacking. In general, and in the most basic sense, four groups of animals are likely to attend veterinary clinics: routine outpatients (predominantly to obtain vaccinations), routine inpatients (elective surgeries and procedures and boarding pets), ill animals that are treated as outpatients and ill animals that are admitted for inpatient treatment.

While these data reveal that most animals will attend a veterinary clinic at least once within a year, it is likely that on any given day the probability of an individual dog attending a veterinary clinic is **low**. This estimate is absolute and it is not possible to obtain a relative estimate for this initial step for all potential routes of acquisition.

3.3.1.2 Route A – Acquisition of MRSA through contact with a MRSA positive owner

The prevalence of MRSA colonisation in human individuals in the general population in the UK has been estimated to be 1.5% (Abudu *et al.*, 2001) and similar estimates (0.8% to 3%) emanate from the USA (Jernigan *et al.*, 2003a; Jernigan *et al.*, 2003b; Kuehnert *et al.*, 2006). Some demographic groups, such as health care workers, are more like to be colonised with MRSA than the general population and it has been estimated that MRSA is carried in the external nares of 6.2% of health care workers in the UK (Eveillard *et al.*, 2004). Similarly, a review of the literature published globally has amalgamated the results of 104 geographically diverse studies to report a nasal carriage rate of 4.1% for health care workers (Albrich and Harbarth, 2008).

A number of other studies, undertaken in various geographical regions, have identified additional demographic groups at greater risk of nasal colonisation. Immunocompromised individuals, injection drug users, the elderly (Kuehnert *et al.*, 2006), in particular those admitted to a nursing home (Jernigan *et al.*, 2003a; Jernigan *et al.*, 2003b), the young, those with a past history of MRSA, those with previous antimicrobial usage, in particular quinolones and macrolides (increasing with number of prescribed antibiotics) (Schneider-Lindner *et al.*, 2007; Tacconelli *et al.*, 2008), and those hospitalised within the previous year (Jernigan *et al.*, 2003a; Jernigan *et al.*, 2003b; Furuno *et al.*, 2006) have been identified to be at increased risk for clonisation with MRSA (Hidron *et al.*, 2005; Furuno *et al.*, 2006; Elston, 2007). Similarly, risk factors for infection with MRSA include: MRSA colonisation, previous hospitalisation, surgery, immunosuppression, use of antibiotics and breach in epidermal integrity (Graffunder and Venezia, 2002; Safdar and Bradley, 2008). However, estimates of the magnitude of these risks, in the form of relative risk estimates, are not available for these groups of individuals.

The distribution of these risk factor groups with respect to dog ownership is unclear. While it has been shown that the presence of permanently sick/disabled persons in a household has been associated with increased odds of owning a dog and that households with adults over 60 years of age are less likely to own a dog (Westgarth *et al.*, 2007), lack of detailed information on dog ownership does not allow stratification with respect to risk factors for carriage of MRSA. Therefore, it must be assumed that dog owners are evenly distributed through risk factor groups.

The number and types of interactions between dogs and their primary owner are difficult to quantify. However, it is likely that all dogs have at least some form of close interaction with their owners on a daily basis, given the dependency of the relationship. Westgarth and colleagues (Westgarth *et al.*, 2008) report close dog-owner interaction, with their questionnaire study of the owners of 279 dogs revealing that 14% of these dogs slept on human beds, 45% lay on owners' laps 'sometimes' or 'often' and that sniffing, nudging with the nose and licking hands were commonly reported to occur 'sometimes' or 'often'.

Risk estimation

These data show that, using an absolute scale (Table 3.1), the probability of exposure to an MRSA positive owner is **low**. When considering risk relative to a median probability of exposure to a source of MRSA within the community, the estimate (Table 3.2) is **high**.

3.3.1.3 Route B – Acquisition of MRSA through contact with a MRSA 'secondary' person

'Secondary' people may be divided into those who are household members and those who are not. The number of secondary contacts that an at-risk dog will have in a given 24 hour period will vary. It has been shown that households that own dogs are associated with a greater number of human occupants than those that do not (Eveillard *et al.*, 2004; Westgarth *et al.*, 2007) and also that, on average, a dog will have contact with between three and fourteen alternative (non-household member) humans per day (Westgarth *et al.*, 2008; Heller *et al.*, 2009).

Again, due to insufficient demographic information, the likelihood that any of these secondary contacts is MRSA positive requires the same consideration as the prevalence information that has been presented for 'contact with a MRSA positive owner'. However, it is expected that a correlation exists between the MRSA status of an owner and a secondary person within the same household (ie if one household member is colonised then the others are more likely to be colonised also) (Noble *et al.*, 1967; Eveillard *et al.*, 2004) but this is unlikely to extend to external contacts. In one of the few studies undertaken that attempts to describe and quantify this correlation, the families of ten MRSA positive health care workers were investigated to find that members of four of the families were also colonised with the same strain of MRSA (Eveillard *et al.*, 2004). Similarly, in another study, the household contacts of eight out of 11 health care workers who were recolonised with MRSA subsequent to initial successful decolonisation were found to have at least one household member that was also colonised with MRSA (Kniehl *et al.*, 2005). Environmental contamination was also found in the latter study and this will be addressed subsequently. In addition, specific subpopulations of pets exist, including those involved in animal assisted interventions in hospitals and long-term care facilities, that are at greater risk of MRSA acquisition (Lefebvre *et al.*, 2009) and, while these groups are likely to be small in number, they should be identified as high risk for exposure to MRSA contaminated persons and environments.

Risk estimation

The probability of exposure to a MRSA positive secondary person is **low** using absolute definitions. However, the probability is reclassified as **high** if they are a household member and **moderate** if they are not when using measures relative to the median level of exposure to a source of MRSA within the community. In addition, it is likely that a dependency exists between the MRSA status of household members.

3.3.1.4 Route C – Acquisition of MRSA through contact with a MRSA positive animal within the community

Little information is available regarding potential contacts between dogs and other animal species within the community. However, small amounts of qualitative and quantitative data are available to define dog-to-dog interactions. It has recently been shown that the

most common number of direct contacts of an at-risk dog with other dogs in a given 24 hour period is between one and ten (Westgarth *et al.*, 2008; Heller *et al.*, 2009). These estimates imply a high frequency of dog-to-dog contact and the common behaviours of 'being playful' and 'sniffing' are reported to occur 'sometimes' or 'often' in 59% and 81% of the dogs surveyed respectively (Westgarth *et al.*, 2008). While it is not possible to quantify the amount of nose-to-nose or other close contact with the data that are available, the nature of the contacts that have been reported is often superficial and it is unlikely that prolonged close contact occurs in many cases. Furthermore, the prevalence of MRSA colonisation in dogs within the general community across a number of countries has been estimated to be between 0 and 1% (Murphy *et al.*, 2006; Rich and Roberts, 2006; Vengust *et al.*, 2006; Hanselman *et al.*, 2008). It is assumed that contact with other animals within the community will be less likely and less intimate than contact with dogs and therefore this species is used to identify the upper limit of contact with other animals within the community.

Recent studies have identified farm animals (pigs and cattle) in The Netherlands and pigs in countries including Canada, Singapore, Germany, Austria and Denmark as having the potential to be colonised with MRSA ST398 (Guardabassi *et al.*, 2007; Sergio *et al.*, 2007; Witte *et al.*, 2007; Khanna *et al.*, 2008; Meemken *et al.*, 2008). Prevalence estimates vary between countries, but are as high as 39% of all slaughter pigs, representing 81% of farms in The Netherlands (de Neeling *et al.*, 2007). While data are not available regarding type and amount of contact between dogs and farm animals, it is possible that dogs housed on farms will have an increased risk of MRSA colonisation, similar to the findings of in-contact humans such as farmers and veterinarians, in these geographical areas (Voss *et al.*, 2005; Wulf *et al.*, 2006; van Loo *et al.*, 2007b). However, it must be noted that MRSA ST398 has not been cultured from a canine source. Similarly, ST398 has not been identified in animals in many countries at the time of writing and there is no evidence that farm animals represent an increased risk for the source of these bacteria outside of the countries named above.

Risk estimation

The probability of exposure to a MRSA positive animal within the community is **low** using absolute and relative definitions.

3.3.1.5 Route D – Acquisition of MRSA through contact with a MRSA positive environment

To the author's knowledge, few estimates exist of the prevalence of environmental contamination with MRSA outside of veterinary and human hospitals. One study, discussed previously with respect to household contact colonisation, screened the household environments of eight MRSA carrying health-care workers who were recolonised after initial eradication regimens, to find seven of the eight households contaminated with MRSA (Kniehl *et al.*, 2005). In this study, effective household decontamination was associated with eradication of carriage status in most cases, and household contamination was implied to be responsible for a number of re-colonisation scenarios (Kniehl *et al.*, 2005). In a potentially less biased study, the households of 35 health-care and non health-care workers in the USA with unknown MRSA status and with pets or children, were screened for *S. aureus* and MRSA (Scott *et al.*, 2008). In this study, MRSA was isolated from 26% of the home environments and an association was found between owning a cat and presence of environmental MRSA, although only univariable analyses were undertaken and sample sizes were small (Scott *et al.*, 2008). Furthermore, a study undertaken in the USA concluded that antimicrobial resistant bioaerosols are commonly found in residential homes, and that resistant strains of *S. aureus* are present in higher concentrations within homes compared to outside (Gandara *et al.*, 2006). To the author's knowledge, no studies of this nature have been undertaken with respect to colonised or infected dogs.

In this risk assessment, exposure to food is considered an environmental source of MRSA for dogs. While it is considered that the role of food in the transmission of MRSA to humans is minimal, it can be asserted that dogs have a very different relationship with food than humans, with far more prolonged and predominantly facial contact with some types of food, including bones and meat-based treats. Some data exist to document pathogen transfer from pet treats to both dogs and their owners (Public Health Agency of Canada, 2006), but this risk appears to be mediated through ingestion and faecal shedding. Contact with and ingestion of raw diets has been shown to be associated with faecal shedding of salmonellae and *E.coli*, but not MRSA (Lefebvre *et al.*, 2008). MRSA has been isolated, at low prevalence, from meat products in Italy (Normanno *et al.*, 2007; Simeoni *et al.*, 2008) and The Netherlands (van Loo *et al.*, 2007a). As such, the risk of MRSA acquisition and shedding in dogs through ingestion can be regarded as negligible, although dogs fed raw

meat diets do have contact with the surface of the meat, and contaminated surfaces such as bowls, which may harbour MRSA and as such the potential for meat to act as an environmental source of the organism should not be discounted.

Risk estimation

The probability of exposure to a MRSA positive environment within the community is **low** using absolute measures and **moderate** using relative definitions. In addition, it is likely that a dependency exists between the MRSA status of household members (owners and secondary persons) and the community environment.

3.3.1.6 Route E – Acquisition of MRSA through contact with a MRSA positive animal within a veterinary hospital

It can be reasonably assumed that the probability of an at-risk dog contacting another dog directly at a veterinary hospital is low (the only situation should be in the waiting room). However, it is likely that the probability of waiting-room contact varies markedly between veterinary clinics and is dependent on the size of the clinic, density of patients and clinic operational procedures. As presented for 'contact with a colonised animal within the community', the prevalence of MRSA colonisation in dogs has been estimated to be between 0 and 1% (Murphy *et al.*, 2006; Rich and Roberts, 2006; Bagcigil *et al.*, 2007; Hanselman *et al.*, 2008) within the general community. Two of these studies report a prevalence of 0 and 0.5% in dogs entering veterinary teaching hospitals in Denmark and Canada, respectively (Bagcigil *et al.*, 2007; Hanselman *et al.*, 2008) and another reports, without describing methods, a prevalence of 0.4% in dogs entering two first opinion veterinary clinics in the UK (Rich and Roberts, 2006). A higher prevalence has been found in dogs sampled whilst hospitalised at a veterinary hospital (up to 8.9%) (Loeffler *et al.*, 2005) although this may represent an outbreak scenario.

Risk estimation

The probability of exposure to a MRSA positive animal within a veterinary clinic is defined as **low** using absolute definitions. Similarly, the probability is also defined as **low**

when using measures relative to the median level of exposure to a source of MRSA within a veterinary clinic.

3.3.1.7 Route F - Acquisition of MRSA through contact with a MRSA contaminated environment within a veterinary hospital

The prevalence of environmental contamination with MRSA has been estimated to be around 10% in two studies of sites within small animal veterinary hospitals (Loeffler *et al.*, 2005; Murphy *et al.*, 2006). However, in a separate study undertaken in a geographical region associated with a low prevalence in veterinary staff, a markedly lower environmental prevalence of 1.4% was found (Heller *et al.*, 2009). As environmental contamination is unlikely to be independent of human and canine colonisation, these estimates are likely to be correlated. Any dog attending a veterinary hospital will come in contact with the environment to some degree, although the extent and nature of contact will vary on a case-by-case basis. In the current model, it is considered that the same risk is present for the clinical environment that will be contacted by an outpatient or inpatient, although the number and length of contacts in any given 24 hour period are likely to be greater for inpatients.

Risk estimation

The probability of exposure to a MRSA positive environment within a veterinary clinic is **low** using absolute and **moderate to high** relative to the median level of exposure to a source of MRSA within a veterinary clinic.

3.3.1.8 Route G - Acquisition of MRSA through contact with MRSA positive veterinary staff within a veterinary hospital

It is considered certain that a dog will come into contact with a member of veterinary staff while at a veterinary hospital, irrespective of whether the dog progresses to be an inpatient

or remains on an outpatient basis. The type and extent of contact, including the number of staff members contacted, will vary depending on the severity of the illness and the nature of the intervention required. Two studies undertaken at international veterinary conferences revealed colonisation rates of 4.4% (Hanselman *et al.*, 2006a) (North America) and 9.8% (Deacon *et al.*, 2006) (UK) in small animal veterinarians, and a cross-sectional study undertaken in staff at a tertiary referral veterinary hospital in London revealed a prevalence of 17.9% (Loeffler *et al.*, 2005). However, a similar study undertaken at the University of Glasgow revealed a markedly lower prevalence of 3.1% in their veterinary staff (Heller *et al.*, 2009). Again, although no objective data exist, it is well documented that, as in human hospitals, MRSA may transfer between veterinary staff and patients, and identical strains of MRSA have been identified in veterinary staff and dogs in a number of studies (Baptiste *et al.*, 2005; Weese *et al.*, 2006b; McLean and Ness, 2008).

Risk estimation

The probability of exposure to MRSA positive veterinary staff within a veterinary clinic is **low** using absolute measures and **high** using measures relative to the median level of exposure to a source of MRSA within a veterinary clinic. In addition, it is likely that a dependency exists between the MRSA status of veterinary staff, patients and veterinary environmental contamination.

3.3.2 Consequence assessment

Definition of the consequence of exposure to a MRSA positive source requires consideration of a) the amount of pathogen that the dog is exposed to and b) the likely dose-response mechanism that occurs after exposure.

3.3.2.1 Quantity of pathogen exposure

Similar to factors considered for zoonotic transmission of disease, the likelihood of transmission of bacterial organisms to animals requires consideration of the number of MRSA positive sites, animals or humans that the dog is exposed to, the quantity of excretion of the bacteria from these sources (human and animal), mode of transmission of

the bacteria, the behavioral characteristics of the animal, the immune response of the animal and existing measures of prevention of pathogen transfer (Chomel and Arzt, 2000; Robinson and Pugh, 2002; Murphy, 2008). The number of positive sites and behavioural characteristics of the at-risk dog may be addressed through knowledge of the number and nature of interactions that have been discussed throughout the exposure assessment. In summary, it is likely that interactions are greater in number and intimacy between dogs and their owners or other household members than with other humans (Westgarth *et al.*, 2008). Similarly, it is likely that intimate interaction exists between dogs and veterinary staff while an animal is hospitalised. The use of mitigation strategies such as hand hygiene in any of these humans prior to dog contact is unknown but it may be postulated that hand washing is far more likely to occur after interacting with a dog than prior to interaction, in both community and veterinary hospital settings.

The likelihood and quantity of excretion of MRSA from a colonised individual is not constant. The main routes of dissemination of MRSA from humans are via touch (primarily or through secondary contact with colonised nasal or other mucosae) and, less commonly, through aerosolisation (Sheretz *et al.*, 1996; Shiomori *et al.*, 2001; Bassetti *et al.*, 2005; Wertheim *et al.*, 2005). The number of MRSA organisms excreted or disseminated is related to the number that are present in the anterior nares (or other area of the body) and increased dissemination has been associated with factors including persistent (as opposed to intermittent) carriage, concurrent perineal carriage and the presence of upper respiratory tract infections and allergies (White, 1961; Sherertz *et al.*, 2001; Wertheim *et al.*, 2005; Bischoff *et al.*, 2006). Similarly, it is widely accepted that use of antimicrobials to which MRSA is resistant will allow the population density of the bacteria to increase in the absence of competitive flora and this should be considered in addition to general individual variation for both human and animal sources (Smith *et al.*, 2002).

Once an animal is exposed to a colonising dose of MRSA, there is a proportional likelihood that those bacteria will be transferred to the animal and establish some form of residence (contamination, colonisation or infection). As with excretion, transfer efficiency can be viewed as heterogeneous and is dependent on factors including the type of source, survival of the organism and nature of contact between the source and the at-risk animal (Rusin *et al.*, 2002). MRSA is known to transfer to humans via the primary route of touch or contact (Wertheim *et al.*, 2005). Other, less likely, routes include aerosolisation, although this route is of greater importance in dissemination of the bacteria as opposed to acquisition (Sheretz *et al.*, 1996; Wertheim *et al.*, 2005), and ingestion (Kluytmans *et al.*,

1995). It must be noted that ingestion is only likely to result in colonisation or infection in exceptional circumstances, due to the protective presence of gastric acid, normal gastrointestinal flora, and a functional immune response, all of which work to prevent colonisation of transient microorganisms such as MRSA (Kluytmans *et al.*, 1995).

3.3.2.2 Dose-response considerations

To the author's knowledge, a dose-response relationship has not been defined for *S. aureus* or MRSA in humans or dogs. In humans, a few studies have attempted to quantify the transfer efficiency of bacteria from various sources, and these report efficiencies of 42% between smooth fomites and the hand (Rusin *et al.*, 2002), and 14-17% between MRSA positive patients and healthcare worker hands (non-gloved and gloved, respectively) (McBryde *et al.*, 2004). However, these studies are only minimally useful in the current assessment and do not allow inference of colonisation or infection secondary to transfer.

It is prudent to consider host-defense mechanisms in the discussion of the potential consequence of exposure of a dog to MRSA. While risk factors for colonisation and infection with MRSA in humans are well defined (discussed in section 3.3.1.2) and many of the MRSA isolates that have been reported from dogs have been obtained from surgical site and wound infections (Tomlin *et al.*, 1999; Leonard *et al.*, 2006; McLean and Ness, 2008), formally defined risk factors are yet to be identified for MRSA infection in dogs, although studies are in progress (Pfeiffer *et al.*, 2005). Risk factors have been identified for MRSA colonisation in horses and these include prior colonisation and antimicrobial administration within 30 days (Weese and Lefebvre, 2007).

Risk estimation

The data available to inform a consequence assessment (presented above) are minimally useful for absolute categorical estimation of risk of organism transfer, given exposure to different sources. However, using the absolute categorical definitions, and taking into account the extent and nature of contact with different sources, it can reasonably be postulated that the probability of transmission of MRSA to an individual dog, given that exposure to an MRSA positive source has occurred may be classed as **moderate** from an owner or veterinary staff, but **low** from all other routes as defined in Figure 3.1. Using

relative measures, the probability of transmission may be classed as **high** for MRSA positive owners and other household members, **moderate** from an MRSA positive environment and **low** from MRSA positive animals and non-household member humans, when compared to the median probability of transfer of MRSA within the community. The relative probability of transmission within veterinary clinics may be classed as **high** from veterinary staff, **moderate** from the environment and **low** from other animals, when compared to the median probability of transfer of MRSA within a veterinary hospital.

3.3.3 Overall Estimation

Taking into consideration all of the evidence presented above, the results of the exposure and consequence assessments are displayed (in absolute and relative qualitative terms) in Table 3.4 and overall estimates, representing matrix-based combinations of these results (Table 3.3), are presented in Table 3.5.

Table 3.4. Qualitative exposure and transmission risk estimations for community and veterinary routes of acquisition of MRSA in the dog.

	Route		Exposure		Transmission	
			Absolute*	Relative*	Absolute	Relative
Community	Goes to the vet		**Low**	**Low**	**N/A**	**N/A**
	MRSA positive Owner (A)		**Low**	**High**	**Moderate**	**High**
	MRSA positive secondary person (B)	Household	**Low**	**High**	**Low**	**High**
		Non-household	**Low**	**Moderate**	**Low**	**Low**
	MRSA positive animal (C)		**Low**	**Low**	**Low**	**Low**
	MRSA positive environment (D)		**Low**	**Moderate**	**Low**	**Moderate**
Veterinary	MRSA positive animal (E)		**Low**	**Low**	**Low**	**Low**
	MRSA positive environment (F)		**Low**	**Moderate - High**	**Low**	**Moderate**
	MRSA positive veterinary staff (G)		**Low**	**High**	**Moderate**	**High**

* Absolute and relative refer to the qualitative risk categories defined in Tables 3.1 and 3.2 respectively

Table 3.5. Qualitative overall risk estimation of acquisition of MRSA in a dog via differing routes.

	Route		Absolute* risk estimation (combined with Low risk of veterinary attendance)	Relative* risk estimation
Community	MRSA positive Owner (A)		**Low**	**Moderate**
	MRSA positive secondary person (B)	Household	**Negligible - Low**	**Moderate**
		Non-household	**Negligible - Low**	**Low**
	MRSA positive animal (C)		**Negligible - Low**	**Negligible - Low**
	MRSA positive environment (D)		**Negligible - Low**	**Low**
Veterinary	MRSA positive animal (E)		**Negligible - Low (Negligible - Low)**	**Negligible - Low**
	MRSA positive environment (F)		**Negligible - Low (Negligible – Low)**	**Low - Moderate**
	MRSA positive veterinary staff (G)		**Low (Negligible - Low)**	**Moderate**

* Absolute and relative refer to the qualitative risk categories defined in Tables 3.1 and 3.2 respectively

3.4 Discussion

This chapter has presented a qualitative assessment of the acquisition of MRSA in a dog over a 24 hour period. This qualitative study provides an opportunity to obtain an estimate of the overall risk of MRSA acquisition, i.e. an exposure assessment for MRSA, in dogs and, more importantly, to attribute qualitative risk rankings to the various routes by which MRSA may be acquired. Additionally and most importantly, the study has identified and prioritised data gaps and potential dependencies for use in future quantitative assessments.

It is of great importance that the results of this chapter are interpreted in light of the specified categorical definitions (Tables 3.1 and 3.2) as these estimates are not intended to stand alone. With that in mind, and focusing initially on the absolute categorical risk estimates defined in Table 3.1, the results show that the overall absolute risk of MRSA acquisition in a dog for any given 24 hour period is likely to vary from low ('may occur in some cases') to moderate ('may occur in all cases'), depending on the route of acquisition. While fine resolution is not possible from these absolute categorical estimates, comparison between locations can be made and it was found that the greatest risk emanates from humans (owners and staff) in both community and veterinary settings and that, even when the probability of attending a veterinary clinic is taken into account, these risks are equally ranked.

The use of relative risk categories in this study facilitates greater resolution of the risk estimates within each of the locations addressed (community and veterinary hospital). While comparison of risk between locations is not possible using relative measures (as the median values vary from setting to setting), these rankings improve discrimination between degrees of risk for different routes within each location. The results of the relative risk analyses revealed that, within the community, household members posed the greatest risk for MRSA acquisition in dogs, followed by non-household members and the environment, while animals posed the least risk. Similarly, within the veterinary clinic, staff were found to pose the greatest risk to dogs, followed by the environment and the least important route was through other animals. These relative measures only have merit for use in combination with an absolute categorical estimate of risk, as they do not provide any insight into the overall measure of risk but simply allow for greater discrimination within absolute categories.

All of the results of this study require interpretation in light of the consequence of acquisition of MRSA for a dog, apart from the direct animal health consequences, and this can be assessed on two levels. Firstly, at a local level, the direct consequence of carriage, colonisation or infection of a dog with MRSA includes the presence of an additional source of environmental contamination within the veterinary hospital and in the community, along with the presence of a potential source of MRSA for in-contact humans. At a more general or global level, colonisation of a dog with MRSA represents the potential for development of a self-perpetuating non-human biological reservoir of MRSA. This has implications for an increased likelihood of exposure and subsequent colonisation or infection for humans and other animals, contamination of the environment and, while it is known that transfer of *mec*A occurs relatively rarely between bacterial species, the provision of a niche that provides the potential for transfer of resistance genes to other staphylococcal organisms, such as *S. pseudintermedius* should not be overlooked (Duquette and Nuttall, 2004).

Although the results of this study may be intuitive, the value of the qualitative absolute measures and the use of a combination matrix in this study are questionable. Within the definitions used for qualitative absolute measures (Table 3.1), the use and interpretation of the term 'may' results in the attachment of a variable component to each category. Probabilistically, if the likelihood of occurrence changes between groups (that is, the quantitative definition of the term 'may' is variable), it is possible that the numerical equivalents of the qualitative groups are, in fact, inverted, otherwise referred to as 'reversed rankings' (Cox, 2005). Furthermore, due to the lack of resolution within categories, the use of qualitative absolute risk estimates in the current assessment does not allow appropriate discrimination between the risk posed by different routes of MRSA acquisition (Cox, 2005). The additional use of relative measures goes some way to addressing this limitation, although these measures do not allow for between-location comparisons. Additionally, while it is acknowledged that it is not possible to obtain truly absolute categories of risk in qualitative studies (Peeler *et al.*, 2004), in this study it was also not possible to obtain a combined categorical estimate of overall risk, as an appropriate algorithm could not be specified for additive combinations of qualitative categories where the categories are not mutually exclusive. Simple adherence to the matrix combination method to produce an overall estimate would have resulted in spurious results in this case. A further criticism of the method lies with the definition of categories. It could be argued that this analysis would have benefited from the inclusion of additional categories such as 'very low' and 'extremely low', as employed by EFSA (2007), in order to provide greater detail and breakdown of risk rankings, particularly as there is clustering

of exposure and transmission estimates within the 'low' category currently. However, differentiating between 'low', 'very low' and 'extremely low' exposure or transmission for MRSA would have been somewhat arbitrary and as such, this breakdown would have added a further level of uncertainty to the model. Finally, the counterintuitive finding that consideration of the probability of attending a veterinary clinic on any given day (assessed as 'low') did not change any of the overall absolute risk estimates requires discussion. This finding is likely to be a result of a loss of information that has occurred with successive levels of qualitative coding and reflects inconsistency in the categorical coding process between steps (Cox *et al.*, 2005). A potentially marked loss of information is reflected in this finding and it highlights that a matrix combination is unable to account for vast variation in estimates if they fall within a single category (in some cases these variations may be in orders of magnitude).

The method used in this study represents a reproducible and repeatable format for qualitative risk assessment procedure that, if utilised exclusively, would allow direct comparison between subsequent qualitative risk assessments (Dufour and Moutou, 2007). However, it is clear that these goals have not been met within the current study and that the prescribed structure is unsuitable for the topic area presented in this chapter, where a non-modular, non-step-wise model has been specified. Other broad areas of study, including food safety and import risk analysis, that can be represented by a modular, sequential process, appear to be more suitable to this method (Moutou *et al.*, 2001; Clough *et al.*, 2006). However, some of the methodological issues that have been highlighted in the current study, including loss of information with successive levels of coding and inability to account for marked variation in estimates within categories, are still relevant for these other areas (Cox *et al.*, 2005).

The key areas of uncertainty that have been identified in this study include contact information for dogs with all potential sources of MRSA, dose-response data for MRSA in humans and dogs, risk factors for MRSA colonisation or infection in dogs and temporal information on MRSA transmission. These areas are important to be aware of prior to undertaking further analyses and should be addressed and quantified where possible in future studies. Similarly, the identification of correlations in the form of dependencies between MRSA status of: a) in-contact humans (particularly household members), b) household members and the community environment to which the dog is exposed and c) veterinary staff, patients and the veterinary environment is important. While it is not

possible to account for these dependencies in this, or any, qualitative study, it is important that this information is used and factored into future quantitative assessments.

In this assessment it has been shown that humans represent the most important source of MRSA for dogs in both community and veterinary hospital settings. The environment was found to be secondary to humans in terms of importance and other animals less still. This study has highlighted some important methodological limitations of a technique that is heavily relied upon for qualitative risk assessments as the first step in the risk assessment process. Given the limitations of the prescribed methods as applied to the problem under consideration, further validation or repudiation of the findings contained herein is called for using a more robust method. A subsequent quantitative assessment applied to the same conceptual model of MRSA transmission is indicated to provide a more defensible appraisal, whilst also accounting for the variability, uncertainty and dependency that has been difficult to assess using the qualitative approach.

CHAPTER 4

FACT FINDING SURROUNDING MRSA IN PET DOGS

4.1 Introduction

The qualitative risk assessment presented in Chapter 3 suggested a number of data gaps that require consideration prior to the implementation of a quantitative risk assessment in the same area. Consideration of these data gaps may take many forms, one of which is dedicated data collection. The funding of this PhD studentship is through a Defra VT0101 research fellowship, the remit of which is not focused on data collection, but rather the development of quantitative epidemiological skills (Defra, 2006). However, as part of the 'capacity building' remit of the VT0101 programme, a number of undergraduate summer students and a graduate MSc student were available to be aligned with the author's research fellowship and it was possible to undertake dedicated data collection through small projects that were supervised by the author. The results of these projects were then analysed independently and included as a form of 'fact finding' within the risk assessment process.

4A: Prevalence and distribution of MRSA within the environment and staff of a university veterinary clinic

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This work was undertaken as part of a summer student project (2006) by Ms Susan Armstrong. All genetic analyses were undertaken at the Scottish MRSA Reference Laboratory, with the exception of the *spa* typing, which was undertaken by Dr Arshnee Moodley, University of Copenhagen.

4A.1 Introduction

The contribution of contamination of the human hospital environment to hospital acquired infection is historically a controversial area (Boyce, 2007; Dancer, 2007). However, there has been increasing attention to the possible role of the environment as a potential reservoir for MRSA infection. Recent studies have demonstrated the presence of MRSA on various environmental surfaces within patient wards, contamination of hands and gloves with infectious doses of MRSA from these surfaces (McBryde *et al.*, 2004) and patient infection subsequent to primary and secondary environmental contacts (Boyce *et al.*, 1997; Hardy *et al.*, 2006). Certain environmental sites including door-handles, computer keyboards, patient bed surrounds and stethoscopes are considered more likely to harbour MRSA than others (Oie *et al.*, 2002; Dancer, 2007).

In one study of environmental contamination with MRSA in a small animal veterinary hospital, researchers sampled 30 environmental sites and reported a 10% prevalence (Loeffler *et al.*, 2005). This finding, along with the high carriage rate of MRSA reported in veterinary personnel and knowledge of the organisms' ability to transfer between humans and animals (Manian, 2003; Weese *et al.*, 2006b), highlights the potential for

environmental contamination to serve as an important reservoir for MRSA contamination, colonisation and infection within a veterinary hospital setting. Given the small sample size and large prevalence reported in the previously quoted study (Loeffler *et al.*, 2005), further quantification of the risk posed by veterinary hospital environmental contamination is required to ensure accurate representation within the quantitative risk assessment model.

The aim of this study was to define the distribution of MRSA within the environment of a small animal veterinary hospital with respect to the departmental regions and colonisation status of the staff.

4A.2 Materials and Methods

Sampling of the hospital environment and staff was conducted at the University of Glasgow Small Animal Hospital on a single day (d1) within working hours. Environmental sampling was repeated 14 days later (d14). No known cases of MRSA infection were present in hospitalised animals on the days of, or for six months prior to, the days of sampling.

4A.2.1 Environmental sampling

A sample size calculation, assuming an infinite 'population' of sample sites and using an estimated prevalence of 10% (Loeffler *et al.*, 2005), with 95% confidence to detect prevalence to within +/-5%, indicated that 138 sites were required. One hundred and forty sites were sampled on d1. The clinic was classified into 14 areas: intensive care unit, pre-theatre, theatre, canine wards (further classified as surgical, medical, chemotherapy and staff dogs/greyhounds), laundry, radiology, consulting rooms (further classified into medical and surgical), client waiting room and corridors. Sites were chosen that were broadly replicable between areas (such as floors, door handles, work surfaces, taps, kennels and drains) to allow comparison. Additional sites relevant and specific to each area were also included.

Samples were collected using single use sterile cotton applicator swabs moistened with sterile distilled water. Each swab was rotated and moved horizontally backwards and

forwards within a $10cm^2$ area (defined by single use sterile cardboard square templates, where possible) for 10 seconds. One sample was obtained for each site.

A second sampling of environmental sites was undertaken on d14 to assess the temporal stability of the initial findings. A total of 60 sites were sampled on this occasion, including all sites positive at d1, along with the remaining number of randomly selected sites that were initially negative.

4A.2.2 Human sampling

Approval was obtained from the University of Glasgow, Faculty of Veterinary Medicine Ethics Committee. It was calculated that, based on a total population of 80 hospital staff and with an estimated prevalence of 10% (Loeffler *et al.*, 2005; Hanselman *et al.*, 2006b) and 95% confidence to detect prevalence to within +/-5%, a sample size of 51 was required.

All staff members and students on clinical rotation at the University of Glasgow small animal hospital were approached to participate in the study via a letter that outlined the study and contained general information on MRSA. Participation was voluntary and signed informed consent was obtained from all participants. Personal information was kept anonymous and information about role (veterinarian, veterinary nurse, veterinary student, administration), department within which the participant worked (medicine, surgery, theatre, other) and whether or not they had had recent contact with human healthcare workers, hospital patients or healthcare institutions, was documented. A sterile cotton applicator swab was issued to each participant and they were instructed to rotate the swab in each nostril for 5 seconds under the supervision of the author.

Results (presence or absence of MRSA) were made available to the participants through sealed letters addressed with the code of each participant. Letters included information about MRSA carriage and advised further discussion with their own medical practitioner if concerned. Positive individuals were invited to return for re-sampling with obvious loss of anonymity to the author.

4A.2.3 Isolation of MRSA

All samples were enriched, cultured, and tested for antimicrobial susceptibility as outlined in sections 2.4.1.1 and 2.4.1.2. Genotypic analysis, PFGE and *spa* typing were also undertaken on each MRSA positive isolate using the methods described in sections 2.4.1.3, 2.4.1.4 and 2.4.1.5.

4A.3 Results

4A.3.1 Environmental sampling

Two environmental sites (2/140, 1.4%; 95% Confidence Interval 1.7-5.1%) were found to be positive for MRSA on d1. These were a foam wedge used for positioning animals for radiography and a door handle located between the waiting room and the corridor leading to the wards. One site (1/60, 1.7%; 95%CI 0.4-8.9%), the same corridor door handle that was positive on d1, was found to be positive for MRSA on d14. The environmental isolates are referred to as E1, E2 and E3 respectively.

4A.3.2 Human sampling

Sixty four of the 69 personnel present on d1 (92.8%) agreed to provide nasal swab samples. These personnel comprised veterinary nurses (n = 20), veterinary nursing students (n = 8), veterinarians (n = 28), veterinary students (n = 5) and receptionists (n = 3). All were working on a daily basis within the small animal clinic at the time. Two samples (3.1%; 95%CI 0.4-8.4%), referred to as H1 and H2, were positive for MRSA. Both of these staff members were veterinarians who worked in the same department and had no known contact with human healthcare workers, hospital patients or healthcare institutions. Neither of the positive staff members presented for re-sampling.

4A.3.3 Phenotypic and genotypic analyses

The results of antimicrobial susceptibility testing and genotypic analyses are displayed in Table 4.1. All MRSA isolates were typed as EMRSA-15 by PFGE, all were positive for the *mec* and *nuc* genes, while E1 was the only isolate positive for PVL. Isolates E2, E3 and H2 had indistinguishable PFGE patterns which differed from E1 by three bands and H1 by two bands (Figure 4.1, Table 4.1). Isolates E1 and H1 differed by one band (Figure 4.1). These findings indicate a close relationship between all strains according to the Tenover criteria (Tenover *et al.*, 1995). Sequence analysis of region X of the *spa* gene resulted in the detection of three distinct *spa* types (Table 4.1). The same *spa* type was observed in two environmental samples and one human isolate. BURP cluster analysis concurred with the PFGE results, further illustrating that all strains are closely related.

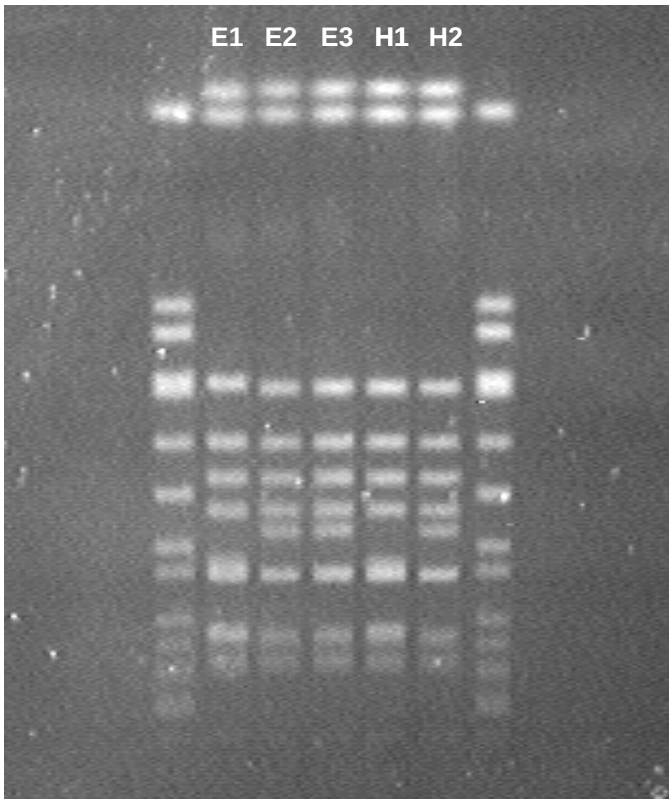


Figure 4.1. PFGE types identified in MRSA isolates from the environment (E1, E2, E3) and in veterinary staff (H1 and H2) from a university small animal veterinary hospital.

Table 4.1. Antimicrobial resistance and genotypic profiles of MRSA isolated from the environment (E1, E2, E3) and staff (H1 and H2) at a university small animal veterinary hospital.

Isolate	Origin	Antimicrobial susceptibility*	*mec*A	*nuc*	*pvl*	SCC*mec*	*spa* type	PFGE profile
E1	Wedge	Pn Cx	P	P	P	IV	t849	PF15-68/PF15-178
E2	Door handle (d1)	Pn, Mt, Cx, Cp	P	P	N	IV	t1977	PF15d/PF15-74/PF15-304
E3	Door handle (d14)	Pn, Mt, Cx, Cp	P	P	N	IV	t1977	PF15d/PF15-74/PF15-304
H1	Human	Pn, Mt, Cx, Cp	P	P	N	IV	t1554	PF15a
H2	Human	Pn, Mt, Cx, Cp	P	P	N	IV	t1977	PF15d/PF15-74/PF15-304

* Resistance shown to listed antimicrobials. All isolates tested against; Cefuroxime (Cx), chloramphenicol, clindamycin, ciprofloxacin (Cp), erythromycin, fusidic acid, gentamicin, kanamycin, methicillin (Mt), mupirocin, neomycin, penicillin (Pn), rifampicin, streptomycin, sulphamethoxazole, tetracycline, tobramycin, trimethoprim, vancomycin.

P: positive result; N: negative result

4A.4 Discussion

This study presents an assessment of the prevalence and distribution of MRSA in the environment and staff of a university referral small animal veterinary hospital.

The estimates of prevalence of MRSA in the environment of this facility (1.4% and 1.7% on d1 and d14 respectively) were lower than have been previously reported (Weese *et al.*, 2004; Loeffler *et al.*, 2005). While it is prudent to consider that the repeat environmental sampling on d14 lacked power and the inclusion of previously positive sites for subsequent sampling may have artificially increased the prevalence on this occasion, estimates of prevalence obtained on d1 and d14 are similar and overlapping confidence intervals imply consistency. The prevalence of MRSA in staff was similar to a single international study that obtained a prevalence of 4.4% in small animal veterinarians (Hanselman *et al.*, 2006b) but lower than previously reported in the UK (Loeffler *et al.*, 2005; Deacon *et al.*, 2006).

Previously published studies assessing environmental prevalence of MRSA in small animal hospital environments have used too few samples to be confident of inferential accuracy (Loeffler *et al.*, 2005; McLean and Ness, 2008). However, it is unlikely that this is also the case for prior estimates of carriage in veterinary staff, which were undertaken using larger sample sizes and in various geographical locations, allowing for increased confidence in interpretation of these results (Loeffler *et al.*, 2005; Deacon *et al.*, 2006). Possible explanations for the disparity in the results of this study with those previously published include absence of MRSA-positive hospitalised animals on the day(s) of sampling and general temporal and/or geographical variation in MRSA colonisation associated with differences in human and animal population densities. Repetition of this study with the inclusion of animal sampling would allow greater understanding of the contribution of these potential biases.

The current study was designed to maximise MRSA yield through the use of enrichment culture to enhance recovery of MRSA isolates (Nahimana *et al.*, 2006; Rich *et al.*, 2007). Similarly, although estimates of sensitivity and specificity are high for the chromogenic media used in this study (MRSA Select; BioRad, France) when used to identify MRSA in samples from humans (Nahimana *et al.*, 2006; Nsira *et al.*, 2006), no objective data exist for accuracy of isolation of MRSA from animals or the environment, where a different spectrum of organisms may result in a change of accuracy for detection of MRSA.

Although these considerations may have an impact on the overall prevalence estimates obtained from the current study, the similarity of the methods used in this study to those previously published allows broad comparative interpretation.

Inference regarding the source of dissemination of the environmental MRSA in this study can be made by considering the location and genotypes of the strains that were identified. Three isolates (one human (H2) and two environmental isolates (E2 and E3) that were obtained from the same site (corridor door handle) but sampled on two different occasions) exhibited indistinguishable resistance patterns, PFGE profiles and *spa* types (Table 4.1). The door handle source of E2 and E3 was inaccessible to animals and is unlikely to have been used by clients, who are infrequently allowed access to the wards (and are escorted by staff on the rare occasions that they are). This doorway was considered to be a highly used thoroughfare for staff, and cleaning of the site was the responsibility of the general cleaners within the University at the time of the study, as opposed to the veterinary nurses, who were responsible for ward cleaning. The staff member colonised with the same strain as E2 and E3 (H2) was a veterinarian. It is unknown whether the door handle was re-contaminated within the 14 days between samplings, or whether the strain remained viable over this period, both of which are plausible in this scenario (Huang *et al.*, 2006).

The two remaining MRSA isolates (E1 and H1) had very closely related PFGE patterns, which only differed from E2, E3 and H2 by two bands (Figure 4.1), implying a close relationship between all the strains (Tenover *et al.*, 1995). The *spa* types and sensitivity patterns of E1 and H1 isolates were different, but they were found to be related using BURP cluster analysis. Additionally, E1 (a foam radiology positioning wedge) was positive for PVL, which has not previously been reported in MRSA isolated from a dog or cat in the UK, but may be a feature of community acquired MRSA strains in humans (Kluytmans-Vandenbergh and Kluytmans, 2006). Although PVL is not commonly found in EMRSA-15, it has been previously reported in Scotland through the Scottish MRSA Reference Laboratory, accounting for <0.1% of the EMRSA-15 isolates submitted to this laboratory (Edwards, 2004).

Taking into consideration the location of the MRSA isolates, along with their phenotypic and genotypic characteristics, it can be noted that a relationship exists between human and environmental samples within this facility. Although it is not possible to rule out animals as a primary source of the H1 and H2 isolates, it is unlikely that any of the three environmental isolates were obtained directly from animals (without a human

intermediary, who may be colonised or contaminated, for the strains that were found on the door handle). As such, it can be postulated that the most likely source for dissemination of the environmental isolates identified in the current study is human, and in particular, a member of staff within the clinic. The two members of staff colonised with MRSA in this study were veterinarians from the same department. Further studies with increased power are required to define whether veterinarians are of greater risk than other staff members for carriage of MRSA.

Although the role of contaminated environmental surfaces in transmission of pathogens associated with hospital acquired infection is not fully elucidated, it is known that transmission of MRSA between contaminated surfaces and healthcare workers' hands may occur independent of contact with infected patients (Boyce *et al.*, 1997). Furthermore, handwashing, along with environmental cleaning, has been associated with a reduction of incidence of hospital acquired infection in human hospitals (Pittet *et al.*, 2000; Rampling *et al.*, 2001) and has been found to be protective for MRSA nasal colonisation in equine veterinarians (Anderson *et al.*, 2008). Therefore, the implications of the findings of the current study are that infection control policies within small animal facilities should continue to focus on hand hygiene and review cleaning protocols to include all environmental 'hot-spot' sites.

These findings contribute to a growing literature and knowledge base on MRSA in companion animals and, more specifically, in people and places closely exposed to these animals. Of particular interest is the important difference in prevalence estimates (environmental and human) compared to previously published reports. This hints at the existence of differing epidemiology of the pathogen in different places and/or settings which demands greater investigation and understanding in order properly to represent and manage the risk in different settings from an animal and public health viewpoint. This putatively differing epidemiology requires consideration when parameterising variables such as these within a quantitative risk assessment. Of importance is the inclusion of an adequate range of input distributions in order to reflect and account for potentially differing epidemiological scenarios that may be encountered, as identified in the current study.

4B: A questionnaire study of interactions between dogs, other animals and humans

This work was undertaken as part of two summer student projects by Ms Britt Hyden and Ms Sarah Bierbaum. The projects were devised and overseen by Ms Jane Heller and data analysis and write-up was undertaken by Ms Jane Heller.

4B.1 Introduction

In order to begin to quantify the risk of pathogen transfer to and from pets, factors including: number of infected, colonised or contaminated sources of the pathogen; extent of infection, colonisation or contamination at the source; mode(s) of transmission of the agent; behavioural characteristics of the animal and/or human and existing measures of prevention must be considered. While many of these factors may be quantifiable for inclusion in risk analysis models, the behavioural interaction between companion animals and humans is poorly described. This information is vital as the potential for transmission between these two populations will vary depending on the pathways available to the pathogen. At the time of conception of this study, no published reports were available regarding type and frequency of pet dog and human interactions. Recently, two studies, using census and non-census questionnaire techniques, have been published by the same authors that report on factors associated with dog ownership and human-dog and dog-dog interactions in a single community in England (Westgarth *et al.*, 2007; Westgarth *et al.*, 2008).

The project outlined in this chapter section aimed to define the nature and extent of interaction between humans (both dog-owners and non dog-owners) and pet dogs in Scotland, and to explore the differences in the dog-owning and non dog-owning population with respect to hygiene and knowledge of zoonotic disease. Importantly, this study aimed to validate the results of previously published work in a different geographical area of the UK and using different sampling and questionnaire techniques (Westgarth *et al.*, 2007; Westgarth *et al.*, 2008).

4B.2 Materials and Methods

A questionnaire was developed for oral interview administration based on recognised techniques (Dillman, 1991), as described in section 2.3, and pre-tested on eight individuals. The results of the pre-test questionnaires were used to modify the questions, include a projected time frame and develop an appropriate preamble for introduction of the questionnaire to members of the public who were approached. A sample size calculation was undertaken and it was found that, to estimate prevalence of any single trait to within 5% accuracy at the 95% confidence level, 385 completed questionnaires were required (calculated for the most conservative estimate of prevalence at 50% and reducing for higher or lower estimates). The questionnaire (Appendix 1) consisted of a total of 25 questions, divided into those for all respondents (N=12), those for dog owners only (N=10) and those for non dog-owners (N=3). The questionnaires were colour coded for easy reference to dog-owner and non dog-owner material. The questions were organised into the five sections of: i) interactions with your dog, ii) interactions with other dogs, iii) hygiene, iv) knowledge of disease and v) responder demographics and were formatted predominantly as 'yes/no' responses and estimations of numbers or proportions. A single open-ended question was included. Two third year veterinary students from the University of Glasgow, Faculty of Veterinary Medicine, were trained to complete face-to-face questionnaire administration. They were advised to use the same preamble on each occasion, to introduce themselves, identify their affiliation with the University of Glasgow and assure participants that their responses would remain anonymous. The interviewers were clearly identified with name badges including picture identification and the official University of Glasgow crest, and a larger image of the academic crest was also on display on a clip-board at all times.

Questionnaires were administered over a 10 day period, in public locations where the density of public was likely to be high, including parkland, shopping districts and town centres in randomly selected towns located within one and a half hours by train from Glasgow city centre. Once identified, towns were randomly selected for each day and interviewing was either undertaken between 9am and 4pm or 12pm and 6pm on both week days and weekends to minimise bias and include as wide a demographic as possible. Permission was obtained for interviewers to conduct interviews on premises where necessary and appropriate.

Data were collated and entered into a database (Microsoft Access 2003, Microsoft Corporation; Seattle, USA). Data entry was cross-checked by the two students and then double-entered by the author to ensure maximum accuracy. Analyses were executed using Microsoft Excel (Microsoft Excel, 2003, Microsoft Corporation; Seattle, USA), Minitab (Minitab release 14.1, Minitab Inc. 2003), StatsDirect (StatsDirect Ltd, 2008) and R (R Development Core Team, 2008) programmes. Proportions were compared using McNemars test for paired and Chi-square test for unpaired observations. Statistical significance was set at $P < 0.05$.

4B.3 Results

A total of 300 questionnaires were completed. Ninety four respondents (31.3%) were dog owners (DO) and of these, 18 (19.1%) also owned cats and 9 (9.6%) owned other pets. Seventy three DO (78.5%) reported that their dogs had regular close contact with other animals; 67 (91.8%) had contact with other dogs, 8 (11.0%) with cats, 6 (8.2%) with rabbits and 6 (8.2%) contacted other species, predominantly reported as 'wildlife'. A median of 3 (range 1-10) dog walks per day were reported by DO and an 'average' walk was reported to last a median of 30 minutes (range 10-120). Within any one walk, a median of 4 (range 0-20) close contacts with other dogs and 5 (range 0-20) close contacts with humans occurred and a median of 6 alternative human contacts per week were also reported (whilst not on a walk). An overall median of 14 (range 0-79) human contacts per day (on walks and elsewhere) and 9 (range 0-60) dog contacts per day (on walks) were calculated.

Patting was the most common interaction between humans and dogs (Figure 4.2). A significantly higher proportion of DO reported that they played with ($P<0.01$), cuddled ($P<0.01$) and fed treats ($P<0.01$) to their own dogs compared to other dogs that they contacted. Conversely, DO were no more likely to play with, feed treats or cuddle ($P>0.05$) a non-owned dog than non-DO. Fifty six (59.6%) DO allowed their dog on the sofa, while 72 (76.6%) dogs were allowed to lick hands, 41 (43.6%) were allowed to lick faces, 39 (41.5%) slept on a household bed and 18 (19.1%) eat from household plates. Significantly lower proportions of non-DO reported that they would allow their dog to undertake the above activities if they owned one. Handwashing practices reported by all respondents are presented in Figure 4.3. No significant difference was found between DO

and non-DO for any handwashing scenario (P>0.05 for all). The proportion of respondents that recognised zoonotic diseases varied depending on the disease process (Figure 4.4a). Subsequent correct identification of the recognised pathogen as zoonotic ranged from 12.8% (Distemper) to 59.6% (Tapeworm) (Figure 4.4b).

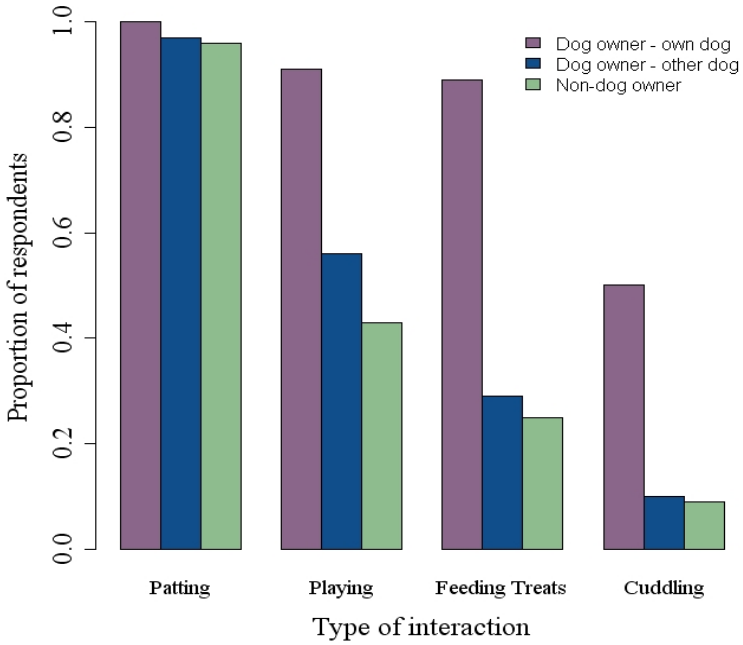


Figure 4.2. Interactions reported between humans and dogs in the human-canine interaction questionnaire.

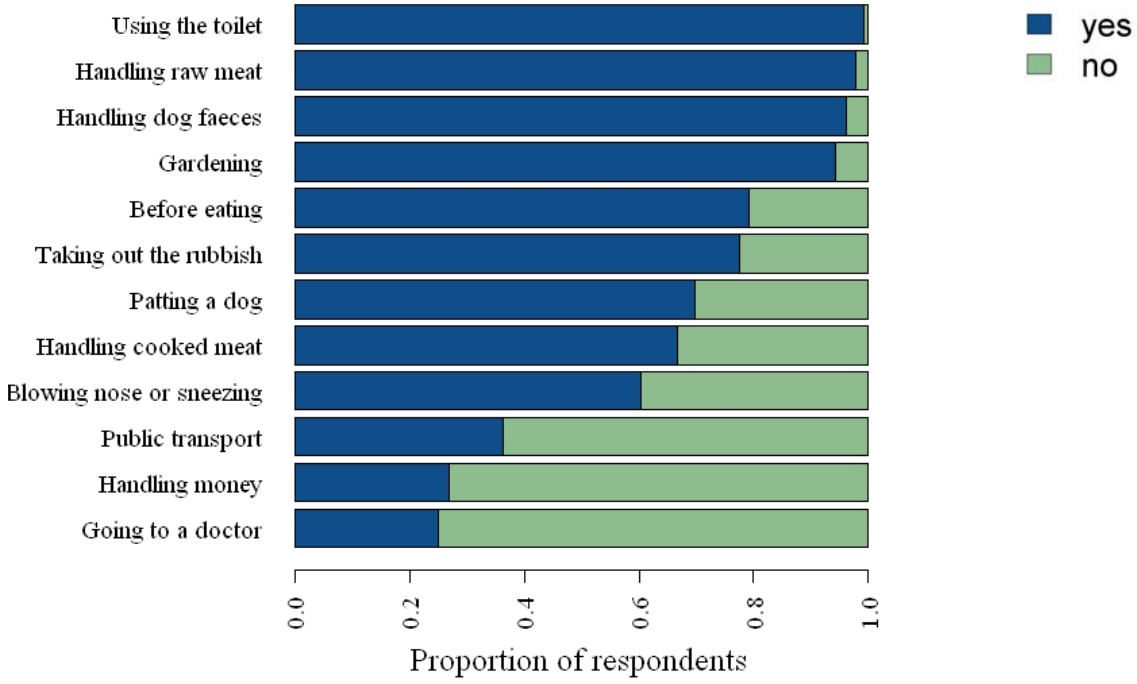


Figure 4.3. Handwashing practices after specified events reported by all respondents (N=300) in the human-canine interaction questionnaire.

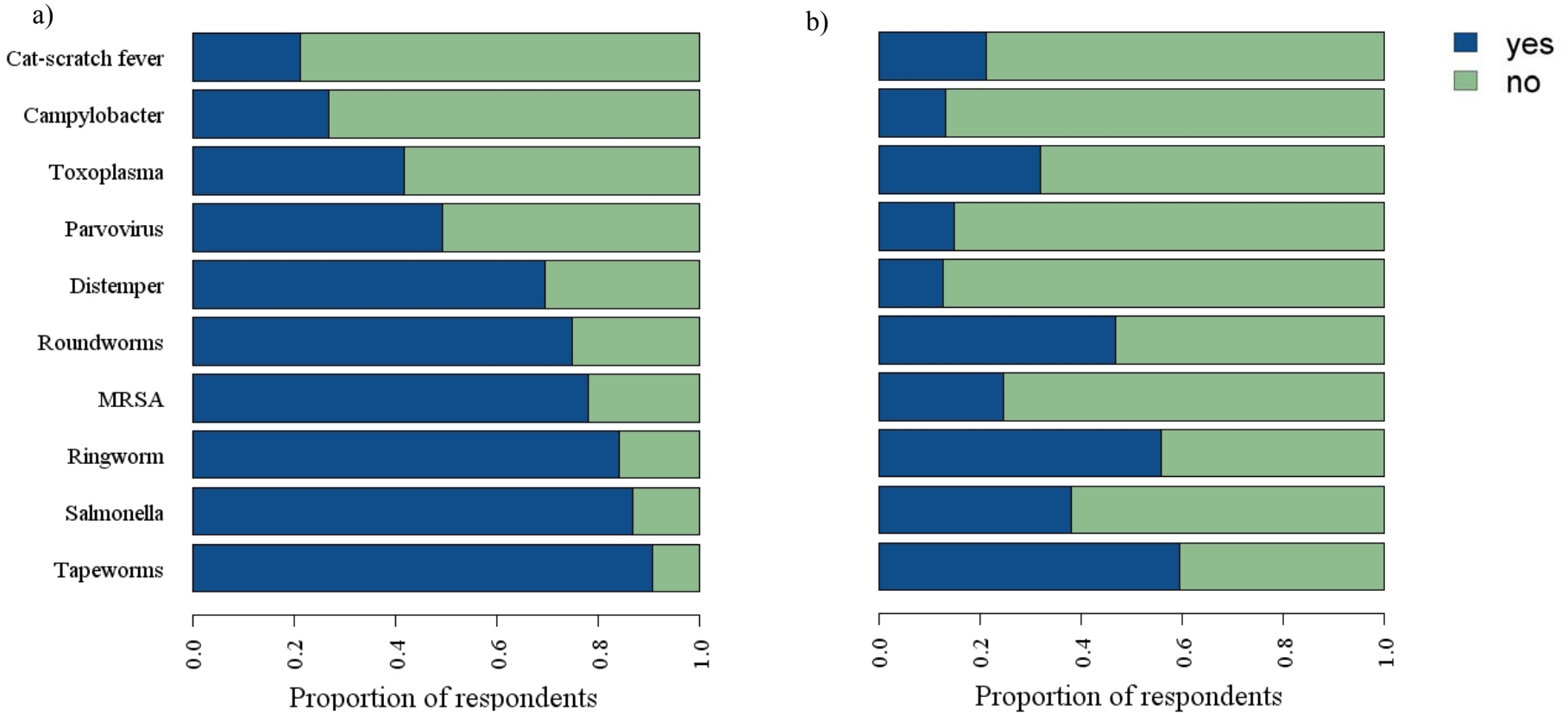


Figure 4.4. a) Recognition of pathogens by all respondents (N=300) of the human-canine interaction questionnaire. b) Correct classification of pathogens as (non)zoonotic by respondents who recognised each organism (refer to Fig 4.4a).

4B.4 Discussion

The results of the study presented in this chapter section require interpretation with respect to the aims of the study: i) validation of pre-existing studies (Westgarth *et al.*, 2007; Westgarth *et al.*, 2008) enabling estimation of dog-dog and dog-human interactions in the UK and ii) comparison of hygiene practices and knowledge of zoonotic disease between DO and non-DO.

The estimate of dog ownership obtained in this study (31.3%) is higher than a previous UK estimate (Westgarth *et al.* 2007 estimated 24%) and commercial (22%, http://www.pfma.org.uk/overall/pet-population-figures-2.htm) and media-reported (19.8% http://news.bbc.co.uk/1/hi/uk/4097716.stm) figures. It is possible that this finding reflects the inclusion of parks and other open areas as sites for questionnaire administration in the current study which is likely to have introduced a selection bias with respect to the members of public that were targeted and approached. Similarly, a response bias is also likely to be present in the current study, and may have affected prevalence estimates, with certain demographic groups more likely to agree to respond to veterinary students in a face-to-face scenario. The findings of the current study should be interpreted with these biases in mind.

The results of this study show that a large number of contacts are likely to occur on a daily basis between most dogs and humans. This study reported a median of 14 (range 0-79) human and 9 (range 0-60) dog contacts per dog per day. While these estimates seem very large, particularly in comparison with previous reports of a median of 3-5 (range 0 – 15+) human and 1-5 (range 0 – 15+) dog interactions per day (Westgarth *et al.*, 2008), one must consider the nature of the 'contacts' reported. While it could be argued that these discrepancies reflect the previously described bias, the 'contacts' estimated within the current study report all individual contacts and do not differentiate between multiple contacts with the same individual (e.g. interactions with dogs or humans that are walked at the same time each day or who are passed on a number of occasions per walk). Therefore, it is possible that these estimates are not conflicting, but rather that they could be interpreted in combination to obtain a crude overall per-human or per-dog contact rate. The median number and length of walks per day were found to be similar between studies. The current study estimated a median of 3 walks per day and a median length of 30 minutes per walk, while Westgarth *et al.* (2008) reported a median of 1-2 walks per day

with each walk reported to last between 16 minutes and an hour. Again, the slightly greater estimates emanating from the current study are likely to reflect selection and response biases and should be interpreted as a maximum estimate.

While Westgarth *et al.* (2008) reported that the frequency of contacts with non-owned dogs was greater for DO compared with non-DO, the current study found that, while closer contact occurred between DO and their own dogs compared with non-owned dogs, there was no appreciable difference in the type of interaction that DO had with non-owned dogs compared with non-DO. This implies that the potential routes for pathogen transfer from non-owned dogs is similar for dog owning and non-dog owning humans, in contrast to the comparatively greater number of routes that are likely to be present between DOs and their own dogs.

The most common interaction between humans and dogs that was reported in this study was patting. Almost 70% of respondents reported that they washed their hands after patting a dog (Figure 4.3), which is slightly higher than the estimate of 58% obtained by Westgarth *et al*. (2008). However, other hygiene estimates were found to be very similar, including 79.3% of respondents reporting handwashing before eating and 96.1% after picking up dog faeces in the current study, compared with 85% and 96% respectively in Westgarth *et al.* (2008). The absence of a significant difference that was found in handwashing practices between DO and non-DO implies that there is no difference in risk perception with respect to zoonotic disease in these two groups. This is supported by the absence of a difference between these groups for both pathogen recognition and zoonotic identification (Figures 4.4a and 4.4b). Correct identification of pathogens as (non)zoonotic was found to be particularly poor in the current study and highlights the lack of knowledge of potential adverse effects of dog ownership in putatively at-risk populations.

The results of this study have broadly supported the results of a larger study undertaken at a similar time in a different geographical region using different questionnaire techniques (Westgarth *et al.*, 2008). Where differences have been found, these can be attributable to selection and response bias that is likely to be present in the current study, along with diminished power associated with a relatively small sample size. The outcome of this questionnaire provides estimates that are of use, in combination with previously published data, in attempting to quantify human-dog and dog-dog exposure for potential infectious disease transfer. It has also identified more intimate interactions between humans and their own dogs compared with those that are not owned and identified no difference between

DO and non-DO with respect to type of interactions with non-owned dogs, hygiene practices and recognition or classification of zoonotic pathogens.

4C: Prevalence of *Staphylococcus aureus* and MRSA in meat and dog treats in Scotland

This work was undertaken as part of two summer student projects by Ms Shona Gorman and Ms Erin Ward. The projects were devised and overseen by Ms Jane Heller and data analysis and write-up was undertaken by Ms Jane Heller. The genetic analyses were undertaken in part at the Scottish MRSA Reference Laboratory.

4C.1 Introduction

The conceptual model that is presented for the risk assessment process in this thesis considers potential routes of transmission of MRSA from humans, dogs and the environment in the community or within veterinary hospital settings (Chapter 3). In this conceptual model, the putative route of transmission through food has not been considered independently to environmental sources (3.3.1.5). While the author is confident of the argument put forward for this categorisation within Chapter 3, the dissemination of ST-398 in animals (van Loo *et al.*, 2007b; Khanna *et al.*, 2008; Wulf and Voss, 2008) and food products (van Loo *et al.*, 2007a; de Boer *et al.*, 2008) in countries outwith the UK (discussed in 1.4.4.1) combined with minimal dedicated surveillance for ST-398 or other MRSA strains in pigs or other farm animals within the UK (European Food Safety Authority, 2007) is an indication to attempt to define the prevalence of MRSA in meat products to which dogs may be exposed. In addition, as pig ear pet treats have previously been identified as sources of infectious pathogens and have been implicated as the point source of an outbreak of Salmonellosis in humans in Canada (White *et al.*, 2003; Public Health Agency of Canada, 2006; Finley *et al.*, 2008), an indication exists to extend an assessment of prevalence of MRSA in meat-based food that dogs may encounter to include this putative pathogenic source.

The aims of this study were to conduct a pilot study to identify if and at what prevalence *S. aureus* and MRSA were present in butcher meat samples and dried pig ear treats in Scotland.

4C.2 Materials and Methods

As this was a pilot study that required the data collection to occur within a short period of time (10 days), the numbers of samples that were obtained were limited by the numbers that could be collected during this time frame. A sample size calculation revealed that, in the absence of prior knowledge of prevalence (and therefore setting prevalence to 50%), with 95% confidence and 5% precision, a sample size of 384 was required for each of the meat and pig ear groups. It was acknowledged prior to the commencement of the study that this sample size would not be reached in the period that was available. Consideration of a lower likely prevalence of 5% reduced the required sample size to 72.

Butchers, pet shops and supermarkets were identified in geographical locations that were randomly selected and within one and a half hours of Glasgow via public transport. Bones (pork and beef), a small portion of steak, a pork chop and two rashers of bacon were requested from each butcher and a single pig ear sample (defined as a single loose, or smallest pre-packaged bag) was obtained from each pet-food site where possible. Information was obtained where possible on the source of the meat and pig ear treats.

Samples were processed identically. A sterile swab was moistened with sterile distilled water and rotated over all surfaces of the specimen for 10 seconds. All samples were enriched and cultured on SBA and MRSA-Select as outlined in section 2.4.2.1. Assessment of growth and subculture was undertaken as described in 2.4.1.1 for the MRSA-Select plates and subculture onto SBA was also undertaken for any isolates with presumptive Staphylococcal colonies, i.e. white or cream colonies 1-3mm in diameter, on the initial SBA plates. All subcultured isolates that displayed Staphylococcal morphology were assessed for catalase production (Section 2.4.2.1) and the pig ear isolates were also gram stained and assessed for the presence of DNase as described in Section 2.4.2.1. All isolates obtained from pig ear samples that were catalase positive, DNase positive and displayed gram positive cocci morphology on gram stain, and isolates from meat that were catalase positive were subjected to PCR for *mecA* and *nuc* as described in section 2.4.1.3.

Isolates that were positive for *mecA* and *nuc* were subjected to antimicrobial susceptibility testing along with further genotypic analysis and PFGE as described in 2.4.2.2. Isolates taken from meat samples that were *mecA* positive and *nuc* negative and all isolates from the pig ear samples that underwent PCR were typed using API ID 32-STAPH (BioMérieux).

4C.3 Results

One hundred meat and 50 dried pig ear samples were obtained. The meat samples were obtained from 36 butchers shops and consisted of 24 pork chops, 33 bacon samples, 27 steak and 16 beef bone samples. The pig ear treats were either loose (26 samples), pre-packaged (15 samples) or packaged on the premises (nine samples) and were obtained from a total of 43 different premises.

After 24 hours growth, three (6%) of the pig ear and 12 (12%) of the meat samples displayed pink colonies on MRSASelect plates. This number increased to 14 (28%) and 40 (40%) respectively after 48 hours of incubation. However, only five of the possible 14 isolates from pig ears and 30 of 40 meat samples were identified as presumptive Staphylococci after subculture on sheep blood agar.

In total, including the isolates plated directly onto SBA, 26 (52%) of the pig ear (with a total of 28 separate isolates when initial SBA and MRSA-Select isolates were combined) and 92 (92%) meat samples (with a total of 117 separate isolates when initial SBA and MRSA-Select isolates were combined) had colonies that were identified as presumptive Staphylococcal isolates based on SBA colony morphology and positive catalase test, along with appropriate results of gram staining for pig ear samples. Seven of the 26 isolates from pig ear samples were also found to be DNase positive and defined as presumptive *S.aureus* isolates. All of the presumptive Staphylococcal isolates from the meat samples and only those that were also DNase positive from the pig ear samples were submitted for PCR.

Two of the pig ear samples contained the *nuc* gene (in the absence of *mecA*) enabling identification as MSSA while the other isolates were negative for both *nuc* and *mecA* genes. Two of the meat samples were found to be *nuc* and *mecA* positive (MRSA) and seven were *nuc* positive and *mecA* negative (MSSA). Seven were *nuc* negative and *mecA*

positive (meticillin-resistant but not *S. aureus*) and typing with API ID 32-STAPH (BioMérieux) identified these as *S. epidermidis*. All of these samples were identified from different original meat samples.

The two meat samples that were identified as MRSA were obtained from a pork chop and a beef steak obtained from the same butcher shop. Further phenotypic and genotypic evaluation revealed that both of these isolates were EMRSA15 but their PFGE profiles differed, one being PF15a and the other PF15c. The antibiograms were the same for each isolate, with resistance recorded to penicillin, meticillin, cefuroxime, erythromycin and ciprofloxacin.

4C.4 Discussion

To the author's knowledge, this pilot study represents the first study undertaken in the UK to assess the prevalence of *S. aureus* and, in particular, MRSA on meat and dried pig ear treats.

In this study, two of the 100 samples (2%, 95%CI 0.2 – 7.0%) of meat from butchers were found to be contaminated with MRSA. These samples were obtained from the same butcher, were isolated on different meat products (beef and pork) and, while they possessed identical phenotypic profiles, differed genotypically, with their reported types, PF15a and PF15c, representing distinguishable pulsotypes by PFGE. Given the strain types of these isolates (EMRSA-15 represents the most common human strain in Scotland), geographical association between them and the similarity of the strains found on different meat products, it can be suggested that they are most likely to represent contaminants of the meat from a human source, rather than true colonisation or contamination of the animal prior to slaughter.

The prevalence of MRSA reported in this study is similar to the findings of a study undertaken by Van Loo *et al.* (2007a) in The Netherlands, but lower than a more recent study that reported a higher prevalence of MRSA in meat in The Netherlands (11.9%) (de Boer *et al.*, 2008). All of the strains isolated by VanLoo *et al.* (2007a) and most of those isolated by de Boer (2008) were ST-398 and therefore likely to have been of animal origin, in contrast to the findings in this study, where the source is most likely to have been human

contamination. ST-398 was not isolated in this study and, to the author's knowledge, has not been isolated from an animal within the UK.

In contrast to the low prevalence of MRSA on butcher meat and absence of MRSA isolated from pig ear treats, MSSA was present in greater numbers, with a prevalence of 7% (95%CI 2.9 – 13.9%) in butcher meat and 4% (95%CI 2.2 – 19.2%) in pig ear treats. As further phenotypic and genotypic analyses were not undertaken on these isolates, their relatedness within and between premises is unknown. Similarly, a large number of non-aureus Staphylococcal isolates with *mecA* genes were found in this study. However, as this study was not designed to isolate and identify all Staphylococcal species, the prevalence of these non-aureus isolates cannot be reported. The results of this study do, however, support prior findings that meat products may be a source of non-pathogenic staphylococcal strains that carry resistance determinants (Simeoni *et al.*, 2008).

The methods used to isolate MRSA and MSSA in this study were employed to optimise the yield of both potential pathogens. The enrichment step was included with the aim of limiting the overgrowth of non-Staphylococcal species whilst enhancing the likelihood of Staphylococcal yield. In the course of this study, it was found that the addition of 2.5% NaCl was inadequate for inhibiting growth of contaminants, so the NaCl concentration was increased to 6.5% in line with other similar studies (de Boer *et al.*, 2008). It is known that increasing the NaCl concentration results in a reduced likelihood of yield of EMRSA-16 strains, which display reduced salt tolerance with respect to other MRSA isolates (Jones *et al.*, 1997). However, these effects must be accounted for within the current study where a compromise has been made by increasing the NaCl concentration to enable a reduction in overgrowth and increased Staphylococcal yield whilst simultaneously acting to partially inhibit growth of EMRSA-16 isolates if they are present.

MRSA-Select and SBA were used in combination (parallel) in an attempt to maximise the sensitivity for identification of meticillin-resistant strains, which may have been missed using SBA alone if a mixed population of Staphylococci grew on the non-selective agar. The subsequent use of catalase testing for both meat and pig ear samples, along with Gram staining and DNase testing allowed targeted application of the PCR and a subsequent increased specificity of the overall methods. While it is known that the PCR for *mecA* and *nuc* genes may produce false positives if the culture contains a mixture of meticillin-resistant coagulase negative Staphylococci and MSSA, PCR-based methods such as those

used herein are reported to perform extremely well when applied to pure cultures as in the current study (Francois and Schrenzel, 2008).

In conclusion, this study found a low prevalence of MRSA in butcher meat and a low prevalence of *S. aureus* in both butcher meat and pig ear treats. This study did not identify MRSA in pig ear treats, although the sample size used lacked power for identification of the organism at very low prevalence. No ST-398 was isolated. In addition, genes encoding for meticillin resistance (*mecA*) were identified in non-pathogenic Staphylococci in butcher meat and pig ear treats. Given the likelihood of a human source of contamination of the meat, the inclusion of food (meat and pet treats) as a part of the general environmental contamination in the risk analysis that has been specified in this thesis seems reasonable, rather than including it as a separate important route. However, future intermittent surveillance of butcher meat should be considered and raw meat should not be negated as a potential source (through contact rather than ingestion) of contamination with MRSA and *S. aureus*.

4.2 Discussion

The results of the three studies presented in this chapter have provided data with which to fill some of the gaps with respect to exposure to MRSA that were identified by the qualitative risk assessment that was presented in Chapter 3. Section 4A has provided a contrasting estimate, with respect to a previously published study (Loeffler *et al.*, 2005), of environmental contamination in a veterinary hospital where the prevalence of staff colonisation or contamination with MRSA was in line with what would be expected within the general population. Section 4B has confirmed previous estimates of dog-dog and dog-human interactions (Westgarth *et al.*, 2008) and obtained greater insight into comparisons between dog-owners and non dog-owners with respect to interactions with dogs and knowledge of zoonotic disease. Section 4C has obtained prevalence estimates for MRSA and MSSA on meat and pig ear treats in Scotland. Although the studies presented in sections 4B and 4C represent pilot studies, the results (specified with surrounding uncertainty) of all three sections allow some inference to be made and input distributions to be extended and modified to encompass a greater range of potential scenarios for subsequent parameterisation of the quantitative risk assessment presented in Chapter 6.

CHAPTER 5

EXPERT OPINION ELICITATION EXERCISE IN THE AREA OF MRSA IN PET DOGS

5.1 Introduction

The difficulty associated with interpretation of the qualitative techniques presented in Chapter 3 along with the inability of these techniques to adequately address the risk assessment considered in this thesis suggested that a quantitative approach might provide a more informative analysis. However, as stated in Chapter 3, the subject area of MRSA acquisition in pet dogs is data sparse and, while quantitative risk assessments are generally based on published data, in the absence of these, data may need to be sourced from elsewhere, including the judgement and opinion of individual experts, in order to adequately populate a defined model (Vose, 2000; Bedford and Cooke, 2001; Van der Fels-Klerx *et al.*, 2005).

The term expert opinion or expert judgement may be used to describe formal and informal use of information from someone skilled in a specific field (Otway and von Winterfeldt, 1992). The use of expert opinion in a risk assessment may range from interpretation of a model output or development of a model structure, in which the analyst or modeller acts as the expert, to elicitation of opinion-based data to populate model parameters using a structured and formalised approach. The field of formal expert opinion elicitation through expert judgement exercises has been recognised for over 15 years and a number of methods have been developed to support its application (Otway and von Winterfeldt, 1992; Clemen and Winkler, 1999; Bedford and Cooke, 2001; Goossens *et al.*, 2008). Formal expert opinion techniques have gained popularity more recently in animal health, and in quantitative microbial risk assessment processes, and have been utilised to populate simulation, mathematical and conceptual models (Stärk *et al.*, 2002; Van der Fels-Klerx *et al.*, 2002; Gallagher, 2005; Gustafson *et al.*, 2005; Van der Fels-Klerx *et al.*, 2005).

A number of data gaps in the conceptual model that was constructed to define the risk of acquisition of MRSA in dogs were identified in Chapter 3 and, while the work described in Chapter 4 attempted to fill some of these directly by collection of new data to augment preexisting information, many of the parameters required for the model remained undefined. The aim of this chapter was to obtain expert estimates for the remaining data gaps identified in Chapter 3.

5.2 Materials and Methods

5.2.1 Expert selection

A multidisciplinary group of experts was selected using pre-defined criteria as follows: At least one expert was selected to represent each paper published in the area of MRSA in animals over the previous 5 years. Additional experts were selected using the delegate lists from the First International Conference in MRSA (2006), the American Society of Microbiology (ASM) Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens (2008), and members of the Scottish Infection Research Network (SIRN). Experts were selected with varied backgrounds and experiences, spanning areas of medical and veterinary microbiology, antimicrobial resistance and epidemiology.

5.2.2 Questionnaire design

A questionnaire was developed using principles outlined by Dillman (1991) in the form of the 'Total Design Method' and Vose (2000). The questionnaire was written as a Microsoft Word 2003 (Microsoft Corporation; Seattle, USA) document and was available for completion by hand after printing, or directly on computer. The questions were developed specifically for the current study and were based on, but not restricted to, data deficiencies that were identified through a qualitative risk assessment (Chapter 3) and were determined to be important for complete parameterisation of the quantitative model presented in Chapter 6.

The questionnaire was formatted as a 21 page A4 document (Appendix 2). The front page introduced the questionnaire as "Expert Opinion Elicitation: MRSA in dogs" and indicated the study's affiliation with the University of Glasgow, Faculty of Veterinary Medicine. A one-page introduction followed the title page with concise instructions for completion of the questionnaire. Assumptions were clearly stated within this introduction as follows:

1. The term **positive** will be used to account for any bodily carriage of the organism (nasal, skin, perineal …). **It does not attempt to distinguish between colonisation** (intermittent or consistent) **and temporary carriage**. This term will also encompass **any infection** with MRSA.

2. Please answer all questions for the average situation expected **within the UK**.

The body of the questionnaire followed and comprised seventeen questions, divided into the subheadings of prevalence of MRSA (6 questions), environmental contamination (5 questions, each with two parts) and transmission of MRSA (5 questions, each with between 4 and 8 parts). Each of the questions (or each part of the question for those with multiple parts), requested the estimation of minimum, maximum and most likely values, along with a level of the expert's confidence in their estimate, between 1 (very unsure) and 10 (absolutely certain). It was stated that the level of certainty would be ascribed as 4 if left blank. All questions were worded to obtain whole number estimates rather than proportions or percentages, consistent with published recommendations and previous expert elicitation exercises (Stärk *et al.*, 2002; Gallagher, 2005) and the minimum and maximum estimates were requested prior to the most likely to avoid adjustment and anchoring bias (Vose, 2000). Two of the questions in the questionnaire did not adhere to the previously defined structure. These were: a) a single question within the prevalence section which requested identification of groups at increased risk of carriage by placing a 'x' on lines (visual analogue scale) next to potential risk groups and b) the final question which requested a self-rating of the expert's specialisation (on a scale of 1 to 10) in 11 areas (veterinary microbiology, human microbiology, small animal medicine, large animal medicine, human medicine, MRSA, public health, zoonotic disease, epidemiology, food safety and communicable disease control). For the final question (question 17), participants were instructed that a rating of 1 represented equivalent knowledge to a member of the general public and a rating of 10 represented the knowledge of a specialist at the top of the particular field. An area for additional comments from experts was also included.

5.2.3 Pre-testing

The questionnaire was pre-tested among a group of 10 (non-expert) veterinary epidemiologists at the University of Glasgow and the feedback used to modify the questionnaire appropriately. The participants were asked to comment on the content, layout, ease of comprehension and time for completion of the questionnaire. All participants reported the time for completion of the questionnaire to be between 15 and 25 minutes, which allowed the inclusion of a projected time frame on the accompanying request for completion. Results of the pre-test questionnaires were not included in the

analyses and none of the participants in the pre-test were subsequently administered the questionnaire.

5.2.4 Implementation

The questionnaire was emailed on two occasions, at time zero to all experts and subsequently at five weeks to non-responders to the previous request. All requests were emailed with a covering letter which was modified for each mailing (Appendix 3). A single additional questionnaire was delivered personally with an accompanying cover letter.

5.2.5 Analysis

All responses were entered into a database (Microsoft Access 2003, Microsoft Corporation; Seattle, USA) and analyses were performed using Microsoft Access 2003, Microsoft Excel 2003 (Microsoft Corporation; Seattle, USA), @RISK 4.5 Professional Edition (Pallisade Corporation; Middlesex, UK) and R (R Development Core Team, 2008).

An initial assessment of expert specialisation was undertaken, based on the results of question 17. Information relating to the expert's level of self-reported expertness or specialisation was graphed to visually assess both within expert and between expert grading and variation, using bar graphs, for each specialty category recorded. For all other questions (excluding question 4), the responses given by each expert (minimum, most likely, maximum and confidence values) were converted to proportional estimates and used to specify individual modified beta distributions (also described as a modified PERT distributions by Vose (2000)). A description of the information that was obtained by the expert opinion questionnaire for each question is presented in Table 5.1.

Table 5.1. Information obtained using the expert opinion questionnaire (Appendix 2) for MRSA in dogs.

Section	Question	Information obtained
Prevalence of MRSA	1	Prevalence of MRSA in general population
	2	Prevalence of MRSA in health care workers
	3	Prevalence of MRSA in veterinarians
	4*	Identification of risk groups for MRSA carriage
	5	Prevalence of MRSA in dogs in general population
	6	Prevalence of dogs hospitalised in veterinary clinics
Environmental Contamination	7	Proportion of houses positive for any environmental contamination with MRSA
	7a	Proportion of environmental sites (10cm x 10cm) contaminated with MRSA within a house with MRSA contamination
	8	Proportion of houses with ≥1 MRSA positive household member that are positive for any environmental contamination with MRSA
	8a	Proportion of environmental sites (10cm x 10cm) contaminated with MRSA within a house with MRSA contamination and ≥1 MRSA positive household member
	9	Proportion of veterinary clinics positive for any environmental contamination with MRSA
	9a	Proportion of environmental sites (10cm x 10cm) contaminated with MRSA within a MRSA positive veterinary clinic
	10	Proportion of veterinary clinics with ≥1 MRSA positive member of staff that are positive for any environmental contamination with MRSA
	10a	Proportion of environmental sites (10cm x 10cm) contaminated with MRSA within a MRSA positive veterinary clinic with ≥1 MRSA positive member of staff
	11	Proportion of veterinary clinics with ≥1 MRSA positive canine patient that are positive for any environmental contamination with MRSA
	11a	Proportion of environmental sites (10cm x 10cm) contaminated with MRSA within a MRSA positive veterinary clinic with ≥1 MRSA positive canine patient
Transmission of MRSA	12a	Proportion of dogs that become MRSA positive after a single average contact with an MRSA positive owner
	12b	Proportion of dogs that become MRSA positive after a single average contact with an MRSA positive human (not owner)
	12c	Proportion of dogs that become MRSA positive after a single average contact with an MRSA positive dog in the household
	12d	Proportion of dogs that become MRSA positive after a single average contact with an MRSA positive dog outwith household
	12e	Proportion of dogs that become MRSA positive after a single average contact with an MRSA positive veterinarian
	12f	Proportion of dogs that become MRSA positive after a single average contact with an MRSA positive veterinary staff member (non-veterinarian)
	12g	Proportion of dogs that become MRSA positive after a single average contact with an MRSA positive household environmental site (10cm x 10cm)
	12h	Proportion of dogs that become MRSA positive after a single average contact with an MRSA positive veterinary hospital environmental site (10cm x 10cm)

Table 5.1(ctd) Information obtained using the expert opinion questionnaire (Appendix 2) for MRSA in dogs

Section	Question	Information obtained
Transmission of MRSA (ctd)	13a	Proportion of humans that become positive for MRSA after a single average contact with a MRSA positive spouse or live-in partner
	13b	Proportion of humans that become positive for MRSA after a single average contact with a MRSA positive non-co-habiting partner
	13c	Proportion of humans that become positive for MRSA after a single average contact with a MRSA positive household member <15 years old
	13d	Proportion of humans that become positive for MRSA after a single average contact with a MRSA positive household member >15 years old
	13e	Proportion of humans that become positive for MRSA after a single average contact with a MRSA positive non-household member
	13f	Proportion of humans that become positive for MRSA after a single average contact with a MRSA positive household environment (10cm x 10cm)
	13g	Proportion of humans that become positive for MRSA after a single average contact with a MRSA positive dog
	13h	Proportion of humans that become positive for MRSA after a single average contact with a MRSA positive food
	14a	Proportion of hospitalised humans that become positive for MRSA after a single average contact with a MRSA positive patient
	14b	Proportion of hospitalised humans that become positive for MRSA after a single average contact with a MRSA positive doctor
	14c	Proportion of hospitalised humans that become positive for MRSA after a single average contact with a MRSA positive (non-doctor) health care worker
	14d	Proportion of hospitalised humans that become positive for MRSA after a single average contact with a MRSA positive visitor
	14e	Proportion of hospitalised humans that become positive for MRSA after a single average contact with a MRSA positive hospital environment (10cm x 10cm)
	14f	Proportion of hospitalised humans that become positive for MRSA after a single average contact with a MRSA positive dog (therapy animal)
	15a	Proportion of transmission of MRSA to humans that occurs by direct contact (humans or animals)
	15b	Proportion of transmission of MRSA to humans that occurs by aerosolisation
	15c	Proportion of transmission of MRSA to humans that occurs by environmental contact
	15d	Proportion of transmission of MRSA to humans that occurs by food
	16a	Proportion of transmission of MRSA to dogs that occurs by direct contact (humans or animals)
	16b	Proportion of transmission of MRSA to dogs that occurs by aerosolisation
	16c	Proportion of transmission of MRSA to dogs that occurs by environmental contact
	16d	Proportion of transmission of MRSA to dogs that occurs by food
	17*	Specialisation level in 11 separate areas

* These questions were the only questions that did not request minimum, maximum and most likely estimates along with a level of confidence

Modified beta distributions (Equation 5.1) were specified using equations 5.2, 5.3 and 5.4, defined by Vose (2000) and implemented through a user-defined R code (Appendix 4).

$$\text{mod}\,PERT(a,b,c) = Beta(\alpha_1,\alpha_2)*(c-a)+a \qquad \text{(Equation 5.1)}$$

$$\mu = \frac{a+\gamma*b+c}{\gamma+2} \qquad \text{(Equation 5.2)}$$

$$\alpha_1 = \frac{(\mu-a)*(2b-a-c)}{(b-\mu)*(c-a)} \qquad \text{(Equation 5.3)}$$

$$\alpha_2 = \frac{\alpha_1*(c-\mu)}{(\mu-a)} \qquad \text{(Equation 5.4)}$$

Where a = minimum, b = most likely, c = maximum estimate, γ = certainty

These individual expert distributions were explored graphically in R and combined using a discrete distribution (Vose, 2000) in the form of: Discrete($\{x_i\},\{p_i\}$), where $\{x_i\}$ are the expert opinions for experts i = 1 to n and $\{p_i\}$ are the weights given to each expert opinion.

Six questions were selected to test the validity of the expert responses. These questions (Questions 1, 2, 3, 5, 6 and 10a) represented variables for which published data were available. The uncertainty distributions of individual experts, along with the combined expert opinion distribution, with each opinion given an equal weighting, were graphed and compared visually to each other and to the distribution specified by the published data for four of the questions (Questions 1, 2, 5 and 10a) in which an appropriate combination algorithm could be defined (Table 5.2).

Table 5.2. Distributions specified from published data used to check validity of expert opinon responses in the expert opinion questionnaire of MRSA in dogs.

Question	Distribution	Inputs	References
1	Beta(s+1,n-s+1)	s = 4 n = 274	Abudu *et al.* (2001)
2	Betapert(min,ml,max)	min = 0.015 ml = 0.065 max = 0.15	Abudu *et al*. (2001) for min, composite published UK estimate for ml (Eveillard *et al.* (2004)) and arbitrary max 10 times greater than general population
5	Discrete({p_1,p_2,p_3},{1,1,1}) (p_i = beta(s_i+1,n_i-s_i+1) for i=1,2,3)	s_1 = 1, n_1 = 255 s_2 = 0, n_2 = 188 s_3 = 2, n_3 = 203	Rich and Roberts (2005) Murphy *et al.* (2006) Hanselman *et al.* (2008)
10a	Discrete({p_1,p_2},{1,1}) (p_i = beta(s_i+1,n_i-s_i+1) for i=1,2)	s_1 = 3, n_1 = 27 s_2 = 2, n_2 = 158	Loeffler *et al.* (2005) Heller *et al.* (2009)

Two separate convergence analyses were undertaken to identify the number of random samples required to be run for each of the specified modified beta distributions to obtain representative expert distributions, and the number of random samples required to be run for the discrete distributions to obtain a representative combination distribution. The convergence analyses were based on the results of a 'moving average', whereby the 'running mean' for successive iterations (random samples) was plotted against the number of iterations used.

The running mean was calculated for the i^{th} iteration using the following formula:

$$rm_i = rm_{i-1} + \frac{x_i - rm_{i-1}}{i}$$

Where *rm* is the running mean, *i* is the i^{th} iteration of the model, resulting the sequence of outcome values $x_1, x_2, ...x_i$.

The overall mean for n iterations in total was calculated using the formula:

$$m = \frac{x_1 + x_2 + ...x_n}{n}$$

Where m is the overall mean, n is the total number of iterations that the convergence analysis is run over, resulting in the sequence of outcome values $x_1, x_2, ...x_n$.

Convergence was assessed visually in comparison with the overall mean for all iterations considered.

5.3 Results

5.3.1 Questionnaire return

Thirty four experts were emailed initially, 17 from the UK, 11 from elsewhere in Europe, three from North America, and one from each of Turkey, Hong Kong and Japan. Of these, one address was incorrect and could not be updated, which reduced the number of experts to 33. Fifteen replies (45%) were obtained prior to the reminder email and two (6%) were obtained afterwards, bringing the total response rate to 52%. Of the responders, seven were from the UK, five from elsewhere in Europe, three from North America and one from each of Turkey and Hong Kong. Two experts contacted the author to explain that they did not have the time to complete the questionnaire and 14 experts did not respond at all. Two of the returned questionnaires were incomplete. One of the incomplete questionnaires was entirely unusable due to partial completion of all questions, while the other had seven useable question responses. The results of both of these questionnaires were excluded from the analyses. The fifteen experts that returned useable questionnaires were coded as experts A through O.

Written comments that accompanied the returned questionnaires varied, but predominantly related to the difficulty of the exercise and the unease that experts felt in completing many of the questions. A sample of quotations is included in Appendix 5. Notably, the expert that returned the unusable questionnaire with partial completion of all questions, intimated that he/she was concerned about misuse of the results and the potential for media

exploitation (justifying his/her non-completion of any 'maximum' estimates). Despite reassurance via return email, the upper ranges were not completed by this expert.

5.3.2 Convergence analysis

The convergence analysis that was undertaken to determine the number of iterations required to accurately represent the expert opinion distributions produced fifteen graphs (one for each expert) for each of the 44 questions that were estimated numerically, resulting in a total of 660 graphs. A single example of a randomly selected expert from a randomly selected question is displayed as Figure 5.1. Similarly, 45 separate graphs were generated for the convergence analysis of expert opinion combination, using equally weighted combinations. A single random example is shown as Figure 5.2. These graphs show clearly that convergence is reached in all instances, for the generation of expert opinion distributions and the combination of these distributions, by 5000 (expert) iterations.

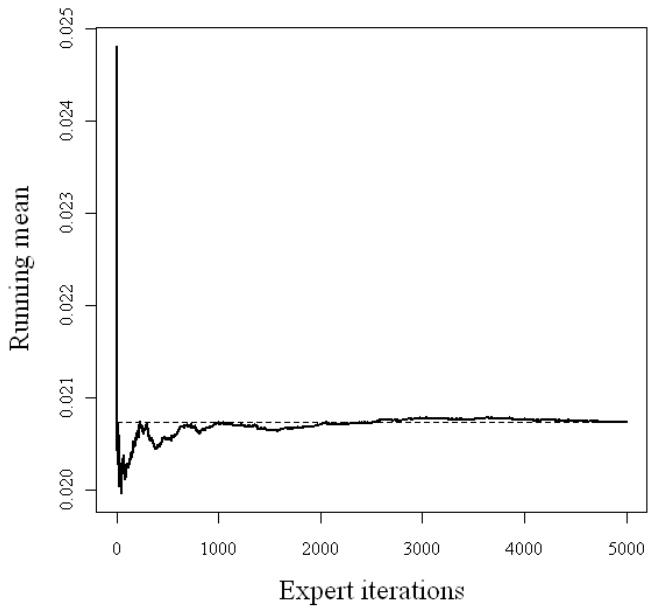


Figure 5.1. Convergence analysis for the number of iterations required to obtain a representative distribution for a single expert and a single question from the Expert Opinion questionnaire for MRSA acquisition in dogs.

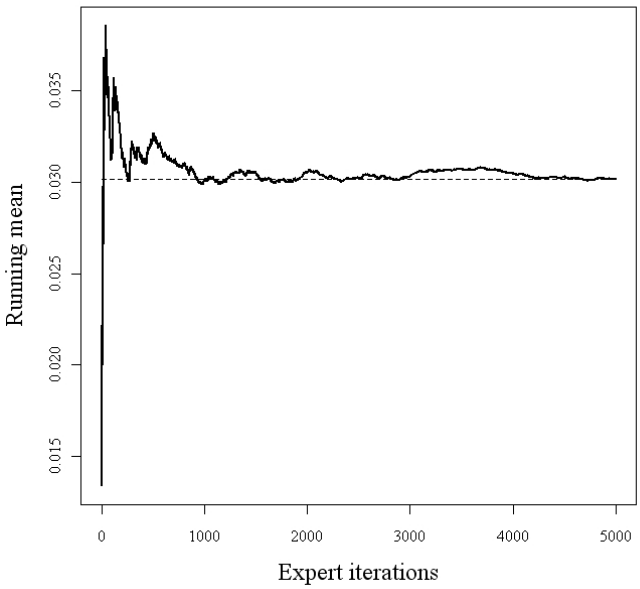


Figure 5.2. Convergence analysis for the number of iterations required to obtain a representative distribution for the combination of the results from all experts of a single question in the Expert Opinion questionnaire for MRSA acquisition in dogs.

5.3.3 Expert profiles

The experts were coded in colour for ease of graphical analysis. Specialist profiles were obtained for each expert based on the eleven specialist fields detailed in Question 17 of the questionnaire. Graphical representations of these profiles for each field and each expert, respectively, are displayed in full in Appendix 6 and examples of each are included as Figure 5.3 and 5.4. As expected, most experts graded themselves very highly in expertise in MRSA, but greater variation was present for more specialist fields beyond the general subject area, such as human medicine (Figure 5.3). When the ratings of each expert are assessed independently, some experts ranked themselves very high in many fields while others ranked themselves much lower across all fields (Figure 5.4).

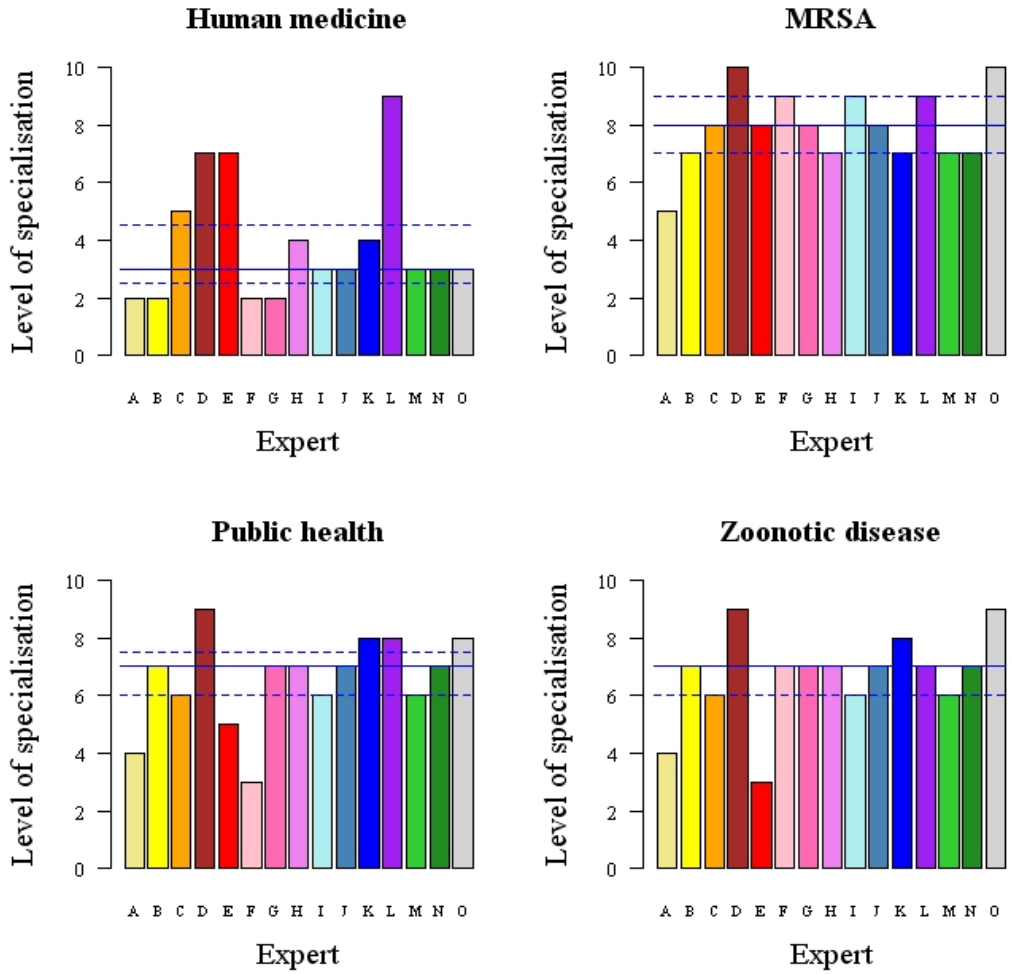


Figure 5.3. Levels of specialisation self-estimated by experts (coded A-O) in areas of human medicine, MRSA, public health and zoonotic disease within the expert opinion questionnaire. The solid horizontal line represents the median and dashed lines represent 25th and 75th percentiles.

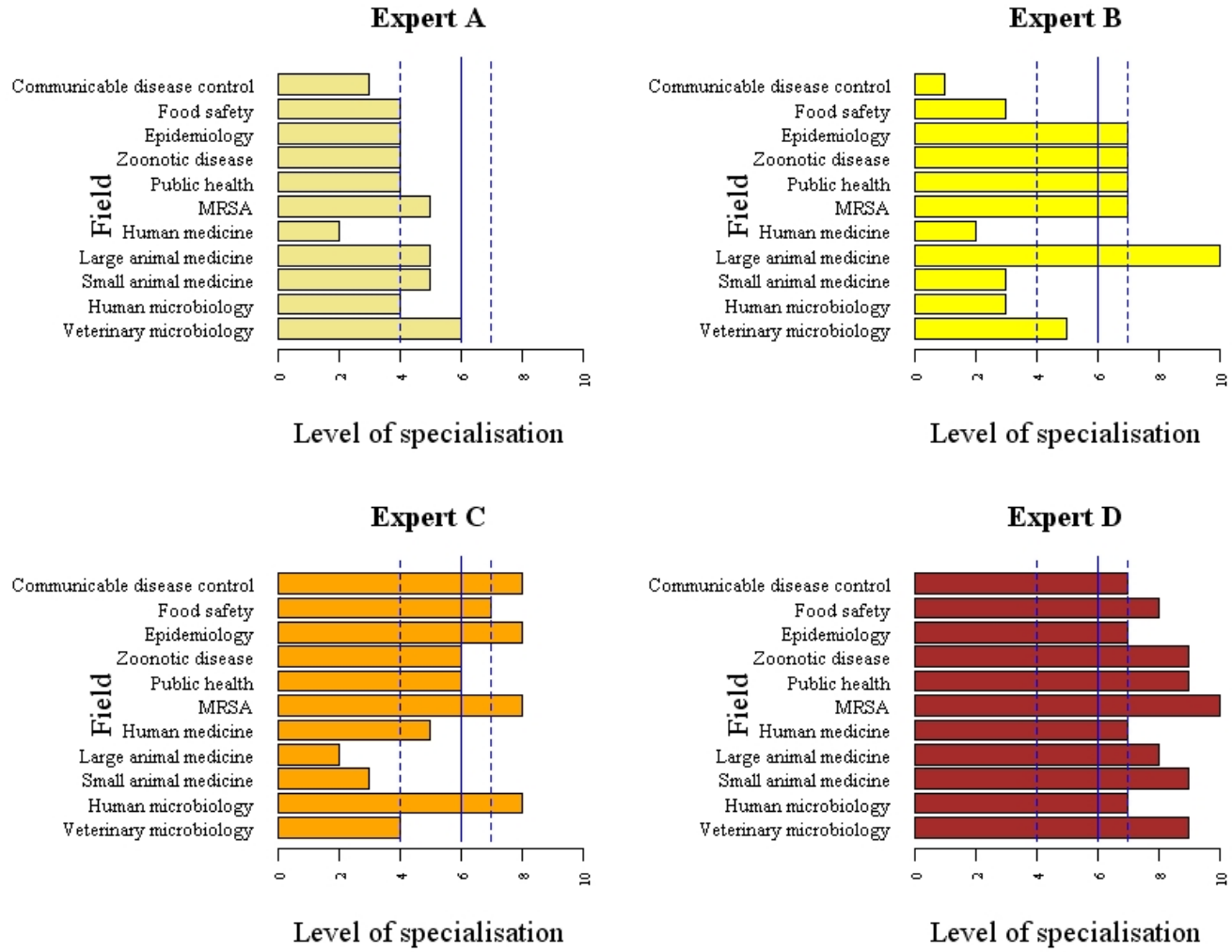


Figure 5.4. Levels of specialisation estimated by four experts (coded A-D) within the expert opinion questionnaire. The solid line represents the median and dashed lines represent 25th and 75th percentiles for all experts.

5.3.4 Expert distributions

5.3.4.1 Validity

The results of the questionnaire for each of the questions on prevalence estimation, for which published data were available, were analysed using a separate graphical procedure and are displayed in Figure 5.5. These graphs show that, for these questions, a single expert (Expert A, coloured beige) consistently provided estimates markedly outwith the range of other expert judgements and, where appropriate combination paradigms were available (questions 1, 2, 5 and 10a), the published data (represented as a black line in the figures). The removal of this expert resulted in a marked reduction in the tail of all of the combined expert distributions (red dashed lines) for each of these questions (Figure 5.6a and 5.6b). As a result of these findings, along with the low confidence estimates of this expert for all questions and low self-perception of 'expertness' (Fig 5.4), this expert was removed from subsequent analyses. All results from hereon will refer to the analysis of Experts B-O.

Comparison of the expert opinion distributions combined using equal weightings with distributions specified from published data are presented for questions 1, 2, 5 and 10a (Figure 5.7). A better fit was found between the expert opinion distributions and published data for question 10a when only the data that were available to the experts (i.e. published) at the time of questionnaire completion was compared (Figure 5.8).

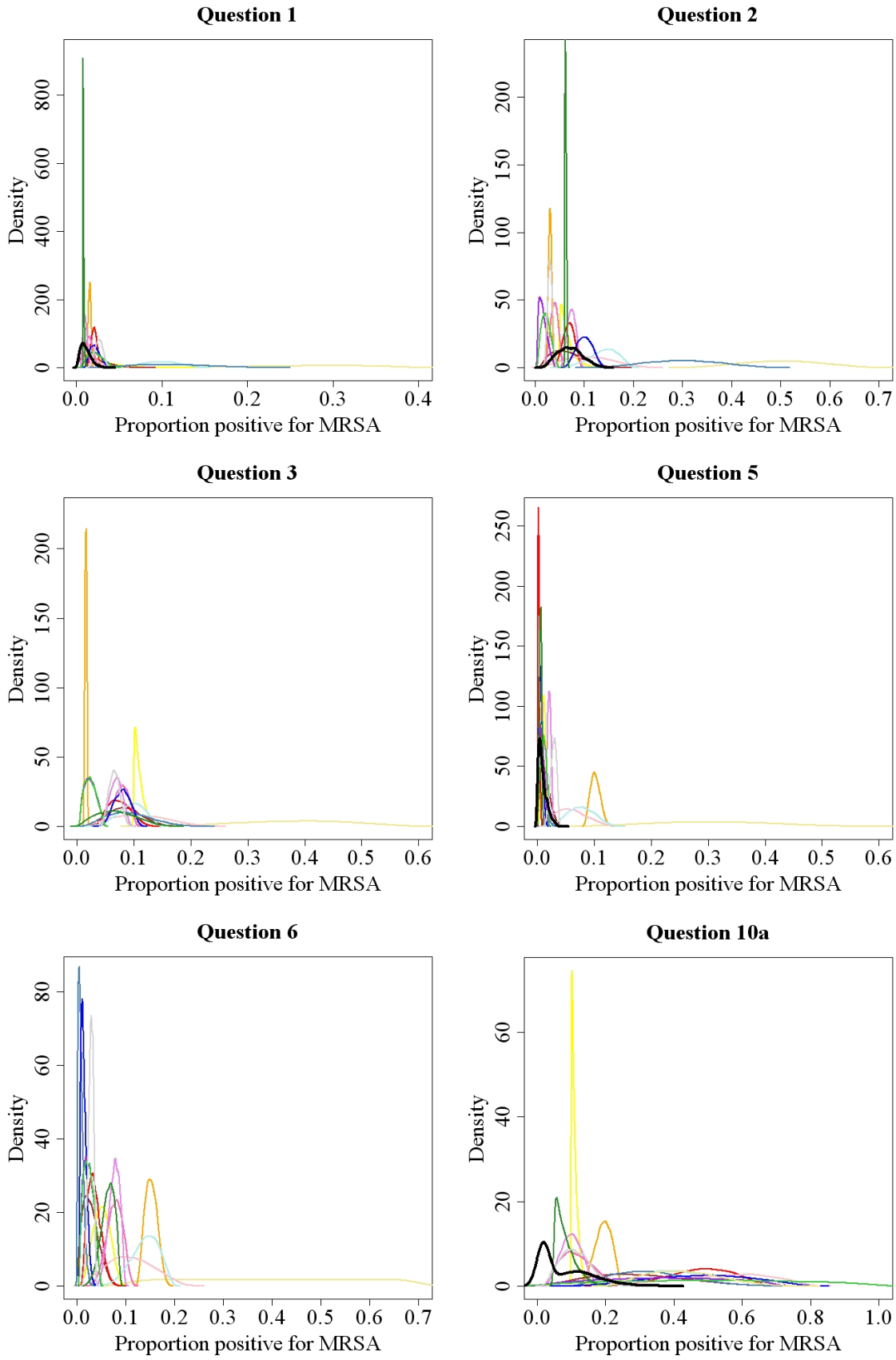


Figure 5.5. Expert uncertainty distributions (coloured lines) for questions for which published data are available. Black lines represent published data (Table 5.2).

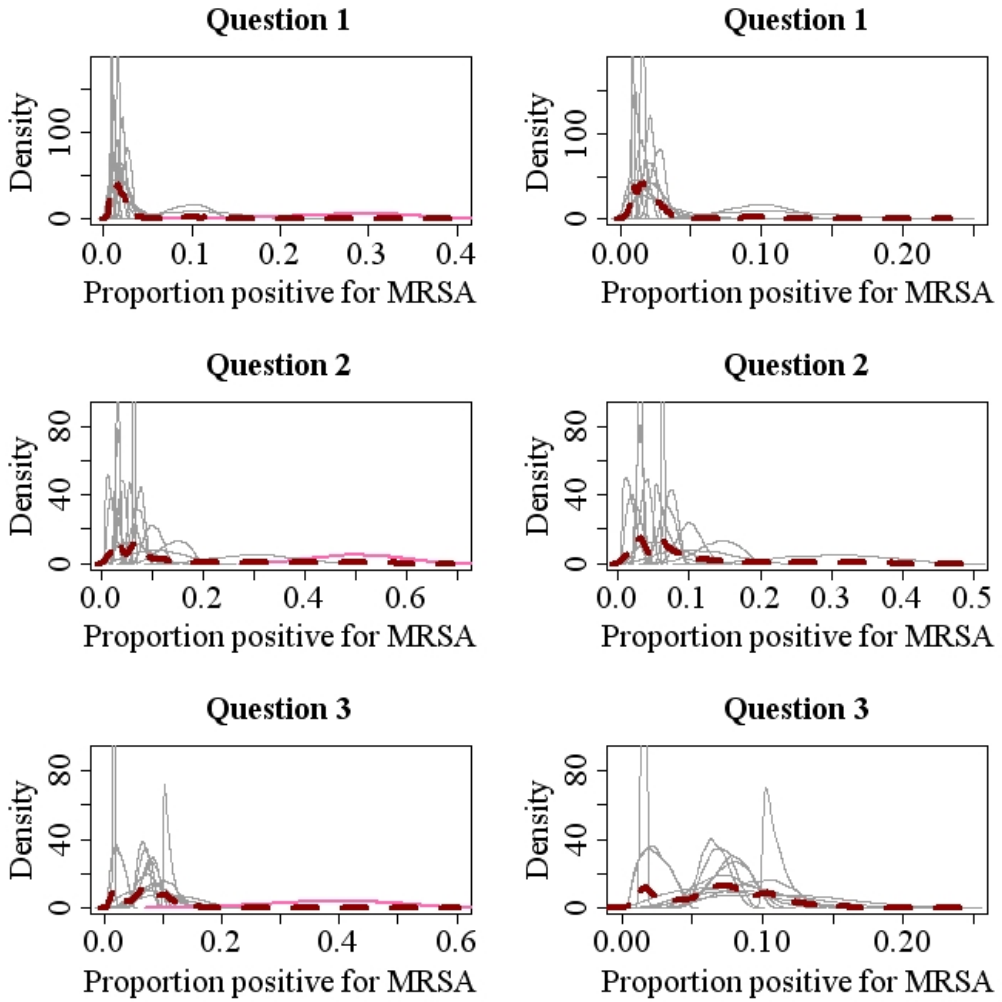


Figure 5.6a) Uncertainty distributions for Experts A (pink) and B-O (grey) for questions testing validity of responses in the Expert Opinion Questionnaire. Red dashed line represents combined expert opinion distribution with (left) and without (right) Expert A.

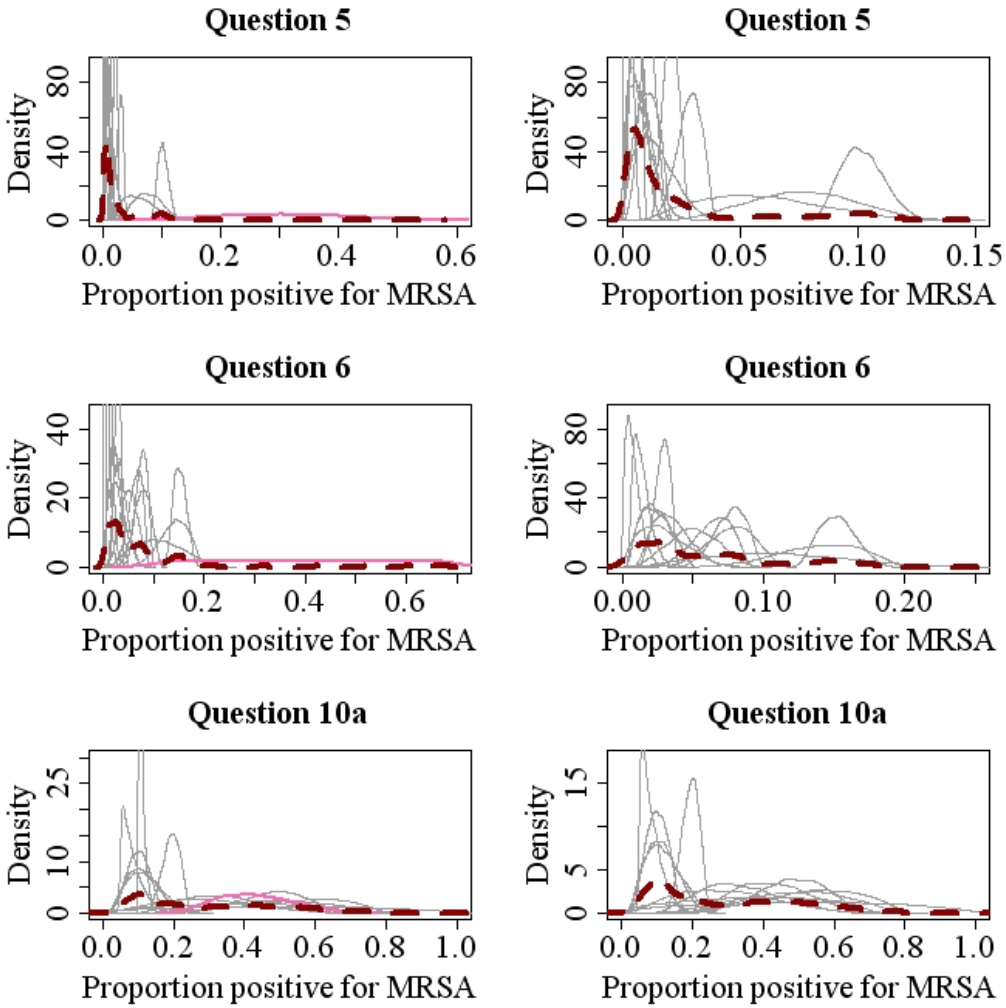


Figure 5.6b) Uncertainty distributions for Experts A (pink) and B-O (grey) for questions testing validity of responses in the Expert Opinion Questionnaire. Red dashed line represents combined expert opinion distribution with (left) and without (right) Expert A.

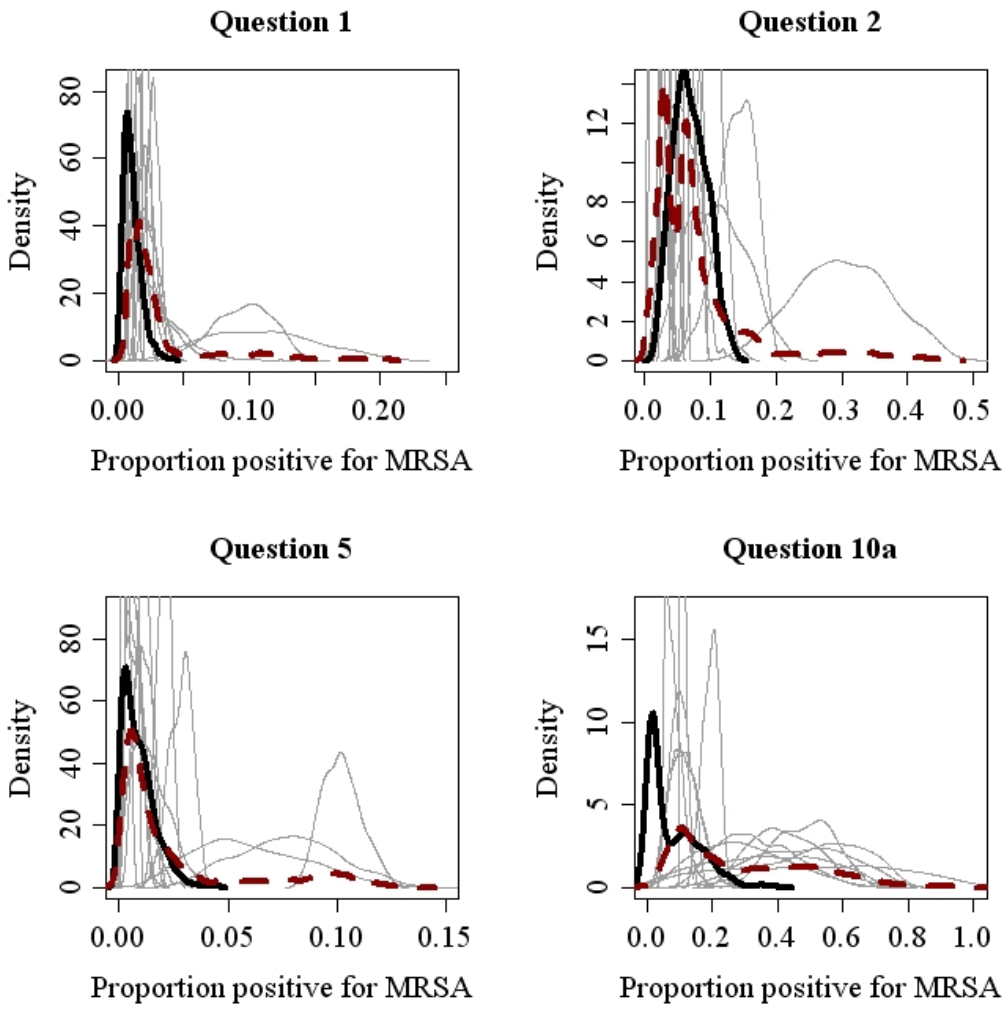


Figure 5.7. Comparison of combined expert distributions (red dashed line) with published or known data (black line) for Expert Opinion questionnaire questions 1, 2, 5 and 10a.

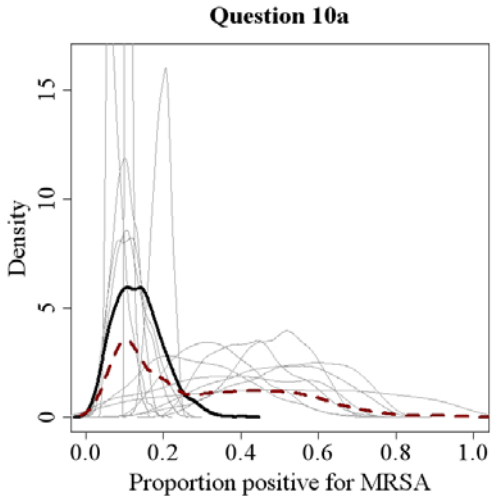


Figure 5.8. Comparison of combined expert distributions (red dashed line) with data published at the time of Expert Opinion questionnaire administration (black line) for Question 10a.

5.3.4.2 Expert estimations

The results of the expert estimations for each expert and each question are presented in full in Appendix 7.

In all sections of the questionnaire (prevalence, environmental contamination and transmission), variation was present between questions for the agreement or similarity between individual expert estimations. Expert confidence also varied markedly within and between questions. Responses for questions estimating prevalence have been presented in section 5.3.4.1 and, in general, showed similarity in estimates between experts, resulting, in most cases, in narrow composite distributions. The responses for questions estimating environmental contamination were found to exhibit marked discrepancy between experts at both the premises level (house or veterinary clinic) and within-premises level (estimated by number of contaminated 10cm x 10cm sites) (e.g. Figure 5.9). Experts displayed greater agreement and demonstrated more confidence in their estimates when asked about 'an average' premesis (e.g. Question 7 and 7a), in contrast with those in which there were occupants that were 'positive' for MRSA (e.g. Question 8 and 8a).

Responses to questions that required estimation of the probability of transmission of MRSA per contact to dogs, (non-hospitalised) humans and hospitalised humans from various sources generally produced estimates with an increasing range of uncertainty and reducing estimates of confidence when considering estimates for dogs and humans respectively (e.g. Figure 5.10). The questions relating to probability of transmission of MRSA to dogs predominantly resulted in composite estimates that were concentrated around very low probabilities (e.g. Figure 5.10 ai), aii))

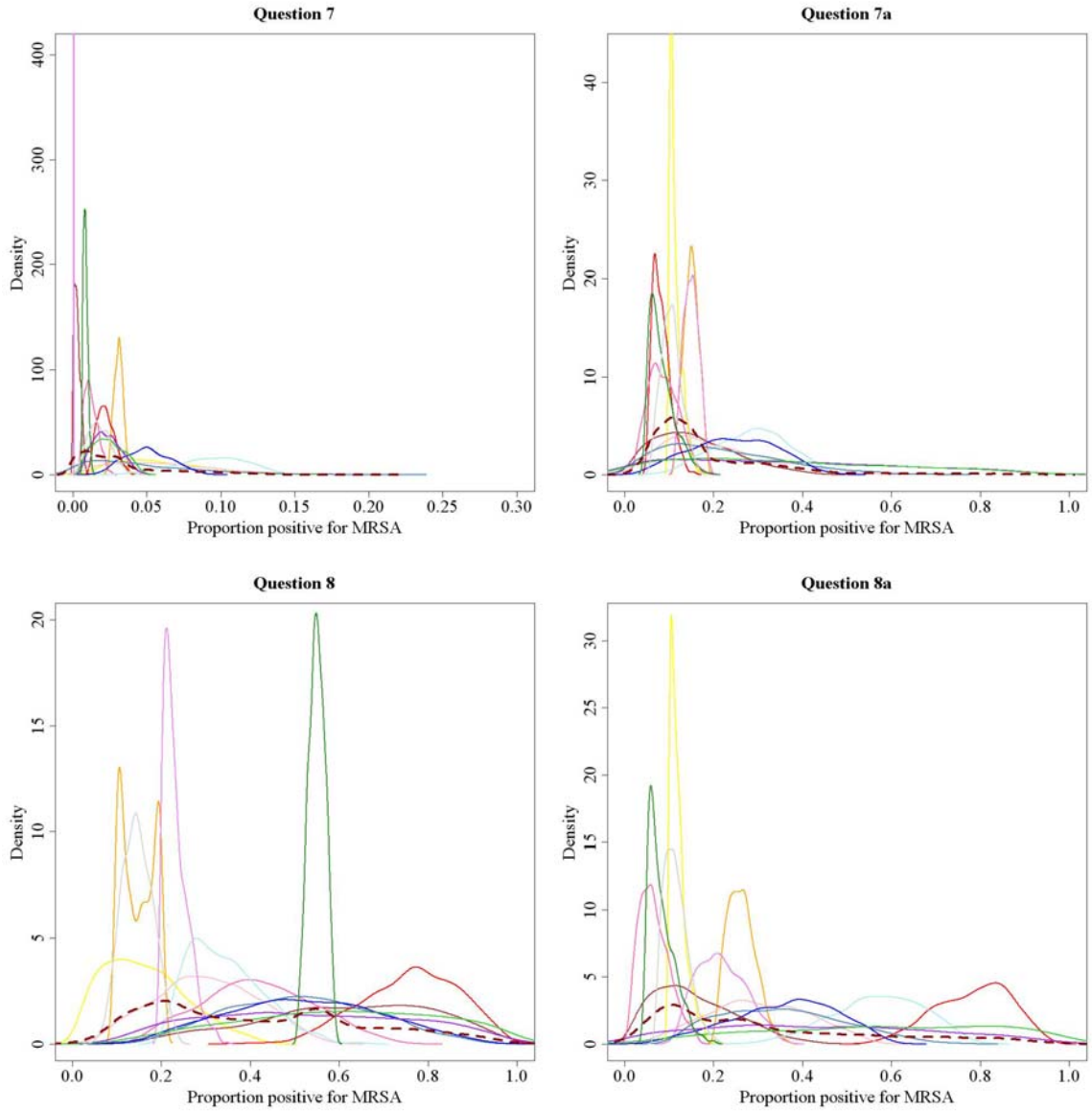


Figure 5.9. Differences in uncertainty, confidence and absolute estimates for questions estimating environmental contamination at the house level (Questions 7 and 8) and within-house site (10cm x 10cm) level (Questions 7a and 8a) and for an average house (Questions 7 and 7a) and those with at least one known MRSA positive occupants (Questions 8 and 8a).

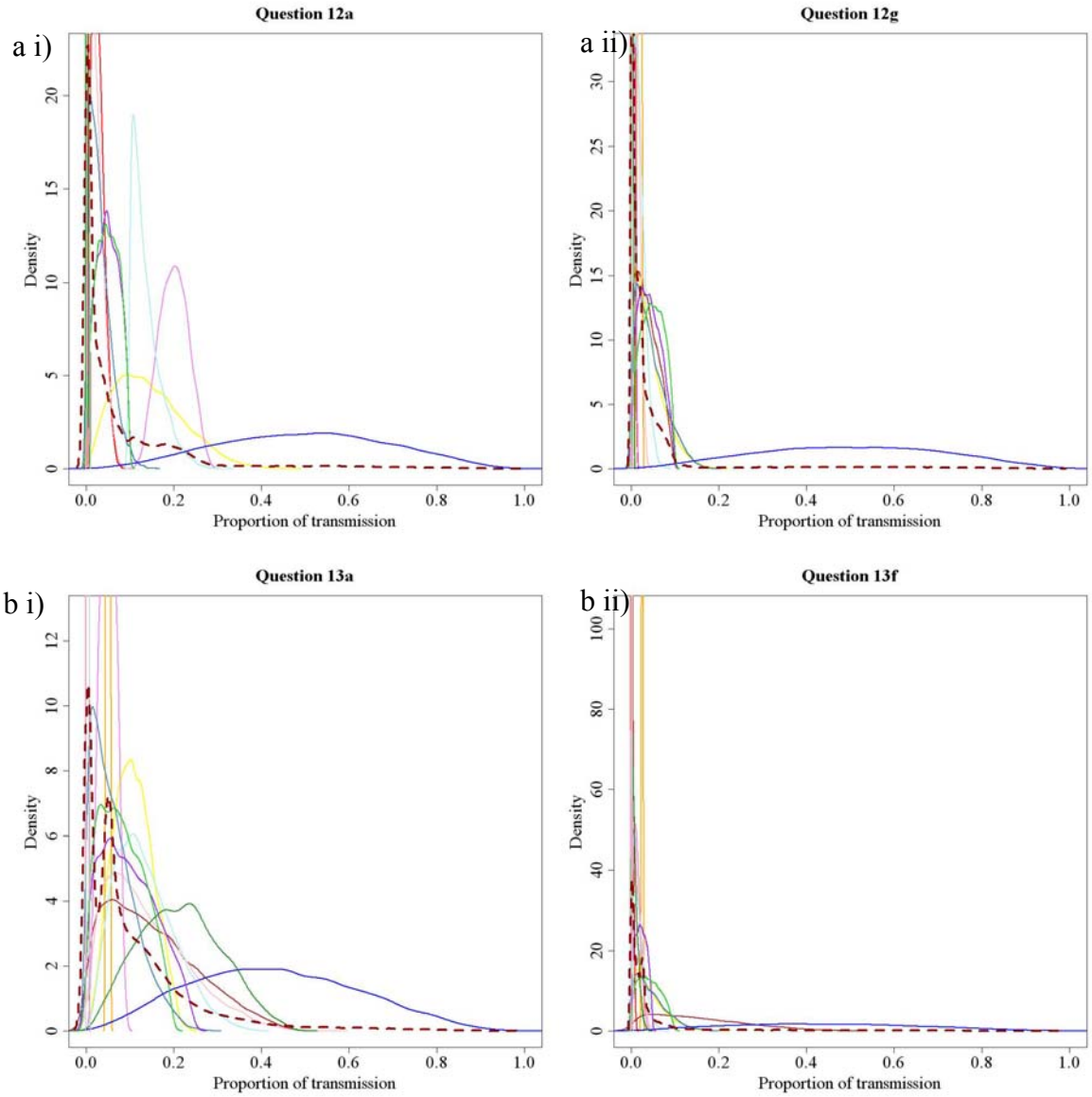


Figure 5.10. Increasing uncertainty and reduced confidence for estimates of probability of transmission per contact from a) a MRSA positive i) owner ii) household environmental site (10cm x 10cm) to a dog, b) an MRSA positive i) spouse or partner ii) household environmental site (10cm x 10cm) to a human.

Questions estimating the routes of transmission of MRSA to humans and dogs obtained responses that showed marked variation in confidence, uncertainty and absolute estimate values, resulting in very wide and poorly informative composite distributions, examples of which are shown in Figure 5.11.

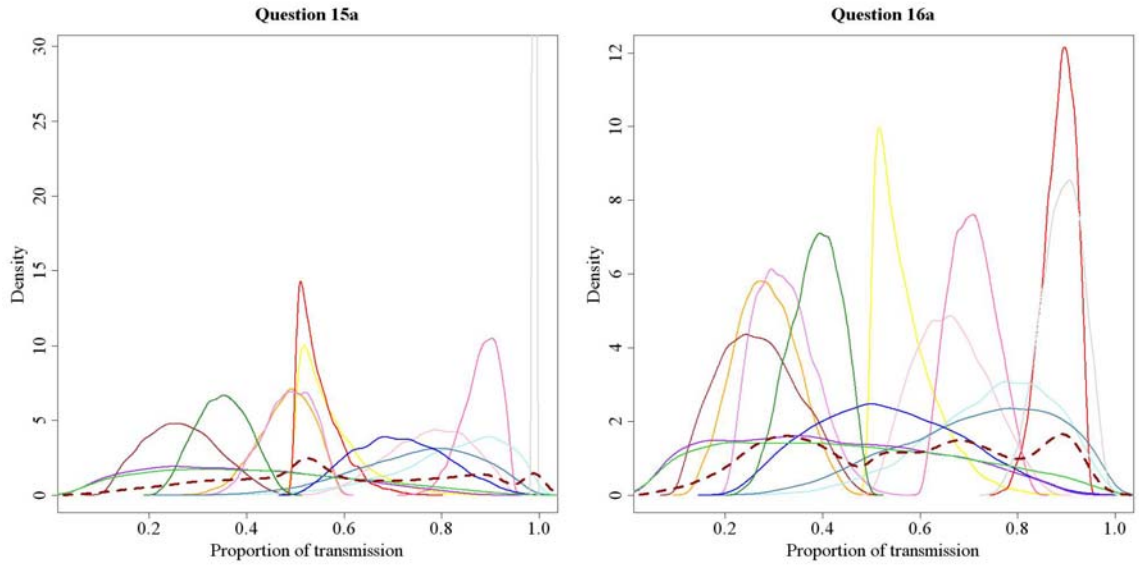


Figure 5.11. Estimations of proportion of transmission of MRSA that occurs by direct contact in a) humans, b) dogs.

A summary table of all of the resulting composite distributions is presented as Table 5.3. This table displays the median, interquartile range and a representation of the shape of the distributions resulting from equally weighted combinations of all expert opinions.

Table 5.3. Summary results for the composite distributions resulting from an expert opinion questionnaire on MRSA in dogs.

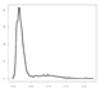
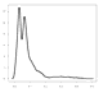
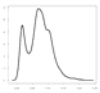
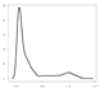
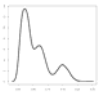
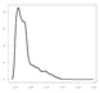
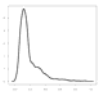
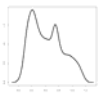
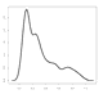
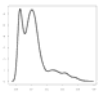
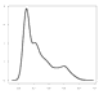
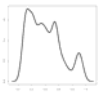
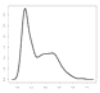
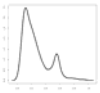
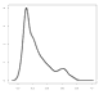
Section	Question[†]	Median	Interquartile range	Distribution shape
Prevalence of MRSA	1	0.020	0.014 - 0.030	
	2	0.062	0.033 – 0.093	
	3	0.072	0.045 – 0.097	
	4*			
	5	0.011	0.006 – 0.028	
	6	0.042	0.021 – 0.081	
Environmental Contamination	7	0.023	0.010 – 0.041	
	7a	0.139	0.097 – 0.231	
	8	0.400	0.220 – 0.583	
	8a	0.243	0.118 – 0.449	
	9	0.096	0.046 – 0.140	
	9a	0.215	0.109 – 0.381	
	10	0.367	0.202 – 0.553	
	10a	0.235	0.115 – 0.460	
	11	0.197	0.116 – 0.345	
	11a	0.199	0.115 – 0.346	

Table 5.3 (ctd) Summary results for the composite distributions resulting from an expert opinion questionnaire on MRSA in dogs

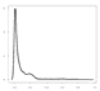
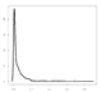
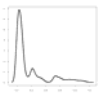
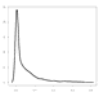
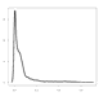
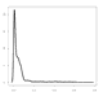
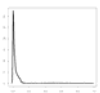
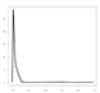
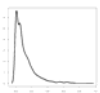
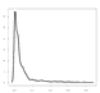
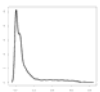
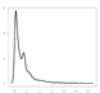
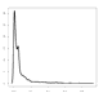
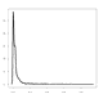
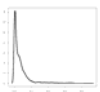
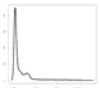
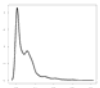
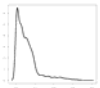
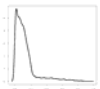
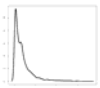
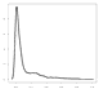
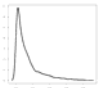
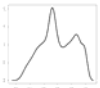
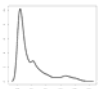
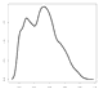
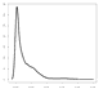
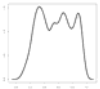
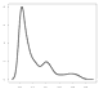
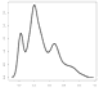
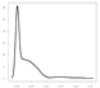
Section	Question[†]	Median	Interquartile range	Distribution shape
Transmission of MRSA	12a	0.026	0.005 – 0.103	
	12b	0.008	0.003 – 0.033	
	12c	0.060	0.025 – 0.215	
	12d	0.017	0.003 – 0.064	
	12e	0.032	0.005 – 0.069	
	12f	0.024	0.004 – 0.060	
	12g	0.014	0.003 – 0.042	
	12h	0.015	0.003 – 0.043	
	13a	0.068	0.019 – 0.156	
	13b	0.038	0.010 – 0.089	
	13c	0.049	0.010 – 0.114	
	13d	0.036	0.006 – 0.080	
	13e	0.017	0.004 – 0.038	
	13f	0.018	0.004 – 0.041	
	13g	0.039	0.010 – 0.089	

Table 5.3 (ctd) Summary results for the composite distributions resulting from an expert opinion questionnaire on MRSA in dogs

Section	Question†	Median	Interquartile range	Distribution shape
	13h	0.003	0.001 – 0.018	
	14a	0.064	0.009 – 0.141	
	14b	0.081	0.028 – 0.151	
	14c	0.088	0.037 – 0.151	
	14d	0.046	0.009 – 0.096	
	14e	0.044	0.011 – 0.147	
	14f	0.075	0.026 – 0.170	
	15a	0.565	0.431 – 0.810	
	15b	0.047	0.018 – 0.130	
	15c	0.303	0.146 – 0.440	
	15d	0.135	0.003 – 0.040	
	16a	0.561	0.354 – 0.760	
	16b	0.059	0.016 – 0.180	
	16c	0.251	0.157 – 0.439	
	16d	0.016	0.001 – 0.048	
	17*			

† Refer to Table 5.1 for question subject

* Distributions could not be specified for these questions

5.4 Discussion

This chapter has presented the results of an expert opinion elicitation exercise that was undertaken to enable parameterisation of the identified data gaps within a risk assessment of acquisition of MRSA in dogs. These gaps were identified in the work described in Chapter 3 and could not be filled by direct collection of new data, as described in Chapter 4. The outcome of this questionnaire was a set of composite distributions (Appendix 7) that represent expert opinion of the parameters that are required to complete the quantitative risk assessment that is presented in Chapter 6.

The technique used in this expert opinion elicitation assessment represents a novel approach adapted from previously published techniques. The use of an emailed expert opinion questionnaire is distinct from the predominantly workshop-based expert opinion elicitation that has been published for prior risk assessments within the veterinary field (Stärk *et al.*, 2002; Hotchkiss, 2004; Gallagher, 2005; Van der Fels-Klerx *et al.*, 2005; Boone *et al.*, 2008). The questionnaire was emailed in this case to enable the inclusion of geographically dispersed experts, allowing greater inclusion of current experts in the field, rather than a convenience sample that may have otherwise been required, given that no dedicated MRSA conferences were scheduled for the period within which this study was undertaken. Additionally, funds were not available to run a workshop specifically for this study. The use of workshop-based exercises undoubtedly presents greater opportunity for open dialogue and interaction between individual experts and between experts and the questionnaire facilitator. Similarly, workshop-based exercises also provide the opportunity to use techniques that result in opinion convergence such as the Delphi or Kaplan's expert information approach, whereby controlled feedback and reassessment and Bayes' theorem applied to rigorous expert-based evidence, are utilised respectively to result in a single combined expert distribution (Kaplan, 1992; Kaplan, 2000; Van der Fels-Klerx *et al.*, 2002). However, the implementation of this questionnaire by email, use of accompanying personalised letter and follow-up reminder after 5 weeks all provided opportunity for expert-facilitator interactions. Furthermore, in this case, expert opinion convergence was not sought. While experts were requested to respond for the situation in the UK, it was anticipated that, given the geographical variation in the prevalence of MRSA, the resulting estimates of prevalence, environmental contamination and transmission would be informed by the literature from or relating to the UK, but would also be updated or tempered with individual regional expert considerations, resulting in potentially important differences in

response. Had a greater number of experts been available, a meaningful comparison of response based on geographical location of the respondents could have been undertaken and trends potentially identified (Stärk *et al.*, 2002).

A small number of variables for which published data already existed have been used in this study to assess the validity of the expert estimates. This is loosely based on the use of 'seed' or calibration variables as defined in the 'Classical model' by Cooke and Goosens (2000) that allow assessment of experts' performance, enable performance-optimised combinations of opinion, and evaluate and validate expert judgement combination. However, the current study differs markedly from the classical model, given that only a few calibration variables could be defined in the area assessed, all of which were based to some degree on published data potentially known to the expert. Therefore, in the current study, the variables used only allow putative validation of expert judgements and combinations. The use of the validation variables in this study identified a single expert (Expert A) to have poor agreement with other experts and previously published data (Figure 5.5). While the omission of divergent opinions should not be the rule, the combination of poor estimation of validation variables, along with low confidence and low self-reported 'expertness' implied that this respondent may have been a source of estimation inaccuracy (Vose, 2000). Consequently, Expert A was excluded from subsequent analyses. With the exclusion of Expert A, assessment of the validation variables showed that there was general agreement between experts and between experts and current knowledge for those variables where prior information was available. Using the assumption that future performance of experts can be judged based on past performance (Cooke and Goossens, 2000), this validation procedure resulted in the acquisition of overall confidence in the validity of subsequent expert estimates.

It is regrettable that an appropriate number of calibration variables were not available to enable performance-optimised expert opinion combination as described in the classical model (Cooke and Goossens, 2000). It has been shown that this method of expert weighting is the least refractory to expert inaccuracy and opinion divergence (Bedford and Cooke, 2001) and its use has been found to result in defensible objectivity in previous expert judgement studies (Van der Fels-Klerx *et al.*, 2005; Boone *et al.*, 2008). However, these recent comparative studies also found that, while self-assessed expertise was unsatisfactory (Boone *et al.*, 2008), equal-weighted combinations performed adequately (Van der Fels-Klerx *et al.*, 2005; Boone *et al.*, 2008). In the current study, while self-assessed expertise was available for eleven areas of specialisation, it was difficult to align

these specialist areas adequately to the expertise required to answer each question, creating further complication of the use of self-assessed expert weighting. Consequently, the use of equally weighted combinations in the form of discrete distributions were used for all composite estimates and it could be argued that, while qualitatively interesting, the addition of question 17 did not provide any additional quantitative information for use within analyses. Future inclusion of targeted expertness on a per-question basis might allow greater utilisation of this information.

Criticisms of the implementation of this questionnaire were indirectly obtained from the recipients, in the form of written comments alluding to the difficulty in completion (Appendix 5). While these comments are valid, it is difficult to consider an easier and less "uncomfortable" way in which to phrase the questions to obtain the intended output and further reduce epistemic uncertainty. In the case of the subject of this questionnaire and in particular the questions relating to transmission, one must consider that there are no published data or prior studies and it is unlikely that the expert would have any data on which to base an estimate, other than their own (expert) experience. While this concept has formed the basis of many prior expert opinion questionnaires, and indeed represents the definition of circumstances in which expert opinion is sought, the lack of confidence reported from the experts, in addition to the marked variation in absolute estimates between experts for many of the questions on transmission (Figure 5.11), highlights the need to avoid over-interpretation or over-reliance on the results as absolute values. Indeed, the results of this questionnaire should, as with other previously completed expert opinion exercises (Stärk *et al.*, 2002), be viewed as the first step in parameter estimation that requires refinement and updating for future use. Notwithstanding this, the use of a discrete distribution with equal weighting to combine individual expert distributions has resulted in the specification of composite outcomes with marked uncertainty and a tendency towards a uniform distribution, in which marked inter-expert discrepancies and large individual uncertainties were reported (e.g. Figure 5.11) As such, while the resulting input distributions for any future quantitative analysis are unlikely to be particularly informative in these cases, they are equally unlikely to be misrepresentative. Perhaps of greater concern is over-reliance of the experts on published data that may or may not be misrepresentative of the real world. This potential bias is evidenced in the putative over-estimation of the proportion of contaminated sites within a veterinary hospital (Figures 5.7, 5.8), whereby a single published estimate based on small sample size and in a non-representative tertiary referral hospital (Loeffler *et al.*, 2005) appears to over-inform the expert estimates. In a data sparse area such as this, only future research and updating or

replacement of the composite estimates will enable accurate assessment of the true representativeness of the results of this elicitation process. However, given the nature of the data gaps, it is difficult to conceive of observational studies or ethical experiments that could provide data in many of the cases.

Otway and von Winterfeldt (1992) reviewed potential biases that may occur in an expert judgement procedure, including unstated assumptions and mindsets, and motivational, structural and cognitive biases. It is likely that unstated assumptions and mindsets were present for the experts that were questioned in this study, an example of which was seen in the unwillingness to include upper estimates from one of the experts, representing a mindset that the results of the questionnaire might be inappropriately analysed and exploited. It is difficult to assess the presence of other mindsets and, similarly, while every effort was made to avoid unstated assumptions, it is not possible to assess whether any remained and if so, what they were, without further expert-facilitator discussion. It is hoped that motivational biases, representing an expert's stake in the outcome, were minimal, given that it was unclear to the expert how the results of this process would influence future research, and that structural biases, representing undue influence of question structure, were also minimal. The use of whole number estimates, e.g., number of dogs out of 1000, were chosen instead of proportions or percentages to avoid structural bias. Nevertheless, while experts were given the option of reporting fractions, it is likely that it was heuristically difficult to estimate a fraction of a person or dog, inadvertently leading to the expert reporting on a confined linear scale, potentially resulting in some loss of information where composite distributions are very narrow, such as the probability of transmission of MRSA to dogs (Figure 5.10 ai) and aii)). Similarly, one expert commented on the likelihood of variation in his/her results if differing time scales had been used to elicit estimates of transmission (Appendix 5). As such, the estimation of a per contact transmission rate may have inadvertently introduced structural bias whilst trying to avoid it, by removing the necessity for experts to estimate average contacts per time period as well as probability of transmission.

Finally, cognitive biases may be expressed in the current study through availability bias, whereby easily recalled events are likely to be overestimated, overconfidence bias, whereby experts are more certain in estimates than their knowledge can justify and anchoring bias, where the expert holds to an original estimate despite new information to the contrary. Availability bias is likely to be reflected through individual experience with MRSA, given that this is a low prevalence pathogen (in dogs) with high emotional,

infection control and public health implications (Leonard and Markey, 2008). While this realisation of availability bias is unlikely to have had a detrimental effect on the results, given that individual expert experience is what was sought, it may be reflected in the increased certainty and confidence displayed in responses for dogs (when compared with humans) for questions on probability of transmission (Figure 5.10). While the expert selection was undertaken with the aim of obtaining a heterogenous panel, it may be that experts were more likely to respond to a veterinary study if they had veterinary interests (response bias), potentially supported by the low overall specialisation scores given for human medicine (Figure 5.3). The availability and use of recently published literature may represent a more tangible form of availability bias that may be exhibited in this study. Recent literature is readily available to all experts, and the effects of its potential influence may result in widespread variation of estimates, an example of which was putatively demonstrated in the estimation of environmental contamination in a veterinary hospital (Figures 5.7, 5.8). The magnitude of the detrimental (or beneficial) effect of this bias is dependent on the quality, power and representativeness of the available published studies which can only be evaluated on a per-study basis and where the likely source is known. Further evidence of availability bias in the current study is potentially found with the increased confidence, certainty and agreement when estimates of environmental contamination were requested for average houses and sites when compared to those with a known MRSA positive occupant (Figure 5.9). It is possible that this reflects the general nature in which prevalence estimates are obtained in experimental studies, although this could also be reflective of the uncertainty surrounding the wording of the question, in which "at least one MRSA positive" dog or human was identified, which represents a source of measurement bias (Dillman, 1991). A further source of bias, overconfidence bias, was limited in the current study by elicitation of measures of expert confidence in addition to upper and lower parameter bounds, which could be reflected in the resulting composite distributions by the use of discrete combination methods (Vose, 2000). However, the question reporting specialisation of respondents does show discrepancy between respondents in terms of self-reported 'expertness' (Figure 5.4) which may affect confidence in question estimates. Without a baseline or non self-reported measure to compare this to, it is not possible to know if overestimation or underestimation due to general over or underconfidence is reflected in the responses. The use of facilitator of other (non-self) expert-based assessment of knowledge may have been beneficial in providing a comparative measure to which the experts' estimates could be compared. Similarly, anchoring bias was limited by provision of minimum and maximum estimates prior to most likely estimates for distribution specification (Vose, 2000). However, this

bias may inadvertently have been present in the overall question structure which requested estimates in different species or environments sequentially, potentially resulting in anchoring of estimates to the response for the initial question. If the questionnaire were to be repeated, questions would be sequenced in an unrelated manner to reduce this bias further.

In conclusion, whilst acknowledging the limitations of a study such as this, this chapter has provided numerous composite expert estimates of prevalence, environmental contamination and transmission variables for parameterisation of a simulation-based quantitative risk assessment model on the acquisition of MRSA in dogs (Chapter 6). The results of this study are not intended to replace the results of dedicated research in the areas explored, but represent the first step in a fully updateable process.

CHAPTER 6

QUANTITATIVE RISK ASSESSMENT OF ACQUISITION OF MRSA IN PET DOGS

6.1 Introduction

Risk analysis is widely accepted and prescribed as a set of technical approaches that can be used to explore the combined effects of multiple factors implicated in a risk pathway. Critically, it is claimed that the first purpose and greatest strength of risk assessment is its ability to rank, in order of importance, the effect of multiple inputs on a single output and thus identify high priority data gaps, or priority candidate mitigation targets (Saltelli, 2000; Vose, 2000). In order to begin to quantify the contributions of dogs and humans to zoonotic transfer of MRSA, an initial assessment of the risk of acquisition of MRSA in dogs will allow the identification of important sources of the pathogen and the overall risk of acquisition of MRSA. Given the data-sparse nature of this field, a further aim was to assess the ability of risk assessment to identify priority data gaps for future research.

While qualitative risk assessment is often used as the first step in assessing the risk from a putative hazard such as MRSA, an initial qualitative risk assessment in this area proved unsatisfactory due to a) failure of conformity of the specified model to sequential stepwise progression through defined events or modules and b) numerous complex dependencies between parameters and permutations that required consideration (Chapter 3). In the absence of a rigorous qualitative assessment, a quantitative risk assessment is now specified with the aim of defining important data gaps and influential areas for future research in this data-sparse field.

6.2 Materials and Methods

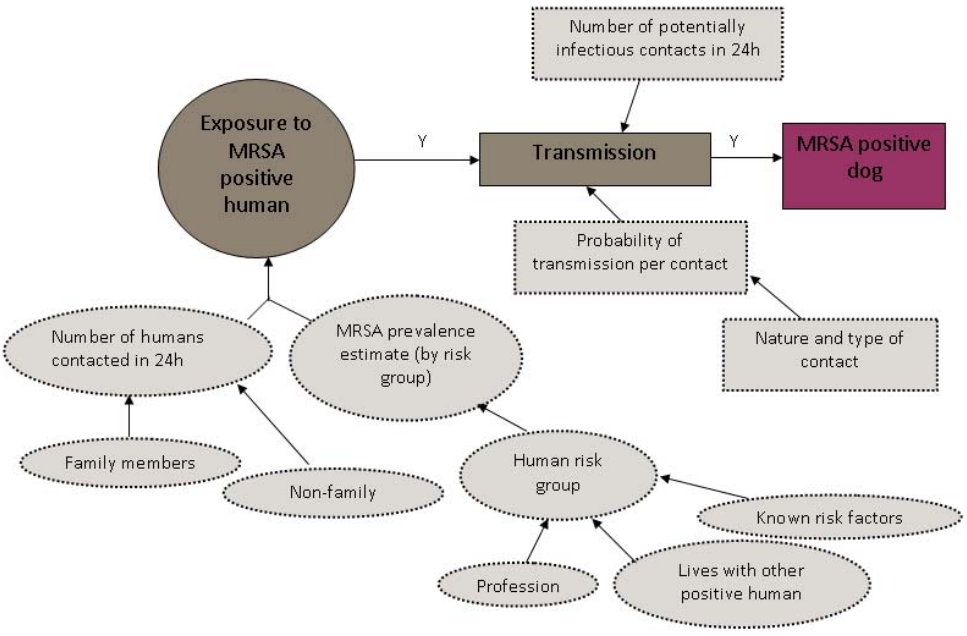
6.2.1 Simulation model

A stochastic simulation model was developed to simulate the proportion of dogs that would acquire MRSA, as carriage, colonisation or infection (defined in the hazard identification in section 3.2.1) over a 24 hour period. The model structure was based on the conceptual model defined and described in detail in Chapter 3 that outlined the likely pathways of acquisition of MRSA for a single dog over a 24 hour time period. Briefly, seven pathways for acquisition were specified and stratified to represent human family member, non-family member or veterinary worker; animal, limited to dogs in the community or at veterinary clinics; and environmental sources of MRSA that could be accessed through community

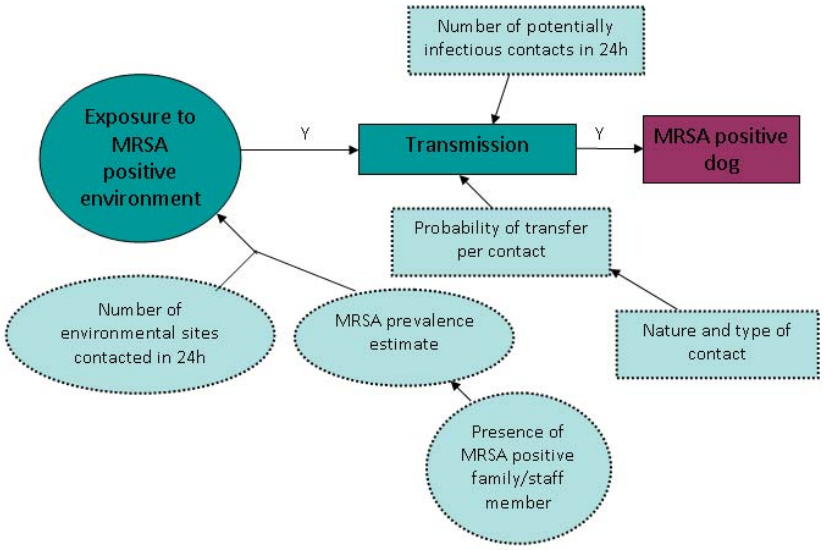
and veterinary hospital routes. The seven separate pathways for acquisition that were specified were considered to be non-sequential and were not mutually-exclusive. A number of dogs were simulated in this study, although these multiple dogs do not represent individual entities, rather multiple realisations of a single dog. The pathways available for each of the dogs that were simulated in the model depended on the result of an initial simulation of whether or not the dog in question attended a veterinary clinic in the 24 hours under consideration. All of the identified potential routes of acquisition of MRSA were accessible if a veterinary clinic was attended in the 24 hours under consideration, but pathways were restricted to the community routes only if a veterinary clinic had not been attended during this period.

Two sub-steps were considered within each pathway: first the risk of exposure to a positive source of MRSA (human, animal or environmental) and, secondly, the risk of transmission of MRSA from that source, given that exposure had occurred. Simplified scenario pathway models showing different factors considered in the risk of MRSA acquisition at each sub-stage of the conceptual model are presented in Figure 6.1.

(a)



(d)



(c)

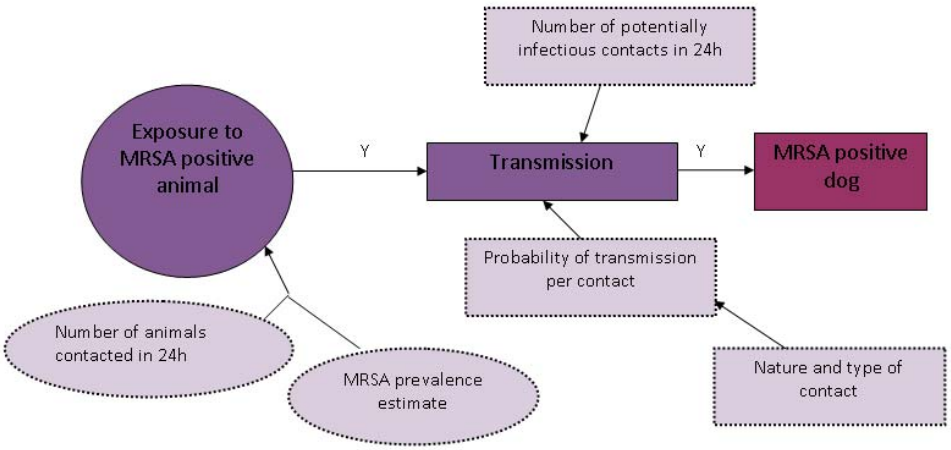


Figure 6.1. Scenario pathway models for a) human, b) animal and c) environmental sources of MRSA

The simulation model was structured as a second-order nested stochastic model, accounting for uncertainty and dog-dependent variability. Variability was accounted for by the use of binomial distributions that were run for each dog using probabilities drawn from independent uncertainty distributions based on prior data (published, unpublished and expert opinion) and specified as beta or modified beta (PERT) distributions (equations 5.1 – 5.4), run over a number of iterations. The probability of transmission (over 24 hours) was calculated using a scenario tree approach (Figure 6.2) and defined by:

$$P(t_{24h}) = 1 - (1 - P(t_{contact}))^{contacts}$$

Where $P(t_{24h})$ is the probability of transmission per 24 hours, $P(t_{contact})$ is the probability of transmission per contact, and contacts is the number of contacts per 24 hours.

The model was repeated, using the same input values, to obtain probabilistic, as opposed to dichotomous, results for colonisation of each dog over a number of 'days' with no variation in environmental factors. As veterinary attendance is a rare event, the model was also re-run in full with the probability of a dog attending a veterinary clinic specified as 1 to obtain results for this population of animals separately.

6.2.2 Parameterisation

The results of the expert opinion elicitation exercise presented in Chapter 5 were used to populate the model at all steps where experimental or observational studies were unavailable. Published material was used where possible and, where numerous published studies were available, these data were combined using expert weightings, either obtained through the results of the expert opinion questionnaire or, where necessary, through the consideration of the author and supervisors. The input distributions used to characterise uncertainty are presented in Table 6.1, while those used to characterise variability are presented in Table 6.2.

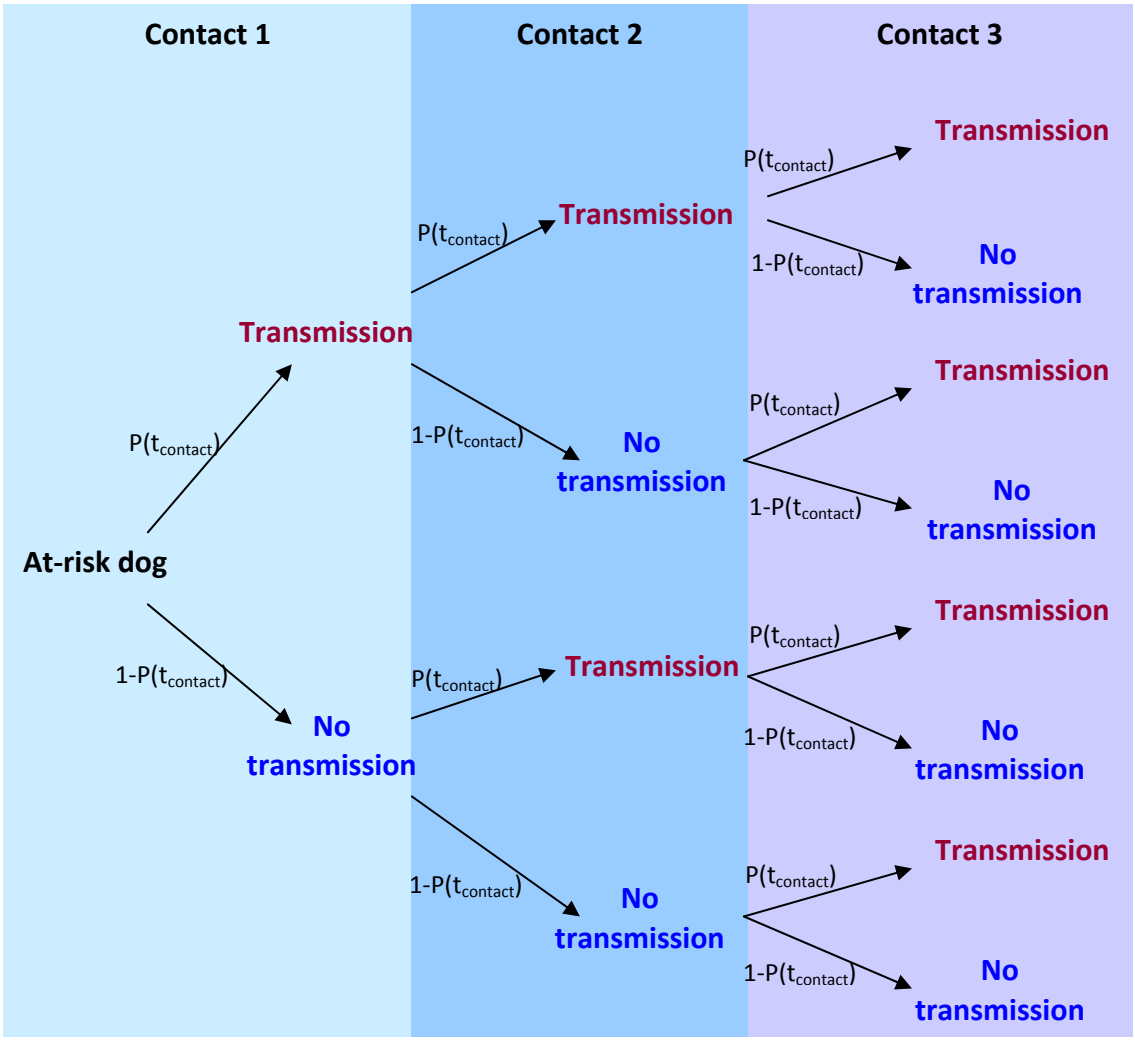


Figure 6.2. Example of a scenario tree for defining the probability of contact over a 24 hour period with three contacts (in this case) with a source of MRSA.

6.2.3 Uncertainty and Sensitivity analyses

An uncertainty analysis was undertaken using summary statistics and graphical analysis of the output of the model. The model was specified as a second order model to take into account both variability (modelled between realisations of dogs) and uncertainty (modelled over iterations) although uncertainty was also unavoidably present within the variability distributions. The model was specified to result in an output that was structured as a three-dimensional matrix, with the number of rows representing realisations of dogs (enabling inclusion of variability) and number of columns representing iterations of the model (reflecting uncertainty), all repeated 50 times to produce a probabilistic outcome from the 1/0 outputs at the dog/iteration level. Uncertainty was assessed by comparing the results

for all realisations of dogs between iterations, while variability was assessed by comparing the results for all iterations across realisation of dogs.

A sensitivity analysis was undertaken using LR to identify the steps of the simulation model associated with the greatest risk of MRSA acquisition. A subset of the model outcome, using the first repeat only to define the model outcome, was obtained by selecting all dogs that became positive for MRSA, classed as cases, and then an equal number of randomly selected entries that did not become positive for MRSA, classed as controls, to enable the sensitivity analysis to be implemented in the form of a case-control study. Two separate LR models were run, assessing exposure and transmission explanatory variables respectively, for each of the simulation models, modelling all animals irrespective of veterinary attendance and only animals that had attended a veterinary clinic over the 24 hours in question, resulting in a total of four regression models. For all regression models, outcome was specified as MRSA positive or negative and explanatory variables considered for inclusion in each model are presented in Table 6.3. The continuous variables were checked for linearity by categorising them and graphing the log odds of the outcome. Those that were not linear were included in the models as factors. Exploration of the data within these subsets was undertaken using two-way tabulation and graphical analysis using mosaic plots. Initial univariate analyses were not undertaken as the purpose was to assess all variables within the multivariable models for the aim of sensitivity analysis. The regression models were fitted using a backwards stepwise algorithm, with reduction in Akaikie's Information Criterion (AIC) used as the criterion for inclusion of variates in the final model.

6.2.4 Assumptions

In the current assessment, the hazard was defined as MRSA. A generalisation of 'MRSA positive' or 'MRSA negative' was used for human, animal and environmental status and refers to a source from which MRSA can be cultured directly and that has the ability to contaminate, colonise or infect others. This definition encompasses any limitations in test sensitivity and specificity that may be reflected in the real world. All assumptions presented in the hazard identification in Chapter 3 (section 3.2.1) hold for the current assessment.

Table 6.1. Parameterisation of uncertain distributions for input to the stochastic simulation model for MRSA acquisition in dogs.*

Model step		Input variable	Distribution	Inputs	Reference
1	i	**P(dog attends vet in 1yr)**	Beta(s+1, n-s+1)	s=234 n=279	Westgarth *et al.* (2008)
	ii	**P(dog attends vet in 24h)**	1-(1- **P(dog attends vet in 1yr)**)^(1/365)		
Exposure					
A / B	i	**P(human is a health care worker)**	Beta(s+1,n-s+1)	s=89 n=2118	Westgarth *et al.* (2008)
	ii	**P(human is a vet)**	Beta(s+1,n-s+1)	s = 15671 n = 37804500	RCVS annual report www.statistics.gov.uk**
	iii	**P(human is in other high risk group)**	Betapert(min,ml,max)	min=0.01 ml=0.02 max=0.05	JH after consideration of literature
	iv	**P(human is in normal risk group)**	1-(**P(human is a health care worker)**+**P(human is a vet)**+**P(human is in other high risk group)**)		
	v	**P(health care worker is MRSA positive)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	vi	**P(veterinary worker is MRSA positive)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	vii	**P(other high risk goup is MRSA positive)**	Betapert(min,ml,max)	min=0.015 ml=0.05 max=0.15	JH opinion after consideration of literature
	viii	**P(general population is MRSA positive)**	Beta(s+1,n-s+1)	s=4 n=274	Abudu *et al.* (2001)
	ix	**P(number of other people in family=0,1,2,3,4,5,6)**	Dirichlet(α_0, α_1, α_2, α_3, α_4, α_5, α_6)	α_0=39 α_1=86 α_2=51 α_3=57 α_4=27 α_5=4 α_6=3	(Westgarth, 2008) (personal communication)

Table 6.1. (ctd) Parameterisation of uncertain distributions for input to the stochastic simulation model for MRSA acquisition in dogs.*

Model step		Input variable	Distribution	Inputs	Reference
	x	**P(person MRSA positive if lives with MRSA positive person)**	Betapert(min,ml,max)	min=0.02 ml=0.1 max=0.5	JH opinion after consideration
C	i	**P(exposure to other dogs in 24h)**	Beta(s+1,n-s+1)	s=246 n=272	(Westgarth, 2008)
		P(community dog is MRSA positive)(1)	Beta(s+1,n=s+1) ($\mathbf{p_{C1}}$)	s=1 n=255	Reported in Rich & Roberts (2006)
		P(community dog is MRSA positive)(2)	Beta(s+1,n=s+1) ($\mathbf{p_{C2}}$)	s=0 n=188	(Murphy *et al.*, 2006)
		P(community dog is MRSA positive)(3)	Beta(s+1,n=s+1) ($\mathbf{p_{C3}}$)	s=2 n=203	(Hanselman *et al.*, 2008)
	ii	**P(community dog is MRSA positive)**	Discrete({$\mathbf{p_{C1}}$,$\mathbf{p_{C2}}$,$\mathbf{p_{C3}}$},{1,1,1})		
D	i	**P(house contaminated with MRSA | at least one positive family member)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	ii	**P(house contaminated with MRSA | no positive family members)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	iii	**P(environmental site contaminated | at least one positive family member)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	iv	**P(environmental site contaminated | no positive family members)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
E		**P(dog at vet is MRSA positive)** (1)	Beta(s+1,n=s+1) ($\mathbf{p_{E1}}$)	s=4 n=200	Loeffler (personal communication)
		P(dog at vet is MRSA positive) (2)	Beta(s+1,n=s+1) ($\mathbf{p_{E2}}$)	s=3 n=287	(Abbott *et al.*, 2006)
		P(dog at vet is MRSA positive) (3)	Beta(s+1,n=s+1) ($\mathbf{p_{E3}}$)	s=7 n=230	(Abbott *et al.*, 2006)

Table 6.1. (ctd) Parameterisation of uncertain distributions for input to the stochastic simulation model for MRSA acquisition in dogs.*

Model step		Input variable	Distribution	Inputs	Reference
		P(dog at vet is MRSA positive) (4)	Beta(s+1,n=s+1) ($\mathbf{p_{E4}}$)	s=4 n=45	(Loeffler *et al.*, 2005)
	i	**P(dog at vet is MRSA positive)**	Discrete({$\mathbf{p_{E1}}$,$\mathbf{p_{E2}}$,$\mathbf{p_{E3}}$,$\mathbf{p_{E4}}$},{1,1,1,1})		
F		**P(veterinary environment contaminated)** (1)	Beta(s+1,n=s+1) ($\mathbf{p_{F1}}$)	s=3 n=27	Loeffler *et al.* (2005) – considered as outbreak data
		P(veterinary environment contaminated) (2)	Beta(s+1,n=s+1) ($\mathbf{p_{F2}}$)	s=2 n=158	Heller *et al.* (2009)
	i	**P(veterinary environment contaminated)**	Discrete($\mathbf{p_{F1}}$,$\mathbf{p_{F2}}$),(0.1,1)		
G		**P(small animal veterinary worker is MRSA positive)** (1)	Beta(s+1,n=s+1) ($\mathbf{p_{G1}}$)	s=12 n=271	Hanselman *et al.* (2006b)
		P(small animal veterinary worker is MRSA positive) (2)	Beta(s+1,n=s+1) ($\mathbf{p_{G2}}$)	s=5 n=51	Deacon *et al.* (2006)
		P(small animal veterinary worker is MRSA positive) (3)	Beta(s+1,n=s+1) ($\mathbf{p_{G3}}$)	s=14 n=78	Loeffler *et al.* (2005) – considered as outbreak data
		P(small animal veterinary worker is MRSA positive) (4)	Beta(s+1,n=s+1) ($\mathbf{p_{G4}}$)	s=2 n=64	Heller *et al.* (2009)
	i	**P(small animal veterinary worker is MRSA positive)**	Discrete({$\mathbf{p_{G1}}$,$\mathbf{p_{G2}}$,$\mathbf{p_{G3}}$,$\mathbf{p_{G4}}$},{1,1,0.1,1})		
Transmission					
A		**P(transmission from MRSA positive family member per contact)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	ii	Number of contacts with family member per 24h	betapert(min,ml,max)	min=0 ml=5 max=20	JH opinion after consideration
	iii	**P(transmission from MRSA positive family member per 24h)**	1-(1- **P(transmission from MRSA positive family member per contact)**)^ Number of contacts with family member per 24h		
B	i	**P(transmission from MRSA positive non-family member per contact)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)

Table 6.1. (ctd) Parameterisation of uncertain distributions for input to the stochastic simulation model for MRSA acquisition in dogs.*

Model step		Input variable	Distribution	Inputs	Reference
	ii	Number of contacts with non-family member per 24h	Betapert(min,ml,max)	min=0 ml=1 max=10	JH opinion after consideration
	iii	**P(transmission from MRSA positive non-family member per 24h)**	1-(1- **P(transmission from MRSA positive non-family member per contact)**)^ Number of contacts with non-family member per 24h		
C	i	**P(transmission from MRSA positive community dog per contact)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	ii	Number of contacts with community dog per 24h	Betapert(min,ml,max)	min=0 ml=1 max=10	Consideration of (Westgarth *et al*., 2008) and results of Chapter 4 to account for intimate contacts
	iii	**P(transmission from community dog per 24h)**	1-(1- **P(transmission from MRSA positive community dog per contact)**)^ Number of contacts with community dog per 24h		
D	i	**P(transmission from MRSA positive home environment per contact)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	ii	Number of contacts with home environment per 24h	Betapert(min,ml,max)	min=0 ml=1 max=100	JH opinion after consideration
	iii	**P(transmission from MRSA positive home environment per 24h)**	1-(1- **P(transmission from MRSA positive home environment per contact)**)^ Number of contacts with home environment per 24h		
E	i	**P(transmission from MRSA positive dog at veterinary clinic per contact)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	ii	Number of contacts with a dog at a veterinary clinic per 24h	Betapert(min,ml,max)	min=0 ml=1 max=10	JH opinion after consideration

Table 6.1. (ctd) Parameterisation of uncertain distributions for input to the stochastic simulation model for MRSA acquisition in dogs.*

Model step		Input variable	Distribution	Inputs	Reference
	iii	**P(transmission from MRSA positive dog at veterinary clinic per 24h)**	1-(1- **P(transmission from MRSA positive dog at veterinary clinic per contact)**)^ Number of contacts with a dog at a veterinary clinic per 24h		
F	i	**P(transmission from MRSA positive veterinary environment per contact)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinon (Chapter 5)
	ii	Number of veterinary environment contacts per 24h	Betapert(min,ml,max)	min=1 ml=10 max=25	JH opinion after consideration
	iii	**P(transmission from MRSA positive veterinary environment per 24h)**	1-(1- **P(transmission from MRSA positive veterinary environment per contact)**)^ Number of veterinary environment contacts per 24h		
G	i	**P(transmission from MRSA positive veterinary staff member per contact)**	Random samples using latin hypercube sampling of length 'iterations' from previously defined combination of expert opinion distributions		Expert opinion (Chapter 5)
	ii	Number of veterinary staff contacts per 24h	Betapert(min,ml,max)	min=0 ml=3 max=20	JH opinion after consideration
	iii	**P(transmission from MRSA positive veterinary staff member per 24h)**	1-(1- **P(transmission from MRSA positive veterinary staff member per contact)**)^ Number of veterinary staff contacts per 24h		

JH = Jane Heller

* Colours within table used for ease of follow through for Table 6.2.

** Population estimate taken from www.statistics.gov.uk – estimate here is 60,975,000 but only 62% are of working age, equating to 37,804,500

Table 6.2. Parameterisation of variability distributions for input to the stochastic simulation model for MRSA acquisition in dogs. Reference within table to uncertainty distributions specified in Table 6.1 (in bold and colours)

Model step	Input variable	Distribution	Inputs	Reference
1	Attendance at veterinary clinic (**attend.vet**)	Binomial(1, **P(dog attends vet in 24h)**)		
Exposure				
A	Owner MRSA status (**O.status**)	Binomial(1,**P(MRSA positive given the specified risk group)**)		
B	Number of non-owner family members (**n.non.O.fam**)	sample(0,1,2,3,4,5,6) with probabilities taken from the dirichlet distribution specified in **Exposure A/B(ix)** (Table 6.1)		
	Number of MRSA positive non-owner family members (**n.pos.non.O.fam**)	Binomial(**n.non.O.fam**, **P(MRSA positive given the specified risk group)**) **OR** binomial(**n.non.O.fam**, **P(person MRSA positive if lives with MRSA positive person)**) depending on if there is a MRSA positive family member present in the house		
	Number of non-family humans exposed to per day (**n.non.fam**)	Betapert(min,ml,max)	min=0 ml=4 mx=15	(Westgarth *et al.*, 2008)
	Number of MRSA positive non-owner family members exposed to per day (**n.pos.non.fam**)	Binomial(**n.non.fam**, **P(MRSA positive given the specified risk group)**)		
C	Is at-risk dog exposed to other dogs in 24h (**exp.dog**)	Binomial(1, **P(exposure to other dogs in 24h)**)		
	Number of dogs in the community that at-risk dog is exposed to in 24h (**n.c.dogs**)	**exp.dog***Betapert(min,ml,max)	min=1 ml=2 max=15	(Westgarth *et al.*, 2008)
	Number of MRSA positive community dogs contacted in 24h (**n.pos.c.dog**)	Binomial(**n.c.dogs**, **P(community dog is MRSA positive)**)		
D	Is house contaminated with MRSA (**house.contam**)	Binomial(1, **P(house contaminated with MRSA | at least one positive family member)**) or binomial(1, **P(house contaminated with MRSA | no positive family members)**) depending on if there is a MRSA positive family member present in the house		

Table 6.2. (ctd) Parameterisation of variability distributions for input to the stochastic simulation model for MRSA acquisition in dogs. Reference within table to uncertainty distributions specified in Table 6.1 (in bold and colours)

Model step	Input variable	Distribution	Inputs	Reference
	Number of sites at-risk dog is exposed to in house (**n.env.sites**)	Betapert(min,ml,max)	min=1 ml=20 max=200	JH opinion
	Number of contaminated sites in house (**n.pos.env.sites**)	Binomial(**n.env.sites**,**P(environmental site contaminated | at least one MRSA positive family member)** OR **P(environmental site contaminated | no MRSA positive family members)**) depending on if there is a MRSA positive family member in the house		
E	Number of dogs contacted at veterinary clinic (**n.vet.dogs**)	**attend.vet***Betapert(min,ml,max)	min=0 ml=1 max=10	JH opinion
	Number of MRSA positive dogs contacted at veterinary clinic in 24h (**n.pos.vet.dog**)	Binomial(**n.vet.dogs**, **P(dog at vet is MRSA positive)**)		
F	Number of veterinary environmental sites contacted in 24h (**n.vet.env**)	**attend.vet***Betapert(min,ml,max)	min=1 ml=10 max=100	JH opinion
	Number of contaminated veterinary environmental sites contacted in 24h (**n.pos.vet.env**)	Binomial(**n.vet.env**, **P(veterinary environment contaminated)**)		
G	Number of veterinary staff contacted in 24h (**n.vet**)	**attend.vet***Betapert(min,ml,max)	min=1 ml=2 max=5	JH opinion
	Number of MRSA positive veterinary staff contacted in 24h (**n.pos.vet**)	Binomial(**n.vet**, **P(small animal veterinary worker is MRSA positive)**)		

Table 6.2. (ctd) Parameterisation of variability distributions for input to the stochastic simulation model for MRSA acquisition in dogs. Reference within table to uncertainty distributions specified in Table 6.1 (in bold and colours)

Model step	Input variable	Distribution	Inputs	Reference
Transmission				
A		Binomial(**O.status**, **P(transmission from MRSA positive family member per 24h)**)		
B		Binomial(**n.pos. non.O.fam**, **P(transmission from MRSA positive family member per 24h)**)		
		Binomial(**n.pos. non.fam**, **P(transmission from MRSA positive non-family member per 24h)**)		
C		Binomial(**n.pos.c.dog**, **P(transmission from community dog per 24h)**)		
D		Binomial(**n.pos.env.sites**, **P(transmission from MRSA positive home environment per 24h)**)		
E		Binomial(**n.pos.vet.dog**, **P(transmission from MRSA positive dog at veterinary clinic per 24h)**)		
F		Binomial(**n.pos.vet.env**, **P(transmission from MRSA positive veterinary environment per 24h)**)		
G		Binomial(**n.pos.vet**, **P(transmission from MRSA positive veterinary staff member per 24h)**)		

JH = Jane Heller

* Colours within table used for ease of follow through from Table 6.1.

Table 6.3. Explanatory variables considered for logistic regression models used in the sensitivity analysis for acquisition of MRSA in dogs (all for a 24 hour time period)

Simulation model	Regression model	
	Exposure Model	**Transmission model**
All dogs	Number of MRSA contaminated environmental sites	P(transmission from environment)
	Number of MRSA positive family members	P(transmission from family member)
	Number of MRSA positive non family members	P(transmission from non family member)
	Number of MRSA positive dogs	P(transmission from dog)
	Attendance at veterinary clinic	P(attendance at veterinary clinic)
Dogs attending veterinary clinics only	Number of MRSA contaminated environmental sites	P(transmission from environment)
	Number of MRSA positive family members	P(transmission from family member)
	Number of MRSA positive non family members	P(transmission from non family member)
	Number of MRSA positive dogs	P(transmission from dog)
	Number of MRSA positive veterinary staff	P(transmission from veterinary staff)
	Number of MRSA contaminated veterinary environmental sites	P(transmission from environment at veterinary clinic)
	Number of MRSA positive dogs at veterinary clinic	P(transmission from dog at veterinary clinic)

6.2.5 Convergence

Prior to model implementation, a convergence analysis was undertaken to define the number of simulation samples required to be taken for the uncertainty distributions to reach convergence. These analyses were undertaken by plotting the running mean for successive iterations against the number of iterations used and convergence was assessed by visual comparison with the overall mean.

All simulation and statistical models were specified, implemented and analysed using R (R Development Core Team, 2008) and statistical significance was set at $P < 0.05$. The complete code for the simulation model is presented in Appendix 8.

6.3 Results

6.3.1 Convergence

The results of the convergence analysis showed that, for the full simulation model, adequate convergence was reached by 1000 iterations (Figure 6.3).

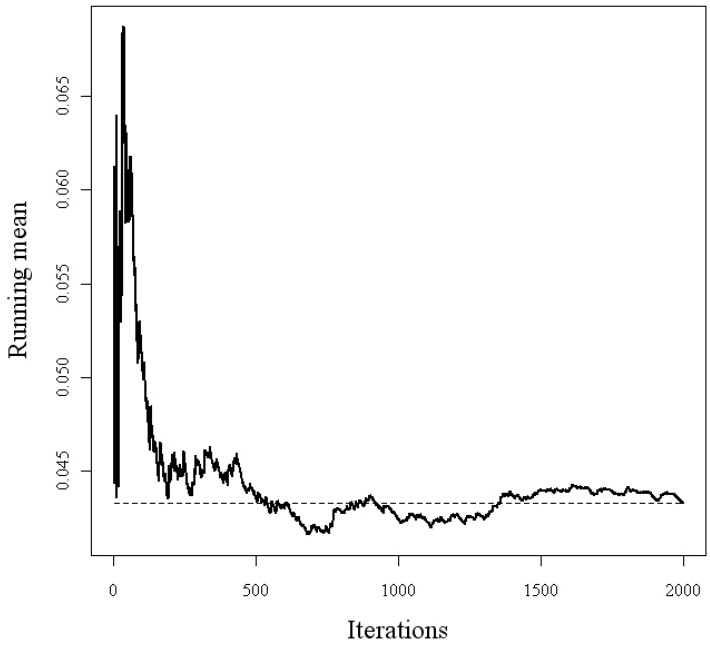


Figure 6.3. Convergence analysis for number of iterations (samples from uncertainty distributions) required for the overall simulation model of MRSA acquisition in dogs.

6.3.2 Overall uncertainty analysis

The model was run for 200 at-risk dogs (realisations of the same dog) over 1000 iterations, using 5000 samples to combine expert opinions, as defined by the convergence analyses (sections 5.3.2, 6.3.1). This model was repeated 50 times using the same input variables for each dog, resulting in an output in the form of a 200*1000*50 (dogs*iterations*repeats) matrix with each entry taking a binary form. Summing across the repeats allowed conversion of binary estimates to proportions.

The outcome of the model predicted the mean proportion of dogs that become positive for MRSA over any given 24 hour period to be 0.042. The uncertainty surrounding this estimate is reflected in Figure 6.4 and is defined by the 95% simulation interval 0.038 -

0.046, and 5th, 50th and 95th percentiles of 0, 0.015 and 0.19, respectively. The variability surrounding this estimate is reflected in Figure 6.5 and is defined by the 95% simulation interval for the mean 0.041 - 0.043 and 5th, 50th and 95th percentiles of 0.031, 0.043 and 0.052, respectively.

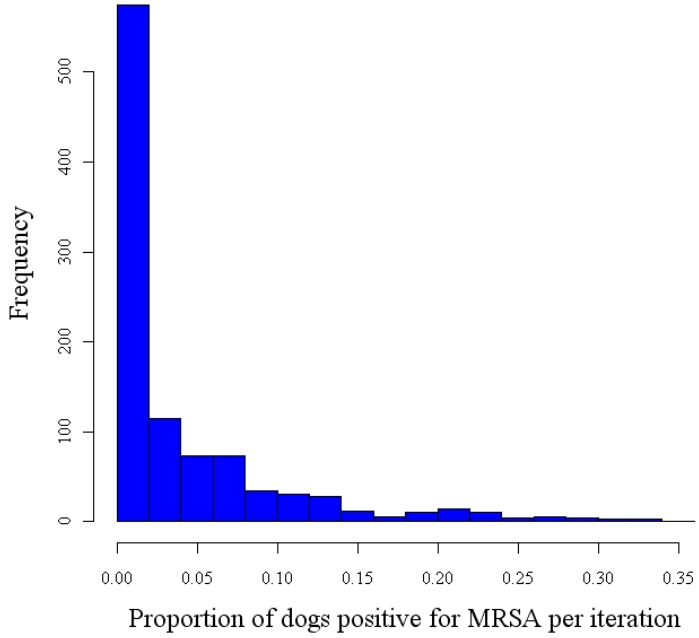


Figure 6.4. Proportion of dogs positive for MRSA in 24 hours for a single model run with 1000 iterations of the simulation model for MRSA acquisition in dogs.

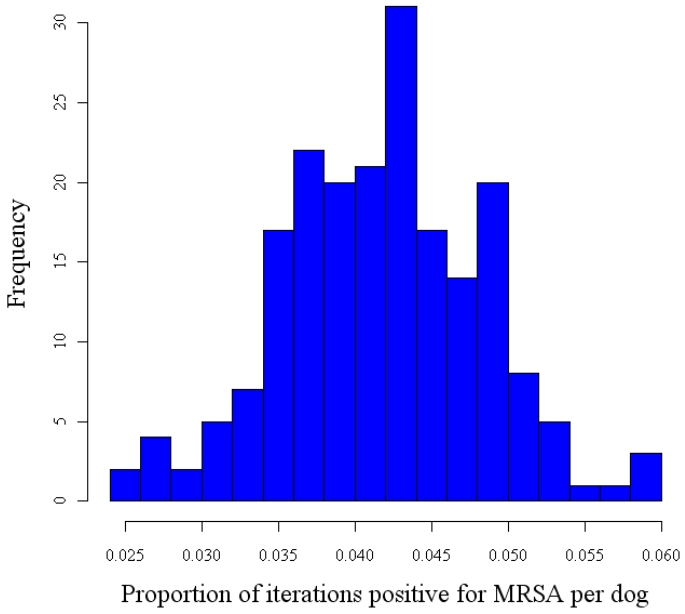


Figure 6.5. Proportion of iterations positive for MRSA in 24 hours for a single model run with 1000 iterations and 200 dogs modelled for the simulation model for MRSA acquisition in dogs.

The uncertainty is plotted in Figure 6.6, using the proportion of repeats of the model in which a dog will be positive for MRSA and showing the probability that a single dog will become positive for MRSA on any given day. Each line on the graph represents one of 100 random samples of iterations (N=1000) and each iteration plots all realisations (N=200) of a single dog, with independent input variables, representing variability, over a given 24 hour time period, averaged across 50 repeats (i.e. the probability that that dog will be MRSA positive on any 24 hour period, given the input variables that are assigned to that iteration).

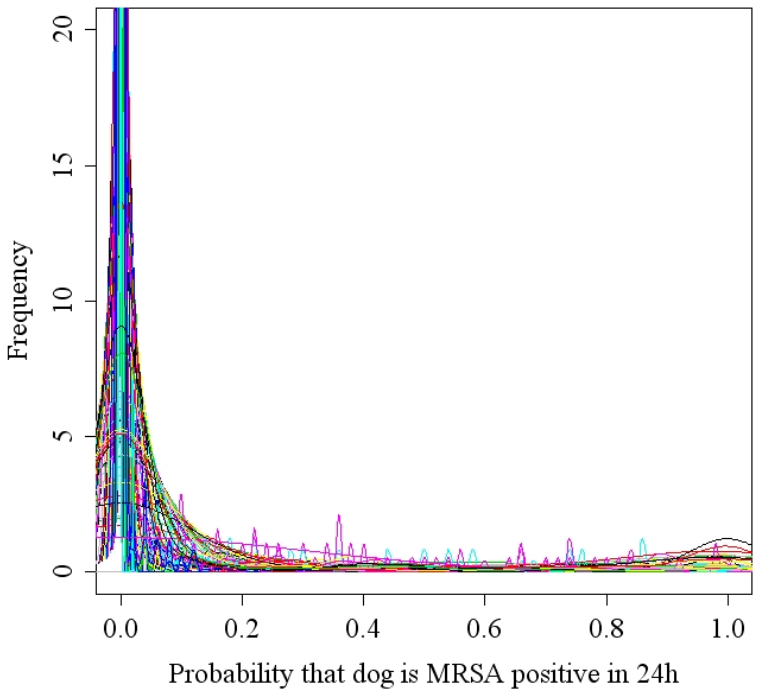


Figure 6.6. Probability that a dog will become MRSA positive on any given day. Each line represents one of 100 random samples of iterations (N=1000) and each iteration plots all realisations (N=200) of a dog (with independent input variables) over a given 24 hour time period, averaged across 50 repeats.

The confidence intervals associated with the uncertainty analysis are displayed as cumulative distribution graphs in Figure 6.7 and 6.8 and show the uncertainty (slope) and variability (width) to increase with an increase in probability of MRSA acquisition. This is similarly reflected in Figure 6.9 which plots the confidence intervals as histograms to allow visualisation of the distribution associated with the confidence intervals.

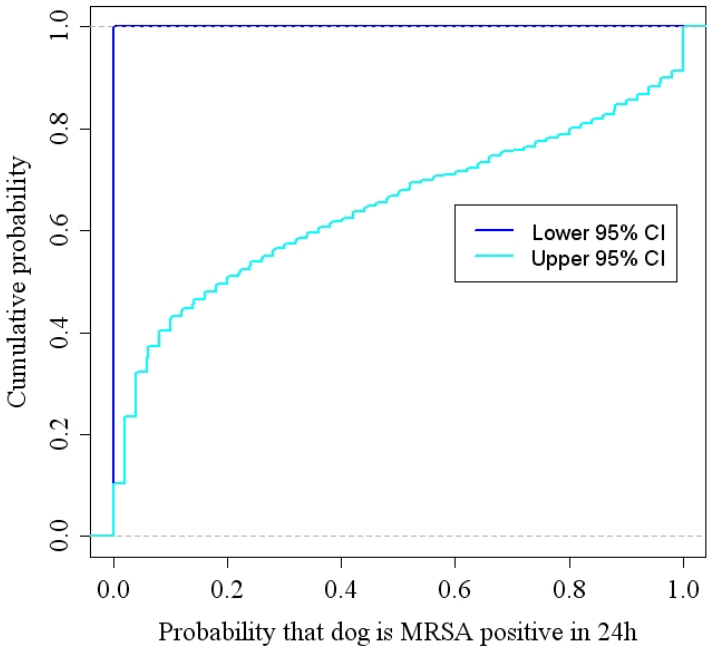


Figure 6.7. 95% Confidence intervals of the distributions representing the probability that a dog will become MRSA positive on any given day, given a certain set of input parameters.

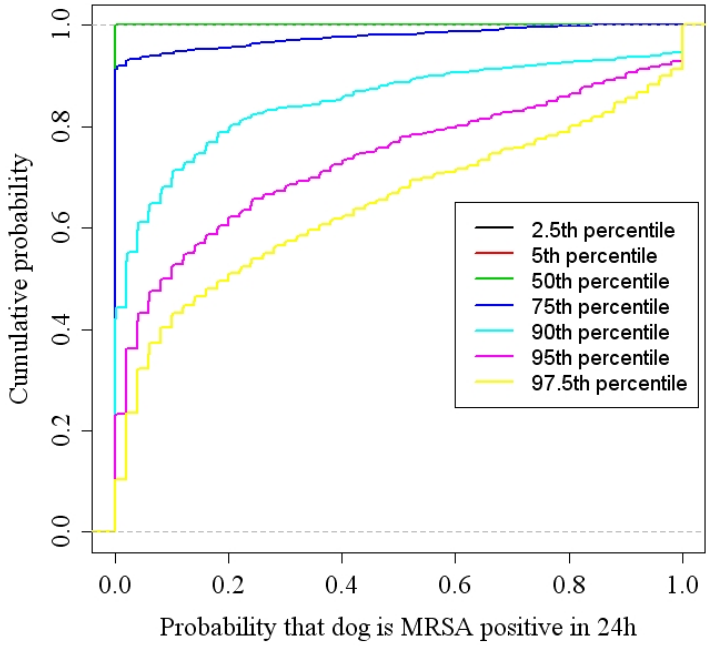


Figure 6.8. Percentile cumulative distributions of the distributions representing the probability that a dog will become MRSA positive on any given day, given a certain set of input parameters.

Figure 6.10 shows the independent uncertainty distributions averaged over realisations of dogs (N=200). Each line represents one of 100 random samples of iterations (N=1000) and each iteration plots all repeats (N = 50) of an iteration, with the same uncertain input variables, over a given 24 hour time period, averaged across 200 dogs. This shows that there is marked variability between some input variables, reflected in differences between distributions for each iteration, and that some variables or variable combinations may be very influential in output scenarios.

6.3.3 Sensitivity analysis

When the simulation model was run for all dogs, and taking into account all realisations of dogs and all iterations, 8446 outcomes (4.2%) were found to be positive for MRSA and when the model was run for dogs that attended a veterinary clinic only, 38194 outcomes (19.1%) were positive for MRSA. Therefore, 16892 and 76388 records were used for the LR models run for all dogs and for those who had attended a veterinary clinic, respectively. The results of the data exploration found that some of the continuous variables were non-linear in the logit. To enable accurate comparison between variables, all transmission variables were included as factors. After assessment of mosaic plots revealed very low numbers of observations in successive categories of exposure variables (e.g. Figures 6.11, 6.12) and to enable ease of comparative interpretation, all exposure variables were dichotomised and included as factors.

The results of the separate exposure and transmission LR models that were specified for all dogs (irrespective of veterinary attendance) are presented in Tables 6.4 and 6.5. The exposure and transmission LR models for dogs that attended a veterinary clinic over the 24 hours are presented in Tables 6.6 and 6.7. The transmission variables were categorised into five levels, the first of which represents a probability of 0 and the other four representing quartiles of the remaining probabilities.

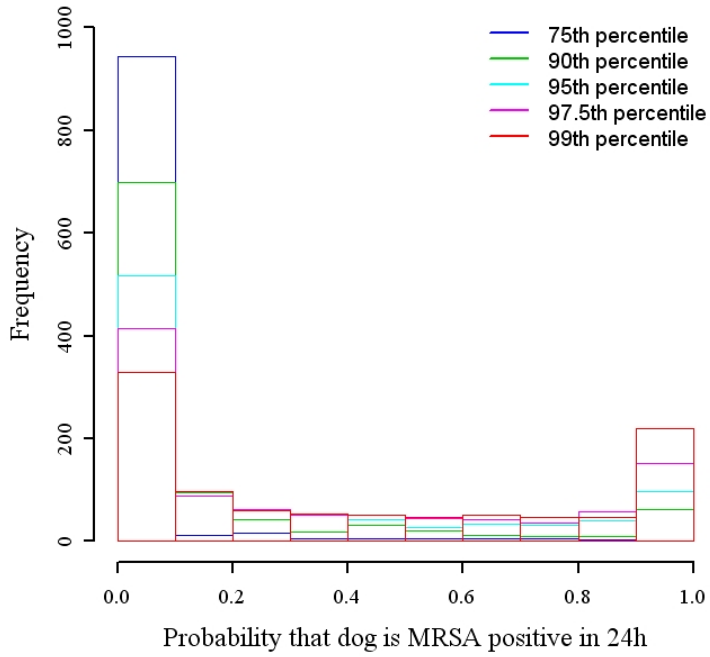


Figure 6.9. Histograms of the confidence intervals of the probability that a dog will become MRSA positive on any given day, given a certain set of input parameters.

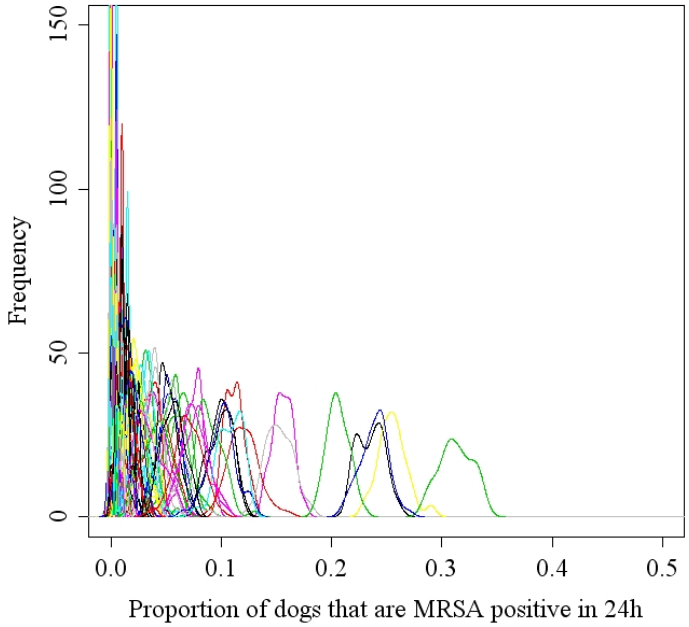


Figure 6.10. Proportion of realisations of a dog (N=200) that will become MRSA positive on any given day. Each line represents one of 100 random samples of iterations (N=1000) and each iteration plots all repeats (N=50) of the model run over a given 24 hour time period, averaged across all realisations of the dog.

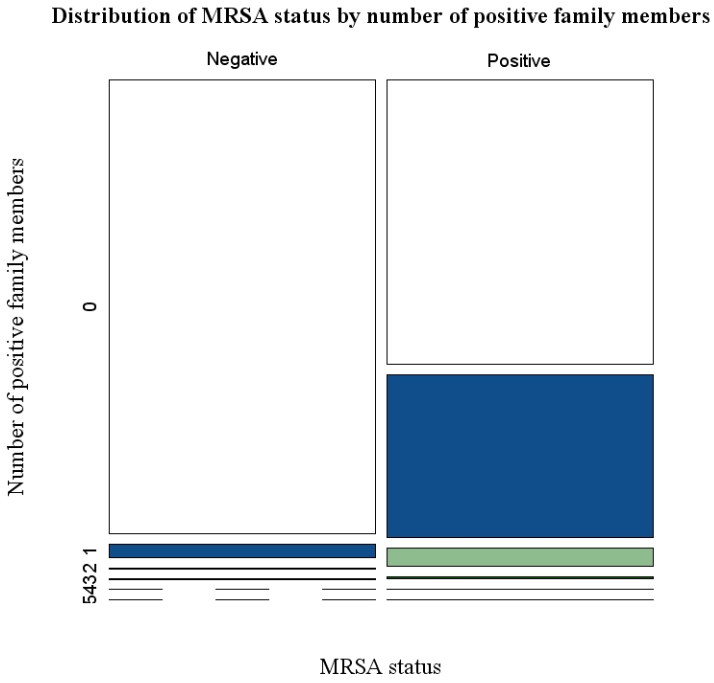


Figure 6.11. Mosaic plot of MRSA status of dogs, for the observations selected from the simulation model for the logistic regression model, stratified by the number of MRSA positive family members that they are exposed to over a given 24 hour period

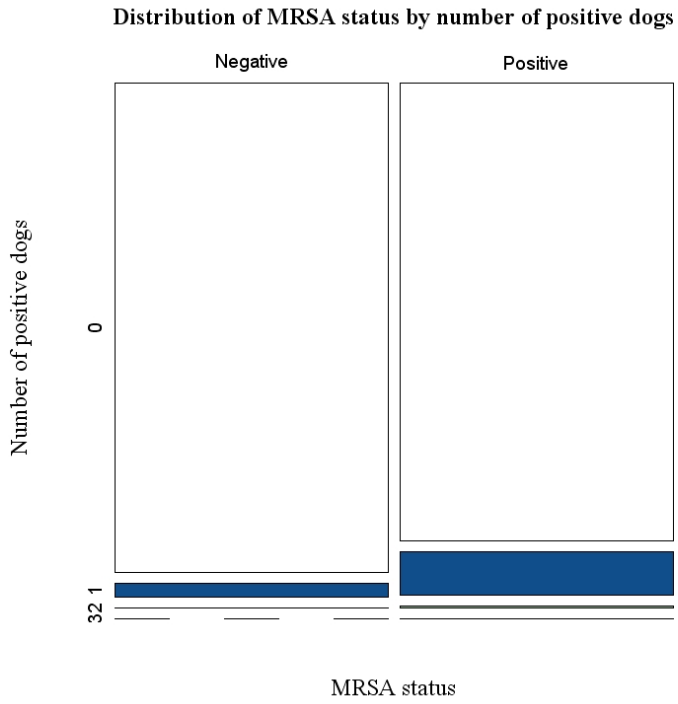


Figure 6.12. Mosaic plot of MRSA status of dogs, for the observations selected from the simulation model for the logistic regression model, stratified by the number of MRSA positive dogs that each is exposed to over a given 24 hour period.

Table 6.4. Results of a multivariable logistic regression model with the outcome specified as MRSA status and explanatory variables specified as exposure variables for all dogs under consideration in the simulation model for acquisition of MRSA in dogs

Model	Explanatory variable	Odds Ratio	95%Confidence Interval	P value
Exposure model	**Number of MRSA contaminated environmental sites**			
	0	1		
	≥1	354.53	292.28 – 430.03	<0.001
	Number of MRSA positive family members			
	0	1		
	≥1	74.85	62.75 - 89.29	<0.001
	Number of MRSA positive non family members			
	0	1		
	≥1	35.56	30.76 - 41.11	<0.001
	Number of MRSA positive dogs			
	0	1		
	≥1	40.13	33.24 - 48.46	<0.001
	Attendance at veterinary clinic			
	No	1		
	Yes	56.94	38.8 - 83.57	<0.001

Log-likelihood = -4288.203
No. of observations = 16892
Akaike's information criterion = 8588.40
Residual deviance = 8576.4 on 16886 df

Table 6.5. Results of a multivariable logistic regression model run with the outcome specified as MRSA status and explanatory variables specified from transmission variables for all dogs under consideration in the simulation model for acquisition of MRSA in dogs.

Model	Explanatory variable	Odds Ratio	95%Confidence Interval	P value
Transmission model	**P(transmission from environment)**			
	0	1		
	0 - 25th %ile	30.67	23.15 - 40.63	<0.001
	25 - 50th %ile	101.81	77.6 - 133.59	<0.001
	50 - 75th %ile	483.39	325.4 - 718.08	<0.001
	75 - 100th %ile	1.70 x 10^{9}	0 - 3.36 x 10^{174}	0.913
	P(transmission from non family member)			
	0	1		
	0 - 25th %ile	0.64	0.36 - 1.16	0.139
	25 - 50th %ile	3.31	2.42 - 4.53	<0.001
	50 - 75th %ile	12.88	10.34 - 16.04	<0.001
	75 - 100th %ile	83.3	67.65 - 102.58	<0.001
	P(transmission from family member)			
	0	1		
	0 - 25th %ile	2.72	1.42 - 5.19	0.002
	25 - 50th %ile	16.2	10.9 - 24.09	<0.001
	50 - 75th %ile	75.41	50.13 - 113.43	<0.001
	75 - 100th %ile	331.8	163.53 - 673.2	<0.001
	P(transmission from dog)			
	0	1		
	0 - 25th %ile	0.55	0.21 - 1.48	0.239
	25 - 50th %ile	3.99	2.54 - 6.27	<0.001
	50 - 75th %ile	15.59	11.1 - 21.89	<0.001
	75 - 100th %ile	109.39	77.43 - 154.53	<0.001
	Attendance at veterinary clinic			
	No	1		
	Yes	28.74	19.78 - 41.75	<0.001

Log-likelihood = -4250.2759
No. of observations = 16892
Akaike's information criterion = 8536.5517
Residual deviance = 8500.6 on 16874 df

Table 6.6. Results of a multivariable logistic regression model with the outcome specified as MRSA status and explanatory variables specified from exposure variables for dogs who have attended a veterinary clinic in the simulation model for acquisition of MRSA in dogs.

Model	Explanatory variable	Odds Ratio	95%Confidence Interval	P value
Exposure model	**Number of MRSA contaminated veterinary environmental sites**			
	0	1		
	≥1	11.07	10.65 – 11.51	<0.001
	Number of MRSA contaminated environmental sites			
	0	1		
	≥1	16.09	14.76 – 17.54	<0.001
	Number of MRSA positive veterinary staff			
	0	1		
	≥1	4.84	4.62 – 5.08	<0.001
	Number of MRSA positive dogs at veterinary clinic			
	0	1		
	≥1	5.16	4.88 – 5.46	<0.001
	Number of MRSA positive family members			
	0	1		
	≥1	2.65	2.43 – 2.89	<0.001
	Number of MRSA positive non family members			
	0	1		
	≥1	1.88	1.77 – 2.00	<0.001
	Number of MRSA positive dogs			
	0	1		
	≥1	1.83	1.66 - 2.01	<0.001

Log-likelihood = -38757.3143
No. of observations = 76388
AIC value = 77530.6286
Residual deviance = 77515 on 76380 df

Table 6.7. Results of a multivariable logistic regression model with outcome specified as MRSA status and explanatory variables specified from transmission variables for dogs who have attended a veterinary clinic in the simulation model for acquisition of MRSA in dogs.

Model	Explanatory variable	Odds Ratio	95%Confidence Interval	P value
Transmission model	**P(transmission from environment at veterinary clinic)**			<0.001
	0	1		
	0 - 25th %ile	1.73	1.56 – 1.91	<0.001
	25 - 50th %ile	8.7	8.14 – 9.29	<0.001
	50 - 75th %ile	27.82	26.05 – 29.71	<0.001
	75 - 100th %ile	167	152.82 – 182.5	<0.001
	P(transmission from veterinary staff)			<0.001
	0	1		
	0 - 25th %ile	0.9	0.77 – 1.05	0.190
	25 - 50th %ile	2.71	2.45 – 2.99	<0.001
	50 - 75th %ile	10.01	9.12 – 10.99	<0.001
	75 - 100th %ile	67.08	60.1 – 74.87	<0.001
	P(transmission from environment)			<0.001
	0	1		
	0 - 25th %ile	2.63	2.19 – 3.17	<0.001
	25 - 50th %ile	14.09	11.89 – 16.68	<0.001
	50 - 75th %ile	72.25	58.588 – 89.12	<0.001
	75 - 100th %ile	1638.78	778.76 – 3448.55	<0.001
	P(transmission from dog at veterinary clinic)			<0.001
	0	1		
	0 - 25th %ile	1.42	1.19 – 1.68	<0.001
	25 - 50th %ile	3.42	3.02 – 3.88	<0.001
	50 - 75th %ile	11.33	10.06 – 12.76	<0.001
	75 - 100th %ile	121.5	102.43 – 144.12	<0.001
	P(transmission from non family member)			<0.001
	0	1		
	0 - 25th %ile	0.69	0.55 – 0.87	0.001
	25 - 50th %ile	1.24	1.07 – 1.43	0.004
	50 - 75th %ile	1.94	1.68 – 2.24	<0.001
	75 - 100th %ile	15.51	13.42 – 17.92	<0.001
	P(transmission from family member)			<0.001
	0	1		
	0 - 25th %ile	0.76	0.52 – 1.12	0.162
	25 - 50th %ile	2	1.56 – 2.54	<0.001
	50 - 75th %ile	14.21	10.94 – 18.45	<0.001
	75 - 100th %ile	138.41	81.4 – 235.34	<0.001
	P(transmission from dog)			<0.001
	0	1		
	0 - 25th %ile	0.6	0.4 – 0.88	0.010
	25 - 50th %ile	1.37	1.07 – 1.75	0.011
	50 - 75th %ile	2.04	1.61 – 2.58	<0.001
	75 - 100th %ile	16.8	13.49 – 20.93	<0.001

Log-likelihood = -25657.32
No. of observations = 76388
AIC value = 51373
Residual deviance = 51315 on 76359 df

6.4 Discussion

The results of this simulation study should be interpreted with care, given the lack of data, reliance on expert opinion estimates and complexity of the simulation model required to represent a largely undocumented biological process. Although iterative communication was maintained with experts in MRSA and risk analysts throughout the model-building process to ensure that any assumptions and parameterisations were well justified, caution in interpretation of outputs is still required. However, the aim of the study in this instance was not to rely heavily on the absolute values of the outputs, but to use the results of the sensitivity analysis to define the most important and influential inputs with respect to the acquisition of MRSA in dogs and to inform the direction of future research activity in what might be considered a priority area.

The output of the full simulation model shows that, on average, 4.2% of dogs will become positive for MRSA (carriage, colonisation or infection) over any given 24 hour period. While there is marked uncertainty associated with this estimate, reflected in the wide 95% uncertain simulation interval (0.038 - 0.046), the estimate for variability is smaller (95% variable simulation interval 0.041 - 0.043). The median estimate for MRSA acquisition in a given 24 hour period is 1.5% and represents a value that is far smaller than the mean and is a more accurate reflection of the model output, given the marked right skew to the output distribution displayed in Figure 6.4. Taking into account the overall output, along with the individual uncertainty distributions in Figure 6.10, the model also is found to predict that, while most dogs will be negative on most occasions, a small proportion will be positive all of the time depending on their input parameters, that is, there are some parameters, corresponding to exposures to and transmissions from certain sources, that are highly associated with the acquisition of MRSA. The graphical analysis of the confidence intervals associated with the uncertainty distributions (Figures 6.7 and 6.8) reveals that the overall uncertainty within this model is clustered at the upper end of the distributions, with both uncertainty and variability increasing as the probability of becoming MRSA positive increases.

It is difficult to corroborate or repudiate the results of this model using data collection, given the temporal specification of the model along with the definition of ‘MRSA positive’ to encompass intermittent carriage. However, the median model output (1.5%) falls within the 95% binomial confidence intervals calculated for four studies of MRSA carriage in

dogs in the community; i) 0.1 – 2.2% reported in Rich and Roberts (2006), ii) 0 – 1.9% (Murphy *et al.*, 2006), iii) 0.1 – 3.5% (Hanselman *et al.*, 2008) and iv) 0 – 1.8% (Vengust *et al.*, 2006) (intervals calculated using biconf.exe as described in Chapter 1). The structure of the simulation model used is dependent on individual estimation of contact and transmission information that was, for some parameters, collected by expert opinion and subsequently likely to encompass marked uncertainty. While this uncertainty is modelled to the best of our ability, the possibility of expert estimates to deviate from reality largely remains and, in the case of probability of transmission, is likely to continue to be unquantifiable given the inability to perform further data collection in this area due to ethical restrictions. Notwithstanding this, the output of the model presented herein is consistent with prior reports, is biologically plausible and allows the implementation of a sensitivity analysis, the output of which is the focus of this work.

The results of the LR models show that the most influential predictors for MRSA acquisition in dogs include exposure to MRSA positive environments, family members and veterinary clinic attendance. Exposure to an MRSA positive home environment was also found to be highly influential for dogs that attended veterinary clinics. For exposures within a veterinary clinic, the greatest level of influence was found to be associated with exposure to the environment at a veterinary clinic, followed by exposure to dogs at a veterinary clinic and then exposure to the veterinary staff. Within the regression models fitted to assess transmission parameters, attendance at a veterinary clinic was found to be highly influential, although all routes of transmission were also found to be influential, particularly when the higher quantiles of the probabilities of transmission were assessed. In comparing the influence of transmission variables, the probability of transmission from the community environment was associated with the greatest influence, followed by transmission from family members, dogs and non-family members. Similarly, for animals that attended veterinary clinics, the probability of transmission from the community environment was also the most influential variable, followed by the probability of transmission from the veterinary clinic environment, dogs at a veterinary clinic and from veterinary staff members, respectively. Transmission from family, non-family and dogs outside of the vet clinic was found to be less influential than the within-clinic factors.

The use of LR for sensitivity analysis as presented in this chapter is a novel application of a technique that is widely used in statistical modelling. Regression analysis has been proposed for use as a tool for sensitivity analysis (Kleijnen, 1987; Kleijnen and Sargent, 2000) and LR, along with linear regression and probit analysis, has been utilised in combination with experimental design methods as a metamodelling technique undertaken

on simulation models previously published in the veterinary literature (Vonk Noordegraaf *et al.*, 2003; de Vos *et al.*, 2006). However, to the author's knowledge, the direct application of a LR model to a simulation model has not previously been reported in the veterinary literature. As previously discussed (Chapter 1), metamodelling describes the use of a regression model as a tool to model the I/O relationships of a simulation model, allowing a formalised approximation of the relationships between input and output factors. The regression models that are applied to the current scenario can not strictly be termed metamodels as they do not attempt to approximate the entire I/O transformation implied by the simulation model, but rather enable the quantification of the relationship between selected intermediate input variables and the output for differing scenarios.

While the regression models that have been implemented are informative in defining inputs to which the output of the model is most sensitive, and therefore most deserving of accurate quantification (Helton and Davis, 2000), this sensitivity analysis could be considered to be incomplete as many of the input variables of the simulation model have not been included in their primary form. This type of analysis does not facilitate inclusion and analysis of distributional or other basic input assumptions and while the effect of all variables are accounted for in the regression models through the inclusion of variables that are composites of many inputs, these inputs themselves cannot be examined. For example, in this study it is not possible to define whether the effect of a variable such as exposure to MRSA positive family members, to which the output is highly sensitive, is evenly distributed across all family members, whether it is greater for the primary owner, or whether it is dependent on owner risk group, all of which are included in the simulation model but cannot easily be included in the LR sensitivity analysis due to their fine resolution, collinearity and potential for confounding. Similarly, interactions between input variables in the LR have not been assessed in this analysis. The notion of global sensitivity analysis encompasses the ability to assess the sensitivity of the model output to each input variable, whilst also considering the potential effects of all other variables within the model. As such, while LR allows the generation of results with respect to the other variables considered in the model, the inclusion of interactions may allow specific consideration of the magnitude of effect, effect modification or confounding, that is conferred by the inclusion of two-way or higher order interaction terms.

The interpretation of the results generated by this LR is difficult. The aim was to define variables to which the output of the model was highly sensitive. However, the inclusion of explanatory variables in the exposure models as dichotomous, whilst satisfying the

assumptions for LR modeling and allowing accurate quantification of the effect size of each variable, results in loss of information regarding the variance of these parameters and, as such, limits the inference that can be made from the model output. Notwithstanding this, the output of a LR model that uses dichotomised input variables allows comparison and ranking of importance of those input variables based on an objective measure. Conversely, the inclusion of categorical predictors with more than one category, as implemented in the transmission models in the current study, results in a loss of ability to compare accurately between explanatory variables whilst maintaining the ability to account for variance in the input factors. Neither of these representations is ideal.

In this study, the finding that both veterinary and non-veterinary routes of acquisition are highly influential for MRSA acquisition in dogs is relevant and highlights the importance of continued research into the interaction between dogs and humans with respect to the zoonotic potential for MRSA. The exposure variables to which the output is highly sensitive provide information about where mitigation strategies might best be targeted, but the use of information about influential transmission parameters in the model raises a more difficult problem. While these variables, e.g. probability of transmission from the environment, are likely to benefit from further study and more accurate quantification, the practicality of undertaking these studies must be considered. The use of expert opinion, on which many of the input distributions for probabilities of transmission are based, resulted in marked uncertainty, reflected in large variation in expert responses, low confidence estimates and written comments imparting low assurance in the validity of the observations (Chapter 5) and is confirmed with the results of the LR models. Given the expert comments (Appendix 5), it is unlikely that this uncertainty is reducible by refinement of the expert opinion technique. Furthermore, observational studies are likely to be inadequate for obtaining microorganism transfer data, and experimental studies are not ethically tolerated and rarely represent real world situations with respect to this biological area. As such, the question is raised: does the identification of highly influential transmission variables merely quantify an unresolvable lack of knowledge?

The quantitative assessment and attendant sensitivity analysis that have been conducted in this data-sparse area have improved on a previous qualitative assessment (Chapter 3) which returned unsatisfactory results due to the complexity of the specified model and lack of modular, sequential form. The approach that has been taken has identified veterinary and non-veterinary routes as important for the acquisition of MRSA in dogs and has allowed the identification of influential exposure, i.e. MRSA positive environments, family

members and veterinary clinic attendance, and transmission, i.e. home and veterinary environment, variables, but has been unable to rank these effects objectively or defensibly, and has raised many questions with respect to the subsequent use of this information. The results of this chapter highlight the need for further research, directed towards expanding the sensitivity analysis presented herein by assessing interactions between the variables in the LR models and by implementing alternative sensitivity analysis techniques, such as factorial analysis, to account for a greater number and finer resolution of input variables. Consideration of the application and results of the current analysis highlights the practical complexity that limits the application of a truly global sensitivity analysis to a biological model such as this.

CHAPTER 7

SENSITIVITY ANALYSES FOR THE QUANTITATIVE RISK ASSESSMENT OF THE ACQUISITION OF MRSA IN PET DOGS

7.1 Introduction

Sensitivity analysis may be defined as the study of how the variation in the output of a mathematical model can be apportioned, qualitatively or quantitatively, to different sources of variation in the input of a model (Saltelli, 2000). The use of sensitivity analysis is considered to be an integral and essential component of the modelling process, the results of which may be used for numerous purposes including model validation, optimisation and calibration (Saltelli, 2000; Trocine and Malone, 2000; Borgonovo, 2008). In risk analysis, sensitivity analysis is primarily used to identify risk-governing parameters, important for both the implementation of mitigation strategies and direction of future research in the form of data collection and/or surveillance (Saltelli, 2000; Frey and Patil, 2002; Saltelli, 2002).

Appropriate selection of sensitivity analysis technique is complex and, as outlined in Chapter 1, may depend on many factors, including the intended goal of the analysis, the structure and methods of the underlying model to which it is applied and the computational cost of its implementation (Saltelli, 2000). The sensitivity analysis that was presented in Chapter 6 represents a novel application to a risk assessment scenario of multivariable LR modelling. While the results of the LR models presented in Chapter 6 were informative, the potential to increase their global capacity may exist through consideration of interaction terms. In addition, consideration of additional sensitivity analysis techniques that assess input variables at a differing resolution within the stochastic model to the LR models will allow comparison of outcomes and consideration of results in parallel.

This chapter presents the results of further sensitivity analyses undertaken on the risk assessment model described and implemented in Chapter 6 and aims to compare and compile the results of different methods of sensitivity analysis when applied to the same scenario.

7.2 Materials and Methods

7.2.1 LR with interactions

The LR models that were completed for transmission parameters in Chapter 6 were repeated with the inclusion of two-way interaction terms between environmental factors and all other possible sources of MRSA (family, non-family and environment) for each of the community and veterinary clinic settings. As described in Chapter 6, two separate LR models were run for each of the simulation models, modelling all animals irrespective of veterinary attendance and only animals that had attended a veterinary clinic over the 24 hours in question. Simulation output data were selected and input variables were categorised as described in Chapter 6. For all regression models, outcome was specified as MRSA positive or negative and explanatory variables considered for inclusion in each model are presented in Table 6.3. The regression models were fitted using a backwards stepwise algorithm, with reduction in AIC used as the criterion for inclusion of variates in the final model.

7.2.2 OAT sensitivity analysis

Twenty eight potentially influential input factors were identified for inclusion in the one-at-a-time sensitivity analysis. The input distributions associated with each of these factors (presented in Chapter 6, Table 6.1 and 6.2) were assessed and each factor was assigned a minimum, most likely and maximum value (Table 7.1).

A OAT analysis was undertaken, using all 28 of the input factors defined in Table 7.1. The model was run initially with all input variables set to their most likely value to obtain a baseline result and then the model was re-run 56 times with each factor changed to their maximum and minimum values in turn, while all other factors were set at their baseline (median) value, resulting in a total of 57 model runs. Each model run was specified for 1000 iterations and the output was defined as the distribution (over 1000 iterations) of proportion of realisations of dogs that became positive for MRSA on that run. The results for each of the variables of the OAT analysis were ranked based on their variation from the baseline mean and 90th percentile values, by dividing the results of each run by the results of the baseline run.

7.2.3 P-B fractional factorial sensitivity analysis

Initially, a P-B fractional factorial design was specified for each of the 28 input parameters specified in Table 7.1. This design was a two-level design, using the minimum and maximum estimates for each factor, described in Table 7.1 and defined herein as '-' and '+'. As the P-B design for k factors requires at least k+1 design points, provided that k+1 is divisible by 4, this analysis required 32 design points, which increased to 64 when a foldover was incorporated (Law and Kelton, 2000; Beres and Hawkins, 2001). This design matrix was specified using DOE++ software (version 1.0.3, Reliasoft corporation, http://www.reliasoft.com/doe/) and is displayed in Table 7.2. However, the results of the analysis using this 28 factor design were unsatisfactory due to the instability of effects found for the within veterinary clinic variables, and, on consideration of this design with respect to the specification of the simulation model, it became apparent that some of the factors specified within the factorial design were mutually exclusive. Consequently, the analysis was re-run for two separate scenarios: 1) All dogs, allowing inclusion of factors F1 to F19, described in Table 7.1 and 2) Dogs that attended a vet in the given 24 hour period, allowing, with the exclusion of F1, assessment of all community and veterinary factors (F2 – F28). The first scenario utilised a 19 factor, 40 design point (foldover of 20 design points) P-B design, while the second utilised a 27 factor, 64 design point (foldover of 32 design points) P-B design, both matrices of which were specified using DOE++ software (version 1.0.3, Reliasoft corporation, http://www.reliasoft.com/doe/) and are displayed in Appendix 9. For design point 1 in the P-B matrix, the simulation model was parameterised according to the + / - combination of variables dictated by the matrix and run for 1000 iterations (as per convergence analysis presented in Chapter 6) of 200 dogs. These were used to derive a distribution of the probability of becoming MRSA positive in 24 hours, given the specified combination of inputs. The mean (and 90th percentile) of the distribution were used as alternative summary output measures for design point 1 in the P-B matrix. This procedure was repeated for all other design points in both P-B analyses.

The main effects for each factor included in the P-B designs were then calculated as described by Beres and Hawkins (2001). The signs from the P-B matrix column associated with that factor were attributed to the output values obtained after running the P-B analysis (as described above), row by row (that is, run by run). Each of these values were then summed and divided by the design size (number of runs) to obtain a P-B main effect value for each variable.

7.2.4 Implementation

All analyses were undertaken using R (R Development Core Team, 2008). To enable the use of maximal iterations with reduced run time, the OAT and P-B analyses were run using a computer cluster utilising 26 x 2.3GHz G5 processors (13 dual processor machines) that were linked via xgrid software (Apple Inc; California, USA, http://www.apple.com/server/macosx/technology/xgrid.html).

Table 7.1. Descriptive statistics for input for sensitivity analysis of the simulation model for MRSA acquisition in dogs.

Factor	Variable*	Minimum	25th Percentile	Median	75th Percentile	Maximum
F1	P(dog attends vet in 24 hours)	4.01×10^{-3}	4.71×10^{-3}	4.96×10^{-3}	5.22×10^{-3}	6.43×10^{-3}
F2	P(health care worker is MRSA positive)	5.24×10^{-3}	3.30×10^{-2}	6.23×10^{-2}	9.02×10^{-2}	0.460
F3	P(veterinary worker is MRSA positive)	3.97×10^{-3}	4.39×10^{-2}	7.12×10^{-2}	9.41×10^{-2}	0.217
F4	P(other high risk goup is MRSA positive)	1.61×10^{-2}	4.18×10^{-2}	5.66×10^{-2}	7.42×10^{-2}	1.61×10^{-2}
F5	P(general population is MRSA positive)	2.09×10^{-3}	1.20×10^{-2}	1.68×10^{-2}	2.22×10^{-2}	4.64×10^{-2}
F6	P(person MRSA positive if lives with MRSA positive person)	2.06×10^{-2}	9.46×10^{-2}	0.142	0.205	0.430
F7	P(transmission from MRSA positive family member per contact)	7.36×10^{-6}	4.91×10^{-3}	2.51×10^{-2}	0.102	0.921
F8	Number of contacts with family member per 24h	0	4	6	9	19
F9	P(transmission from MRSA positive non-family member per contact)	4.77×10^{-7}	3.00×10^{-3}	8.09×10^{-3}	3.46×10^{-2}	0.446
F10	Number of contacts with non-family member per 24h	0	1	2	3	9
F11	P(community dog is MRSA positive)	3.71×10^{-5}	3.32×10^{-3}	6.94×10^{-3}	1.31×10^{-2}	5.02×10^{-2}
F12	P(transmission from MRSA positive community dog per contact)	9.05×10^{-6}	3.37×10^{-3}	1.83×10^{-2}	6.42×10^{-2}	0.373
F13	Number of contacts with community dog per 24h	0	1	2	3	9
F14	P(house contaminated with MRSA | at least one positive family member)	1.23×10^{-2}	0.218	0.388	0.575	0.995
F15	P(house contaminated with MRSA | no positive family members)	4.29×10^{-5}	9.59×10^{-3}	2.33×10^{-2}	4.20×10^{-2}	0.181
F16	P(environmental site contaminated | at least one positive family member)	6.06×10^{-3}	0.119	0.250	0.471	0.983
F17	P(environmental site contaminated | no positive family members)	1.27×10^{-3}	9.53×10^{-2}	0.140	0.237	0.953
F18	P(transmission from MRSA positive home environment per contact)	7.48×10^{-6}	3.37×10^{-3}	1.47×10^{-2}	1.47×10^{-2}	0.908
F19	Number of contacts with home environment per 24h	0	6	14	14	95
F20	P(dog at vet is MRSA positive)	1.95×10^{-3}	1.66×10^{-2}	2.87×10^{-2}	5.30×10^{-2}	0.248
F21	P(transmission from MRSA positive dog at veterinary clinic per contact)	1.1×10^{-4}	2.73×10^{-2}	6.04×10^{-2}	0.211	0.968
F22	Number of contacts with a dog at a veterinary clinic per 24h	0	1	2	3	9
F23	P(veterinary environment contaminated)	1.17×10^{-3}	1.15×10^{-2}	1.83×10^{-2}	2.77×10^{-2}	0.460
F24	P(transmission from MRSA positive veterinary environment per contact)	1.69×10^{-6}	3.16×10^{-3}	1.54×10^{-2}	4.30×10^{-2}	0.928
F25	Number of veterinary environment contacts per 24h	1	8	11	14	25
F26	P(small animal veterinary worker is MRSA positive)	5.72×10^{-3}	3.95×10^{-2}	5.68×10^{-2}	9.09×10^{-2}	0.255
F27	P(transmission from MRSA positive veterinary staff member per contact)	4.97×10^{-6}	4.14×10^{-3}	3.04×10^{-2}	6.55×10^{-2}	0.647
F28	Number of veterinary staff contacts per 24h	0	3	5	7	19

* P(..) refers to probability

Table 7.2. Design matrix for a two-level, 28 factor, 32 design point, Plackett-Burman design with foldover.

	Factor																												
Design point	**F 1**	**F 2**	**F 3**	**F 4**	**F 5**	**F 6**	**F 7**	**F 8**	**F 9**	**F 10**	**F 11**	**F 12**	**F 13**	**F 14**	**F 15**	**F 16**	**F 17**	**F 18**	**F 19**	**F 20**	**F 21**	**F 22**	**F 23**	**F 24**	**F 25**	**F 26**	**F 27**	**F 28**
1	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+
2	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+
3	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+
4	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-
5	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-
6	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+
7	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-
8	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+
9	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+
10	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+
11	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-
12	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+
13	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+
14	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-
15	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-
16	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+
17	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+
20	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-
21	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-
22	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-
23	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-
24	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+
25	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-
26	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+
27	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+
28	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-
29	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-
30	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-
31	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+
32	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+

Table 7.2 (ctd). Design matrix for a two-level, 28 factor, 32 design point, Plackett-Burman design with foldover.

	Factor																											
Design point	**F 1**	**F 2**	**F 3**	**F 4**	**F 5**	**F 6**	**F 7**	**F 8**	**F 9**	**F 10**	**F 11**	**F 12**	**F 13**	**F 14**	**F 15**	**F 16**	**F 17**	**F 18**	**F 19**	**F 20**	**F 21**	**F 22**	**F 23**	**F 24**	**F 25**	**F 26**	**F 27**	**F 28**
33	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-
34	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-
35	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-
36	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+
37	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+
38	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-
39	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+
40	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-
41	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-
42	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-
43	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+
44	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-
45	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-
46	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+
47	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+
48	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-
49	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+
50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
51	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-
52	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+
53	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+
54	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+
55	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+
56	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-
57	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+
58	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-
59	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-
60	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+
61	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+
62	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+
63	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-
64	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-

7.3 Results

7.3.1 LR with interactions

The results of the analysis of deviance are presented in Table 7.3 and 7.4 and show that significant interactions exist between: i) probability of transmission from the home environment and probability of transmission from family members, dogs and non-family members, respectively when all dogs are considered and ii) probability of transmission from the veterinary clinic environment and veterinary staff and dogs at a veterinary clinic, respectively. Significant interactions were also present between the home environment and family and non-family members for dogs that attend veterinary clinics during the 24 hours in question. The pattern of the interactions was assessed by graphical representation of the proportion of observations in each cell of the interaction table that are positive for MRSA. Graphs representing each interaction term are presented in Appendix 10 and an example of one for each analysis are displayed in Figures 7.1 and 7.2. These graphs show that the probability of transmission from the environment reduces as the magnitude of the positive association between the proportion of dogs that become MRSA positive and probability of transmission from each of the explanatory variables considered, i.e. humans and dogs in the community and hospital setting respectively, increases. The full outcomes of the LR models are presented in Tables 7.5 and 7.6.

Table 7.3. Analysis of deviance table to assess significance of model terms for the multiple logistic regression model implemented with the outcome 'acquisition of MRSA' and explanatory variables representing transmission variables with two-way environmental interactions run for all dogs.

Explanatory variable	Df	Deviance	Residual Df	Residual deviance	P value
Null			16891	23417.3	
P(transmission from environment)	4	8687.9	16887	14729.4	<0.001
P(transmission from non family member)	4	2569.7	16883	12159.7	<0.001
P(transmission from family member)	4	1642.2	16879	10517.4	<0.001
P(transmission from dog)	4	1658.9	16875	8858.6	<0.001
Attendance at veterinary clinic	1	358.0	16874	8500.6	<0.001
P(transmission from environment)* P(transmission from family member)	14	91.1	16860	8409.5	<0.001
P(transmission from environment)* P(transmission from dog)	11	32.5	16849	8377.0	<0.001
P(transmission from environment)* P(transmission from non family member)	13	30.7	16836	8346.3	0.004

Table 7.4. Analysis of deviance table to assess significance of model terms for the multiple logistic regression model implemented with the outcome 'acquisition of MRSA' and explanatory variables representing transmission variables with two-way environmental interactions run for dogs that visit a veterinary clinic.

Explanatory variable	Df	Deviance	Residual Df	Residual deviance	Pvalue
Null			65534	89045	
P(transmission from environment at veterinary clinic)	4	22712	65530	59452	<0.001
P(transmission from veterinary staff)	4	6881	65526	59452	<0.001
P(transmission from environment)	4	6598	65522	52854	<0.001
P(transmission from dog at veterinary clinic)	4	6581	65518	46274	<0.001
P(transmission from non family member)	4	1452	65514	44822	<0.001
P(transmission from family member)	4	1187	65510	43635	<0.001
P(transmission from dog)	4	676	65506	42958	<0.001
P(transmission from environment at veterinary clinic)* P(transmission from veterinary staff)	14	857	65492	42101	<0.001
P(transmission from environment at veterinary clinic)* P(transmission from dog at veterinary clinic)	14	637	65478	41465	<0.001
P(transmission from environment)*P(transmission from family member)	14	107	65464	41357	<0.001
P(transmission from environment)*P(transmission from non family member)	13	31	65451	41326	0.004

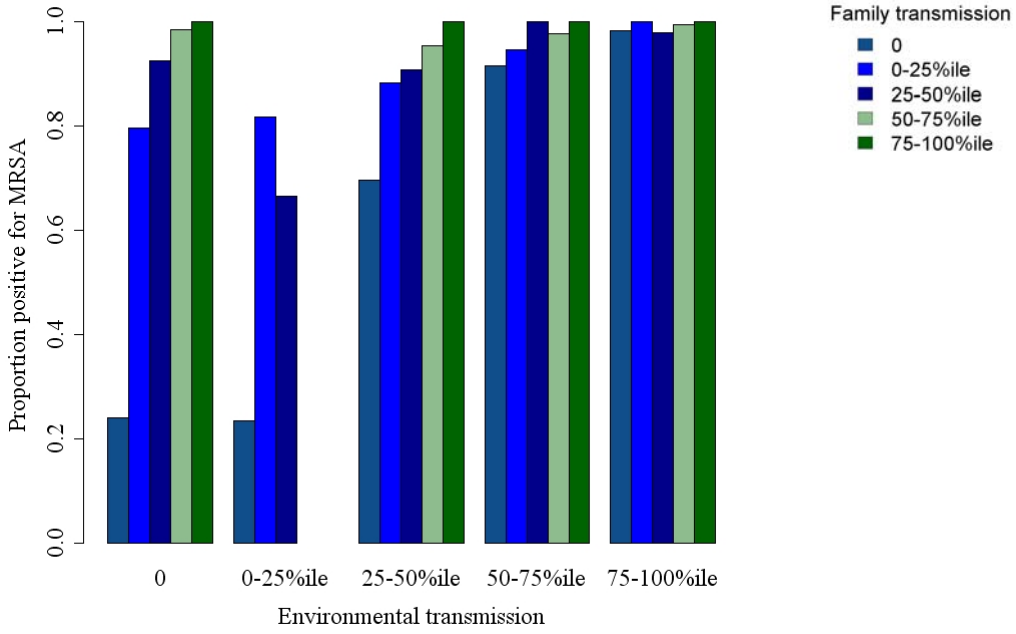


Figure 7.1. Graphical representation of the relationship between the probabilities of transmission from family members and from the environment with regards to MRSA acquisition in a dog.

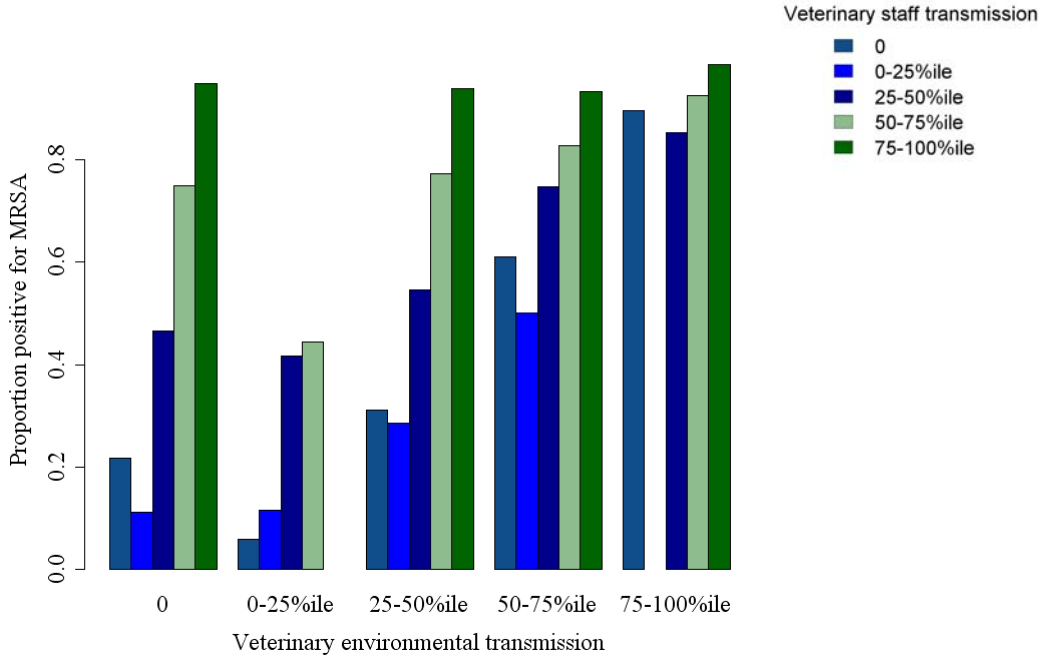


Figure 7.2. Graphical representation of the relationship between the probabilities of transmission from veterinary staff members and from the veterinary environment with regards to MRSA acquisition in a dog.

Table 7.5. Output for multiple logistic regression model implemented with outcome 'acquisition of MRSA' and explanatory variables representing transmission variables with two-way environmental interactions run for all dogs.

Explanatory variable*	Odds Ratio	95%Confidence Interval	P value
P(transmission from environment)			
0	1		
0 - 25th %ile	36.42	26.96 – 49.19	<0.001
25 - 50th %ile	131.82	97.90 – 177.48	<0.001
50 - 75th %ile	726.11	156.38 – 1155.24	<0.001
75 - 100th %ile	1.22×10^{9}	8.04×10^{-128} – 1.88×10^{145}	0.896
P(transmission from non family member)			
0	1		
0 - 25th %ile	0.67	0.37 – 1.22	0.187
25 - 50th %ile	3.53	2.57 – 4.84	<0.001
50 - 75th %ile	13.77	11.04 – 17.19	<0.001
75 - 100th %ile	88.45	71.68 – 109.14	<0.001
P(transmission from family member)			
0	1		
0 - 25th %ile	3.46	1.80 – 6.66	<0.001
25 - 50th %ile	22.99	15.07 – 35.06	<0.001
50 - 75th %ile	102.44	64.94 – 161.60	<0.001
75 - 100th %ile	554.81	228.22 – 1348.78	<0.001
P(transmission from dog)			
0	1		
0 - 25th %ile	0.45	0.15 – 1.38	0.163
25 - 50th %ile	4.50	2.87 – 7.05	<0.001
50 - 75th %ile	16.59	11.79 – 23.36	<0.001
75 - 100th %ile	121.02	20.63 – 43.58	<0.001
Attendance at veterinary clinic			
No	1		
Yes	29.99	20.63 – 43.58	<0.001
P(transmission from environment) * P(transmission from non family member)			
25-50th%ile * 0 - 25th %ile	0.024	0.01 – 0.412	0.017
0 - 25th %ile * 25-50th%ile	0.092	0.028 – 0.296	<0.001
25-50th%ile * 25-50th%ile	0.038	0.010 – 0.145	<0.001
50 - 75th %ile * 25-50th%ile	0.014	0.002 – 0.114	<0.001
0 - 25th %ile * 50 - 75th %ile	0.043	0.012 – 0.156	<0.001
50 - 75th %ile * 50 - 75th %ile	0.006	0.002 – 0.024	<0.001
25 - 50th %ile * 75 - 100th %ile	0.006	0.001 – 0.035	<0.001
50 - 75th %ile * 75 - 100th %ile	0.005	5.50×10^{-4} – 0.045	<0.001
P(transmission from environment) * P(transmission from dog)			
25-50th%ile * 25-50th%ile	0.028	0.004 – 0.18	<0.001
25-50th%ile * 50 - 75th %ile	0.035	0.004 – 0.298	0.002
25-50th%ile * 75 - 100th %ile	0.009	0.001 – 0.068	<0.001
50 - 75th %ile * 75 - 100th %ile	0.003	4.27×10^{-4} – 0.027	<0.001
P(transmission from environment) * P(transmission from family member)			
50 - 75th %ile * 50 - 75th %ile	0.036	0.008 – 0.162	<0.001
25 - 50th %ile * 75 - 100th %ile	0.026	0.003 – 0.197	<0.001
50 - 75th %ile * 75 - 100th %ile	0.016	0.002 – 0.124	<0.001

* Non-significant interaction terms are not displayed
AIC = 8458.3
Log likelihood = -4173.163
Residual deviance = 8346.3 on 16836 df

Table 7.6. Output for multiple logistic regression model implemented with outcome 'acquisition of MRSA' and explanatory variables representing transmission variables with two-way environmental interactions run for dogs who visit a veterinary clinic.

Explanatory variable	Odds Ratio	95%Confidence Interval	P value
P(transmission from environment at veterinary clinic)			
0	1		
0 - 25th %ile	2.53	2.23 – 2.86	<0.001
25 - 50th %ile	16.35	15.05 – 17.76	<0.001
50 - 75th %ile	52.70	48.63 – 57.10	<0.001
75 - 100th %ile	3.4.78	275.30 – 337.41	<0.001
P(transmission from veterinary staff)			
0	1		
0 - 25th %ile	1.13	0.88 – 1.45	0.324
25 - 50th %ile	5.89	5.16 – 6.73	<0.001
50 - 75th %ile	23.12	20.72 – 25.80	<0.001
75 - 100th %ile	130.09	114.86 – 147.33	<0.001
P(transmission from environment)			
0	1		
0 - 25th %ile	3.33	2.67 – 4.16	<0.001
25 - 50th %ile	25.44	20.97 – 30.86	<0.001
50 - 75th %ile	122.67	97.86 – 153.76	<0.001
75 - 100th %ile	3579.11	1482.47 – 8640.98	<0.001
P(transmission from dog at veterinary clinic)			
0	1		
0 - 25th %ile	2.40	1.89 – 3.05	<0.001
25 - 50th %ile	7.23	6.16 – 8.49	<0.001
50 - 75th %ile	26.20	22.82 – 30.08	<0.001
75 - 100th %ile	243.42	200.71 – 295.24	<0.001
P(transmission from non family member)			
0	1		
0 - 25th %ile	0.76	0.60 – 0.96	0.021
25 - 50th %ile	1.23	1.06 – 1.43	0.008
50 - 75th %ile	2.02	1.73 – 2.37	<0.001
75 - 100th %ile	24.03	20.63 – 28.00	<0.001
P(transmission from family member)			
0	1		
0 - 25th %ile	1.16	0.78 – 1.74	0.466
25 - 50th %ile	3.19	2.38 – 4.29	<0.001
50 - 75th %ile	30.84	22.58 – 4.21	<0.001
75 - 100th %ile	229.12	129.29 – 406.04	<0.001
P(transmission from community dog)			
0	1		
0 - 25th %ile	0.63	0.42 – 0.94	0.024
25 - 50th %ile	1.37	1.06 – 1.76	0.016
50 - 75th %ile	2.09	1.64 – 2.67	<0.001
75 - 100th %ile	25.14	20.03 – 31.56	<0.001

Table 7.6 (ctd) Output for multiple logistic regression model implemented with outcome 'acquisition of MRSA' and explanatory variables representing transmission variables with two-way environmental interactions run for dogs who visit a veterinary clinic.

Explanatory variable	Odds Ratio	95%Confidence Interval	P value
P(transmission from environment at veterinary clinic)* P(transmission from veterinary staff)			
0 - 25th %ile*25 - 50th %ile	0.48	0.30 – 0.77	0.003
25 - 50th %ile*25 - 50th %ile	0.24	0.19 – 0.30	<0.001
50 - 75th %ile*25 - 50th %ile	0.21	0.16 – 0.28	<0.001
75 - 100th %ile*25 - 50th %ile	0.17	0.08 – 0.35	<0.001
25 - 50th %ile*50 - 75th %ile	0.15	0.12 – 0.20	<0.001
50 - 75th %ile*50 - 75th %ile	0.07	0.06 – 0.09	<0.001
75 - 100th %ile*50 - 75th %ile	0.04	0.02 – 0.05	<0.001
25 - 50th %ile*75 - 100th %ile	0.04	0.02 – 0.09	<0.001
50 - 75th %ile*75 - 100th %ile	0.03	0.02 – 0.04	<0.001
75 - 100th %ile*75 - 100th %ile	0.03	0.02 – 0.04	<0.001
P(transmission from environment at veterinary clinic)* P(transmission from dog at veterinary clinic)			
0 - 25th %ile*0 - 25th %ile	0.55	0.36 – 0.83	0.005
25 - 50th %ile*0 - 25th %ile	0.41	0.27 – 0.63	<0.001
25 - 50th %ile* 25 - 50th %ile	0.20	0.15 – 0.26	<0.001
50 - 75th %ile*25 - 50th %ile	0.15	0.11 – 0.22	<0.001
75 - 100th %ile*25 - 50th %ile	0.02	0.01 – 0.08	<0.001
25 - 50th %ile*50 - 75th %ile	0.11	0.08 – 0.16	<0.001
50 - 75th %ile*50 - 75th %ile	0.08	0.06 – 0.11	<0.001
75 - 100th %ile*50 - 75th %ile	0.04	0.02 – 0.06	<0.001
25 - 50th %ile*75 - 100th %ile	0.04	0.01 – 0.2	<0.001
50 - 75th %ile*75 - 100th %ile	0.02	0.01 – 0.03	<0.001
75 - 100th %ile*75 - 100th %ile	0.02	0.01 – 0.04	<0.001
P(transmission from environment)* P(transmission from family member)			
0 - 25th %ile*0 - 25th %ile	0.10	0.02 – 0.37	<0.001
0 - 25th %ile*25 - 50th %ile	0.33	0.16 -0.65	0.001
25 - 50th %ile*25 - 50th %ile	0.13	0.06 – 0.29	<0.001
50 - 75th %ile*25 - 50th %ile	0.05	0.01 – 0.17	<0.001
0 - 25th %ile*50 - 75th %ile	0.38	0.17 – 0.85	0.019
25 - 50th %ile*50 - 75th %ile	0.11	0.06 – 0.21	<0.001
50 - 75th %ile*50 - 75th %ile	0.06	0.02 – 0.17	<0.001
25 - 50th %ile*75 - 100th %ile	0.07	0.01 – 0.32	<0.001
P(transmission from environment)* P(transmission from non family member)			
75 - 100th %ile*50 - 75th %ile	0.02	0.002 – 0.16	<0.001
25 - 50th %ile*75 - 100th %ile	0.12	0.04 – 0.37	<0.001
75 - 100th %ile*75 - 100th %ile	0.01	0.002 – 0.12	<0.001

AIC = 49627
Log likelihood = -24729.75
Residual deviance = 49459 on 76304 df

7.3.2 OAT sensitivity analysis

When the simulation model was run with all factors set to their baseline value, the mean probability that a dog would become positive for MRSA in a 24 hour period was 0.0014 (95%SI 9.31 x 10^{-4} - 0.0019). The results of the OAT analysis are presented in Table 7.7. The effect on the outcome was greatest for factors representing transmission from family members, non-family members, home environment and dogs within the community, in decreasing order. The number of contacts with the home environment, the probability of house contamination given the presence absence of MRSA positive family members, the number of contacts with MRSA positive family members and non family members along with the probability that a member of the general population was MRSA positive, and the probability that a healthcare worker was MRSA positive were also found to be influential to the outcome. The only veterinary factors that were found to be influential were the probability that the veterinary environment is contaminated and the probability of transmission from an MRSA positive veterinary environment.

Although the results of this initial analysis were plausible, on closer consideration of the conceptual model, along with the initial spurious results of the P-B analysis using 28 factors (section 7.2.2), it became apparent that the same issues that were encountered with the P-B design, i.e., mutual exclusivity, was likely to be present in the OAT analyses. In addition, within-veterinary clinic factors were poorly represented, due to the low prevalence of veterinary attendance. To overcome these limitation, the OAT analyses were re-run twice, using the factors F1 – F19 to assess main effects for all dogs (irrespective of veterinary attendance) in the given 24 hours and F2 – F28 for those that attended a veterinary clinic during the 24 hours under consideration. When the models were run with all factors set to their baseline value, the mean probability that a dog would become positive for MRSA in a 24 hour period was 1.16 x 10^{-3} (95%SI 1.02 x 10^{-3} – 1.30 x 10^{-3}) and 9.69 x 10^{-3} (95%SI 9.26 x 10^{-3} – 10.12 x 10^{-3}) respectively. The results of the final OAT analyses are presented in Table 7.8 and Table 7.9. The results of the OAT analysis run for all dogs and assessing community variables only were qualitatively identical to the initial OAT analysis run with 28 factors (Table 7.8). However, specification of the model for only those dogs that attended a veterinary clinic revealed that the most influential within veterinary clinic factors were those associated with the probability of transmission from the veterinary environment and the probability that the veterinary environment was contaminated. The next most influential variables were probability of transmission from

veterinary staff and the probability of transmission from a dog at a veterinary clinic. The probability of transmission from family members was still influential for animals that attended a veterinary clinic, along with the probability of transmission from non family members.

Table 7.7. Results of the OAT sensitivity analysis for the simulation model for acquisition of MRSA in dogs.

Factor[†]	Level	Mean probability (x 10^{-3})	90th percentile	Mean probability/Baseline mean probability (rank[#])	90th percentile/Baseline 90th percentile
F1	-	1.04	0.005	1.05	1
	+	1.23	0.005	1.24	1
F2	-	1.04	0.005	1.05	1
	+	2.13	0.005	2.14* (**10**)	1
F3	-	1.02	0.005	1.03	1
	+	1.11	0.005	1.12	1
F4	-	1.17	0.005	1.17	1
	+	1.17	0.005	1.18	1
F5	-	5.15	0	0.52	0
	+	3.12	0.01	3.14* (**8**)	2*
F6	-	1.14	0.005	1.15	1
	+	1.16	0.005	1.17	1
F7	-	0.55	0.005	0.55	1
	+	28.05	0.045	28.19* (**1**)	9*
F8	-	0.60	0.005	0.60	1
	+	3.27	0.01	3.29* (**7**)	2*
F9	-	0.97	0.005	0.97	1
	+	25.79	0.04	25.91* (**2**)	8*
F10	-	0.89	0.005	0.89	1
	+	2.50	0.005	2.51* (**9**)	1
F11	-	1.13	0.005	1.14	1
	+	1.68	0.005	1.68	1
F12	-	1.10	0.005	1.10	1
	+	5.29	0.01	5.31* (**4**)	2*
F13	-	1.11	0.005	1.12	1
	+	1.30	0.005	1.31	1
F14	-	1.01	0.005	1.01	1
	+	1.49	0.005	1.50	1
F15	-	0.90	0.005	0.90	1
	+	4.61	0.01	4.63* (**6**)	2*
F16	-	1.10	0.005	1.11	1
	+	1.10	0.005	1.10	1
F17	-	1.10	0.005	1.07	1
	+	1.07	0.005	1.08	1
F18	-	0.75	0.005	0.75	1
	+	16.08	0.03	16.16* (**3**)	6*
F19	-	0.84	0.005	0.84	1
	+	4.82	0.01	4.84* (**5**)	2*
F20	-	1.13	0.005	1.14	1
	+	1.35	0.005	1.36	1
F21	-	1.15	0.005	1.16	1
	+	1.25	0.005	1.26	1
F22	-	1.07	0.005	1.07	1
	+	1.02	0.005	1.02	1
F23	-	0.95	0.005	0.95	1
	+	2.09	0.005	2.10* (**11**)	1
F24	-	0.99	0.005	0.99	1
	+	2.03	0.005	2.04* (**12**)	1
F25	-	1.04	0.005	1.05	1
	+	1.23	0.005	1.24	1
F26	-	1.01	0.005	1.01	1
	+	1.29	0.005	1.29	1
F27	-	1.05	0.005	1.05	1
	+	1.51	0.005	1.52	1
F28	-	1.23	0.005	1.23	1
	+	1.27	0.005	1.27	1

[†] With reference to Table 7.1; * Factors that increased or reduced the outcome by a factor of two or greater;
[#] Rank of factors marked with *

Table 7.8. Results of the OAT sensitivity analysis run for all dogs for the simulation model for acquisition of MRSA in dogs.

Factor[†]	Level	Mean probability (x 10^{-3})	90th percentile	Mean probability/Baseline mean probability (rank[#])	90th percentile/Baseline 90th percentile
F1	-	1.91	0.005	1.64	1
	+	2.13	0.005	1.83	1
F2	-	1.63	0.005	1.40	1
	+	2.8	0.01	2.41* (**10**)	2*
F3	-	1.68	0.005	1.44	1
	+	1.84	0.005	1.58	1
F4	-	1.67	0.005	1.44	1
	+	1.81	0.005	1.56	1
F5	-	1.43	0.005	1.23	1
	+	3.85	0.01	3.32* (**8**)	2*
F6	-	1.81	0.005	1.56	1
	+	1.84	0.005	1.59	1
F7	-	1.19	0.005	1.02	1
	+	28.43	0.045	24.51* (**1**)	9*
F8	-	1.29	0.005	1.11	1
	+	3.90	0.01	3.36* (**7**)	2*
F9	-	1.68	0.005	1.44	1
	+	26.23	0.04	22.61* (**2**)	8*
F10	-	1.53	0.005	1.31	1
	+	3.35	0.01	2.88* (**9**)	2*
F11	-	1.80	0.005	1.55	1
	+	2.58	0.005	2.22* (**11**)	1
F12	-	1.74	0.005	1.50	1
	+	5.85	0.015	5.04* (**4**)	3*
F13	-	1.82	0.005	1.56	1
	+	2.17	0.005	1.87	1
F14	-	1.82	0.005	1.56	1
	+	2.16	0.005	1.86	1
F15	-	1.64	0.005	1.41	1
	+	5.33	0.01	4.59* (**6**)	2*
F16	-	1.74	0.005	1.50	1
	+	1.77	0.005	1.53	1
F17	-	1.75	0.005	1.51	1
	+	1.74	0.005	1.50	1
F18	-	1.58	0.005	1.36	1
	+	16.51	0.03	14.23* (**3**)	6*
F19	-	1.51	0.005	1.30	1
	+	5.78	0.015	4.98* (**5**)	3*

[†] With reference to Table 7.1; * Factors that increased or reduced the outcome by a factor of two or greater;
[#] Rank of factors marked with *

Table 7.9. Results of the OAT sensitivity analysis run for dogs that have attended a veterinary clinic in the 24 hours under consideration only for the simulation model for acquisition of MRSA in dogs.

Factor[†]	Level	Mean probability (x 10^{-3})	90th percentile	Mean probability/Baseline mean probability (rank[#])	90th percentile/Baseline 90th percentile
F2	-	9.99	0.02	1.03	1
	+	10.65	0.02	1.10	1
F3	-	9.97	0.02	1.03	1
	+	9.58	0.02	0.99	1
F4	-	10.53	0.02	1.09	1
	+	10.18	0.02	1.05	1
F5	-	9.32	0.02	0.96	1
	+	11.95	0.0205	1.23	1.025
F6	-	9.74	0.02	1.00	1
	+	10.54	0.02	1.09	1
F7	-	9.42	0.02	0.97	1
	+	37.18	0.055	3.84* (**5**)	2.75*
F8	-	9.42	0.02	0.97	1
	+	11.935	0.025	1.23	1.25
F9	-	10.12	0.02	1.04	1
	+	34.08	0.05	3.52* (**6**)	2.5*
F10	-	10.11	0.02	1.04	1
	+	11.25	0.02	1.16	1
F11	-	9.56	0.02	0.99	1
	+	10.61	0.02	1.09	1
F12	-	10.29	0.02	1.06	1
	+	14.48	0.025	1.49	1.25
F13	-	10.06	0.02	1.04	1
	+	10.00	0.02	1.03	1
F14	-	9.85	0.02	1.02	1
	+	10.39	0.02	1.07	1
F15	-	9.69	0.02	1.00	1
	+	13.76	0.025	1.42	1.25
F16	-	9.93	0.02	1.02	1
	+	10.13	0.02	1.04	1
F17	-	10.19	0.02	1.05	1
	+	10.07	0.02	1.04	1
F18	-	9.47	0.02	0.98	1
	+	24.34	0.04	2.51* (**8**)	2*
F19	-	9.58	0.02	0.99	1
	+	13.69	0.025	1.41	1.25
F20	-	8.51	0.015	0.88	0.75
	+	23.99	0.0355	2.48* (**9**)	1.775
F21	-	9.31	0.02	0.96	1
	+	46.09	0.065	4.76* (**4**)	3.25*
F22	-	8.84	0.02	0.91	1
	+	17.08	0.03	1.76	1.5
F23	-	3.94	0.01	0.41* (**10**)	0.5*
	+	228.62	0.265	23.59* (**2**)	13.25*
F24	-	3.18	0.01	0.33* (**7**)	0.5*
	+	228.55	0.27	23.59* (**1**)	13.5*
F25	-	4.08	0.01	0.42* (**12**)	0.5*
	+	23.12	0.035	2.39* (**11**)	1.75
F26	-	9.09	0.02	0.94	1
	+	16.12	0.025	1.66	1.25
F27	-	8.84	0.02	0.91	1
	+	93.81	0.12	9.68* (**3**)	6*
F28	-	8.57	0.015	0.88	0.75
	+	15.95	0.03	1.65	1.5

[†] With reference to Table 7.1; * Factors that increased or reduced the outcome by a factor of two or greater;
[#] Rank of factors marked with *

7.3.3 P-B fractional factorial sensitivity analysis

The results of the P-B analysis implemented for all dogs, run for 27 variables and using 32 design points, resulting in 64 runs when a foldover was applied are presented in Table 7.10. These results show that the five most influential variables for all dogs identified by this technique were, in decreasing order, the probability that a community dog is MRSA positive (F11), the number of contacts with a non family member (F10), the probability that the general human population is MRSA positive (F5), the probability of transmission from a community dog (F12) and the number of contacts that the dog has with family members (F8). The results of the P-B analysis for dogs who attended a veterinary clinic only, run for 19 variables and using 20 design points, resulting in 40 runs when a foldover was applied are presented in Table 7.11. These results identify the five most influential within veterinary clinic factors to be, in decreasing order, the probability of transmission from the veterinary environment (F24), the probability that the veterinary environment is contaminated (F23), the number of contacts with dogs at a veterinary clinic (F22), the probability that a home environmental site is contaminated given that there are no MRSA positive family members (F17) and the probability that a dog at a veterinary clinic is MRSA positive (F20). The results are displayed for main effects calculated using the mean outcomes of the P-B analysis and also using the 90^{th} percentile of the outcomes.

The amalgamated results of the LR, OAT and P-B analyses are presented in Table 7.12.

Table 7.10. Main effects of a P-B sensitivity analysis run for all dogs for the simulation model for acquisition of MRSA in dogs.

Factor code	Factor description	P-B main effect (using mean values)	Rank†	P-B main effect (using 90th percentiles) (rank*)
F1	P(dog attends vet in 24 hours)	0.022	12	0.025 (12)
F2	P(health care worker is MRSA positive)	0.013	14	0.014 (14)
F3	P(veterinary worker is MRSA positive)	-0.014	13	-0.019 (13)
F4	P(other high risk group is MRSA positive)	0.009	15	0.007 (18)
F5	P(general population is MRSA positive)	0.055	**3**	0.058 (**3**)
F6	P(person MRSA positive if lives with MRSA positive person)	0.009	16	0.010 (16)
F7	P(transmission from MRSA positive family member per contact)	0.040	**6**	0.045 (**6**)
F8	Number of contacts with family member per 24h	0.044	**5**	0.052 (**5**)
F9	P(transmission from MRSA positive non-family member per contact)	0.032	**9**	0.036 (**8**)
F10	Number of contacts with non-family member per 24h	0.068	**2**	0.080 (**1**)
F11	P(community dog is MRSA positive)	0.070	**1**	0.079 (**2**)
F12	P(transmission from MRSA positive community dog per contact)	0.048	**4**	0.053 (**4**)
F13	Number of contacts with community dog per 24h	0.034	**7**	0.036 (**9**)
F14	P(house contaminated with MRSA | at least one positive family member)	-0.003	19	-0.006 (19)
F15	P(house contaminated with MRSA | no positive family members)	0.028	**10**	0.029 (11)
F16	P(environmental site contaminated | at least one positive family member)	0.008	17	0.007 (17)
F17	P(environmental site contaminated | no positive family members)	-0.006	18	-0.010 (15)
F18	P(transmission from MRSA positive home environment per contact)	0.034	**8**	0.037 (**7**)
F19	Number of contacts with home environment per 24h	0.027	11	0.031 (10)

† Ranked on absolute mean main effect values
* Ranked on 90th percentile main effect values

Table 7.11. Main effects of a P-B sensitivity analysis run for dogs that attended a veterinary clinic in the 24 hours under consideration only for the simulation model for acquisition of MRSA in dogs.

Factor code	Factor description	P-B main effect (using mean values)	Rank†	P-B main effect (using 90th percentiles) (rank*)
F1	P(dog attends vet in 24 hours)		NA	NA
F2	P(health care worker is MRSA positive)	0.022	16	0.027 (14)
F3	P(veterinary worker is MRSA positive)	-0.011	22	-0.011 (23)
F4	P(other high risk group is MRSA positive)	0.001	27	0.003 (25)
F5	P(general population is MRSA positive)	0.023	14	0.023 (16)
F6	P(person MRSA positive if lives with MRSA positive person)	-0.036	**10**	-0.038 (**10**)
F7	P(transmission from MRSA positive family member per contact)	-0.013	17	-0.013 (19)
F8	Number of contacts with family member per 24h	0.013	18	0.017 (17)
F9	P(transmission from MRSA positive non-family member per contact)	-0.028	13	-0.029 (13)
F10	Number of contacts with non-family member per 24h	0.012	20	0.015 (18)
F11	P(community dog is MRSA positive)	0.057	**7**	0.060 (**7**)
F12	P(transmission from MRSA positive community dog per contact)	0.008	23	0.011 (22)
F13	Number of contacts with community dog per 24h	-0.013	19	-0.013 (20)
F14	P(house contaminated with MRSA | at least one positive family member)	-0.002	26	-0.002 (26)
F15	P(house contaminated with MRSA | no positive family members)	-0.012	21	-0.012 (21)
F16	P(environmental site contaminated | at least one positive family member)	-0.053	**8**	-0.060 (**8**)
F17	P(environmental site contaminated | no positive family members)	0.076	**4**	0.079 (**4**)
F18	P(transmission from MRSA positive home environment per contact)	-0.003	25	-0.001 (27)
F19	Number of contacts with home environment per 24h	0.022	15	0.025 (15)
F20	P(dog at vet is MRSA positive)	0.064	**5**	0.070 (**5**)
F21	P(transmission from MRSA positive dog at veterinary clinic per contact)	0.033	11	0.032 (12)
F22	Number of contacts with a dog at a veterinary clinic per 24h	0.087	**3**	0.093 (**3**)
F23	P(veterinary environment contaminated)	0.380	**2**	0.368 (**2**)
F24	P(transmission from MRSA positive veterinary environment per contact)	0.407	**1**	0.401 (**1**)
F25	Number of veterinary environment contacts per 24h	-0.006	24	-0.005 (24)
F26	P(small animal veterinary worker is MRSA positive)	0.060	**6**	0.061 (**6**)
F27	P(transmission from MRSA positive veterinary staff member per contact)	0.031	12	0.033 (11)
F28	Number of veterinary staff contacts per 24h	0.047	**9**	0.049 (**9**)

† Ranked on absolute mean main effect values

* Ranked on 90th percentile main effect values

Table 7.12. Combined table of ranks of results for the LR, OAT and P-B sensitivity analyses for the simulation model for acquisition of MRSA in dogs.

	Variables	All dogs			Dogs that attend veterinary clinics		
		OAT rank^	P-B rank§	LR rank^^	OAT rank^	P-B rank§	LR rank^^
F1	P(dog attends vet in 24 hours)			#	NA	NA	NA
F2	P(health care worker is MRSA positive)	10		*			*
F3	P(veterinary worker is MRSA positive)			*			*
F4	P(other high risk goup is MRSA positive)			*			*
F5	P(general population is MRSA positive)	8	3	*			*
F6	P(person MRSA positive if lives with MRSA positive person)						
F7	P(transmission from MRSA positive family member per contact)	1	6	2	5		=4
F8	Number of contacts with family member per 24h	7	5				
F9	P(transmission from MRSA positive non-family member per contact)	2	9	4	6		=5
F10	Number of contacts with non-family member per 24h	9	2				
F11	P(community dog is MRSA positive)		1			7	
F12	P(transmission from MRSA positive community dog per contact)	4	4	3			=5
F13	Number of contacts with community dog per 24h		7				
F14	P(house contaminated with MRSA | at least one positive family member)						
F15	P(house contaminated with MRSA | no positive family members)	6	10				
F16	P(environmental site contaminated | at least one positive family member)			1**			1**
F17	P(environmental site contaminated | no positive family members)					4	
F18	P(transmission from MRSA positive home environment per contact)	3	8		7		
F19	Number of contacts with home environment per 24h	5					
F20	P(dog at vet is MRSA positive)	NA	NA	NA	8	5	
F21	P(transmission from MRSA positive dog at veterinary clinic per contact)	NA	NA	NA	4	9	3
F22	Number of contacts with a dog at a veterinary clinic per 24h	NA	NA	NA		3	
F23	P(veterinary environment contaminated)	NA	NA	NA	2	2	
F24	P(transmission from MRSA positive veterinary environment per contact)	NA	NA	NA	1	1	2**
F25	Number of veterinary environment contacts per 24h	NA	NA	NA	9		
F26	P(small animal veterinary worker is MRSA positive)	NA	NA	NA		6	
F27	P(transmission from MRSA positive veterinary staff member per contact)	NA	NA	NA	3	10	=4
F28	Number of veterinary staff contacts per 24h	NA	NA	NA		8	

P(..) = Probability; ^ Ranking of the first 10 factors that have a positive effect on the baseline ≥ a factor of two; § Ranking of the first 10 factors that have a positive main effect; ^^ Ranked based on odds ratios of main effects. Boxes denote all input variables that are included in the estimate of composite input factor;
Not included in ranking as dichotomous variable; * Also included in non family member composite factor; ** When P(transmission) from all other routes = 0

7.4 Discussion

This chapter has presented three methods of sensitivity analysis applied to the same stochastic model (described in full in Chapter 6).

The inclusion of interaction terms to the LR model presented in Chapter 6, for the goal of sensitivity analysis, has enabled extension of the main effects LR models. The main effects LR analyses presented in Chapter 6 examined the effect of varying each input variable away from a baseline value whilst maintaining all other variables at their baseline value, representing a defensible sensitivity analysis technique. However, the LR analyses presented in the current chapter also address the effect on the outcome of varying more than one variable at the same time, enabling a more global application of the LR method (Campolongo *et al.*, 2000b). Whilst this technique as specified is globally appropriate, the interpretation of the LR model outputs are not intuitive, and the difficulty associated with interpretation of interaction terms for non-dichotomous categorical predictors is well documented (Hosmer and Lemeshow, 2000; Dohoo *et al.*, 2003; Norton *et al.*, 2004). If the LR models presented in this chapter were to be used for predictive purposes, thorough quantitative interpretation of the results of all of the significant interaction terms would be imperative. However, for the purposes of this study, the interaction terms were included in an attempt to enhance the global capacity of the analysis and as such, interpretation of interactions qualitatively, along with quantitative interpretation of the main effects within the models that account for interaction terms, is adequate and appropriate (Frey and Patil, 2002).

The interactions that were specified in the LR models presented in this chapter reflect the potentially plausible interactions with respect to the underlying simulation model. It was found that, while the fit of the LR models presented in Chapter 6 is improved by the inclusion of interaction terms (AIC values and deviance decrease), the ranking of the main effects reported for each of the transmission LR models in both chapters is identical. This finding was expected, given that this ranking is with respect to the effect of all other variables in the model when they are set at their baseline value, in this case zero, and, in contrast, it has been shown that the magnitude of effect of the probability of environmental transmission when the other variables in the model are not zero reduces. That is, the probability of transmission from the environment is not independent of probability of transmission from other sources and the magnitude of effect of probability of transmission

from the environment reduces in the presence of increased probability of transmission from other sources. This is illustrated by the graphs (Figures 7.1 and 7.2) and results of the LR models (Tables 7.5 and 7.6) but could also be illustrated by the main effects that would be obtained by varying the referent categories for non-environmental variables and re-running the LR models. The implication is that, while the environment may be the most influential source of MRSA when no other sources are present, its effect reduces in the presence of other sources of the pathogen and given that, within the stochastic model, environmental contamination is dependent on MRSA status of humans and (potentially, but not explicitly modelled) animals, it is unlikely that this source plays a large independent role in MRSA acquisition in dogs, but may be highly influential as a reservoir of the pathogen. These results support a number of published studies that identify the environment as a potentially important reservoir of MRSA in the hospital and the home (Wagenvoort *et al.*, 2000; Kniehl *et al.*, 2005; Dancer, 2008) and also concurs with the small number of studies that have been undertaken at veterinary clinics where a putative collinearity appears to exist between human and environmental prevalence estimates (Loeffler *et al.*, 2005; Heller *et al.*, 2009). Given the propensity for prolonged environmental survival of MRSA organisms, and particularly in outbreak strains (Wagenvoort *et al.*, 2000), further elucidation of the role of the environment in transmission of MRSA should be explored.

The use of LR modelling for sensitivity analysis presents advantages over local sensitivity analysis models which include; i) the ability to evaluate the sensitivity of individual model inputs while taking into account the simultaneous impact of other model inputs on the result (Helton and Davis, 2000; Frey and Patil, 2002) and ii) the ability to assess the input variables over a wide range, particularly when continuous variables are included. However, the reliance on a functional form of the relationship between the input and output variables, and the requirement for the key assumptions of regression to be met, represent potential limitations of the application of this method (Frey and Patil, 2002). In the case of the study presented in this chapter, an additional limitation was the necessity to categorise the continuous input variables that represented probabilities in order to avoid collinearity and to satisfy the assumption of linearity in the logit. While the results of the analyses as specified are interpretable and robust, it is somewhat more difficult to relate these results to the original input variables given that the specifications for the LR models are in terms of percentile estimates. Finally, it should be noted that the large sample size that was used for the LR analyses resulted in an inability to assess or rank the models with respect to their p value estimates, given that these are a function of the sample size (McCarthy *et al.*, 1995).

In the models presented in Chapters 6 and 7, the importance of a variable was assessed with respect to the magnitude of its coefficient(s) and resulting odds ratio(s).

In addition to the LR models, two alternative sensitivity analysis techniques are presented in this chapter. One-at-a-time analyses are referred to as 'typical' model sensitivity experiments (Henderson-Sellers and Henderson-Sellers, 1996). This technique assesses the impact of changing the values of each of the chosen factors in turn and is a local form of sensitivity analysis (Henderson-Sellers and Henderson-Sellers, 1996; Campolongo *et al.*, 2000a; Saltelli, 2000). In the OAT analysis presented in this chapter, the results of the 'baseline' run of the analysis, in which all factors are set at their most likely value, returned markedly different results when compared to the overall output of the model as described in Chapter 6. This discrepancy is likely to have occurred due to the pronounced right skew that is present for many of the variables that are included in the simulation model. This finding further supports the marked uncertainty that is present in the overall system and that was also reflected by the discrepancy between the median and mean output values of the simulation model in Chapter 6. However, while this discrepancy is noted and discussed, the danger of making inference by replacing a probability distribution by its mean or other summary value is acknowledged and thus excess weight should not be placed on this absolute result (Law and Kelton, 2000). The results of the OAT analyses are representative of a largely deterministic implementation of the simulation model and, while the weaknesses of an analysis such as this, including the inability to consider interactions, unsuitability to non-linear models and inability to resolve uncertainties of different orders of magnitude, have long been recognised (Henderson-Sellers and Henderson-Sellers, 1996; Campolongo *et al.*, 2000a; Saltelli, 2000), these techniques continue to be commonly applied.

The final technique, a P-B fractional factorial design reflects a method that allows for main effects and also for factor interactions by varying the levels of multiple factors simultaneously (Campolongo and Saltelli, 2000; Campolongo *et al.*, 2000b). The use of a full factorial experimental design to assess all potential interactions, using two levels for each of k factors, would require 2^k model runs. However, a fractional factorial design can reduce the number of runs required by accounting for only some of the possible interactions, as described in Chapter 1 (Campolongo and Saltelli, 2000). The P-B design, with a foldover to enable reduction of aliasing, requires "2 times that multiple of 4 which is next greater than the number of parameters [factors]" (Beres and Hawkins, 2001) and thus,

in the scenario where 28 input factors are considered, reduces the number of model runs from the computationally impractical 2.6×10^8 (2^{28}) to 64 (Beres and Hawkins, 2001).

The overall results of the three analyses were found to be markedly different (Table 7.12). While many of the same factors were identified by the OAT and P-B analyses to be within the ten most influential, the ranking varied between techniques and, in particular, for the community factors when the models were run for all dogs. A greater similarity was found for the OAT and P-B analyses run for dogs that attended veterinary clinics only, with the probability of transmission from the veterinary environment and probability that the veterinary environment is contaminated with MRSA defined as the two most influential factors in both analyses. While only the main effects are assessed for both the OAT analyses and P-B analyses, it possible to compute two-way interaction terms within the P-B analyses (Beres and Hawkins, 2001). However, the resolution of the P-B design does not support reliable estimation of two-way interactions as these effects are potentially confounded with each other (Law and Kelton, 2000) and consequently, the application of a P-B design matrix does not represent a global sensitivity analysis technique. While the results of the OAT analysis may be described as more intuitive than the results of the P-B analysis, the knowledge that the stochastic model is non-linear and that the uncertainties associated with each factor are not always within the same order of magnitude, reduces confidence in the OAT results. Conversely, the P-B results are less intuitive, but it is known that the P-B design is more robust to the non-linearity and variation in input factor magnitudes that exist within the specified stochastic model (Beres and Hawkins, 2001).

All of the analyses indicate that the probability of transmission from the veterinary clinic environment is a highly influential factor for acquisition of MRSA by dogs that attend a veterinary clinic. Consequently this area is strongly identified as deserving of future research efforts and more accurate quantification. The probability of transmission of MRSA from the home environment was also identified as highly influential in the LR models as previously discussed (for all dogs and those that attend a veterinary clinic) and OAT analysis but was ranked lower in the P-B analysis. This may reflect the effect of the interaction between this factor and other probabilities of transmission that have been discussed above and that may have been identified by the P-B design, resulting in a reduction of overall effect for this factor. It is interesting that the same reduction in effect was not evident in the P-B analysis for the veterinary environment factors although a similar interaction was identified with respect to the probability of transmission from the veterinary environment as was identified for the home environment in the LR models. The

difference in effect on the P-B results indicates that the effect of the veterinary environment interactions is likely to be smaller than that of the home environment interactions and this finding is confirmed by the higher odds ratios for the interaction terms for veterinary environmental transmission (Table 7.6). While this previous statement is counterintuitive, one must remember that the interpretation of interaction term odds ratios for categorical predictors is as a ratio of a ratio. As an example, if the first interaction term in the model specified for all dogs is considered (Table 7.5), one finds that when the probability of transmission from the environment is within the 25^{th} – 50^{th} percentile, and the probability of transmission from a non family member is in the 0-25^{th} percentile, a dog is 0.024 times more likely (41.6 times less likely) to become MRSA positive from environmental transmission than when the probability of transmission from the environment is within the 25^{th} -50^{th} percentile and the probability of transmission from a non family member is 0 (the referent category). In the veterinary model, the reduction is less pronounced, with odds ratios maintaining generally greater values (Table 7.6).

While the results of the P-B analyses can be interpreted with greater ease than the results of LR analyses, are more accurate than the OAT analyses and provide a useful adjunct to the LR outputs for models, some ambiguity is found with respect to the ranking of input variables within the P-B output. Many of the variables that represent the same overall area received conflicting ranks. The upper and lower ('+' and '-') values that were selected for use in these analyses represent the maximum and minimum values of the distributions respectively. While these values were chosen to enable maximum assessment of the parameter space (Beres and Hawkins, 2001), it is possible that, given the marked uncertainty present in this model, the resulting variable combinations represented highly unlikely scenarios in many or all cases, resulting in the potential for inaccurate estimates of the main effects. Re-running these models with more modest upper and lower estimates would potentially address this issue, but may also result in failure to detect and account for the effect of extreme values which, in an area with such marked uncertainty, may be of importance. In addition, relationships and conditional dependencies exist between the some of the input variables that cannot be represented in the P-B or OAT designs, and indeed may be inaccurately represented, i.e., overridden by design-based variable combinations. It is also possible that the input variables are so similar in some cases that a non-explicitly modelled dependence may be present in the data and simulation model that is ignored in the P-B design, whereby differing combinations of upper and lower values are forced. Similarly, the finer resolution of input variables, that is, the representation of the data that were input into the simulation model rather than composite values obtained from

the simulation model, were included purposely in the OAT and P-B analyses in an attempt to facilitate a more targeted approach to future research and reduction of uncertainty. However, counterintuitively, the results of the P-B and OAT analyses would be likely to be less ambiguous and more comparable with the LR model outputs if coarser resolution of input variables (composite representations of the finer resolution variables) were used. Repetition of the P-B analysis in particular using the same input variables that were used for the LR models would further validate these points.

In conclusion, this study found that the chosen technique and resolution of input variables is likely to have a marked effect on the output of any sensitivity analysis undertaken, particularly when applied to a complex non-linear system. The stochastic model to which the sensitivity analyses were applied in this chapter was non-linear with input factors that are associated with numerous explicit and non-explicit dependencies, and uncertainties that vary in magnitude. As a result of these model-dependent factors, the outcomes of the three sensitivity analysis methods varied. The results of the OAT analyses, whilst plausible, were not robust, while the results of the P-B analyses were, to some degree, ambiguous, but were in agreement with the interaction terms within the LR models. The results of the LR models were difficult to interpret and differentiate between but allowed consideration of putative interactions between input factors.

Given the complexity of the simulation model to which the sensitivity analyses have been applied and the limitations encountered as discussed above, the results as presented in Chapter 6 remain valid, but are tempered by the inclusion of interaction terms. That is, both community and veterinary routes are important for the acquisition of MRSA in dogs with the influence of transmission from family members of greatest importance and the role of the environment identified as an area that may be of importance as a non-independent reservoir and deserving of greater quantification. However, the explicit differentiation between 'important' variables and those that may be less important within community and veterinary settings was not possible using the techniques presented herein, given the marked uncertainty associated with the modelled system.

CHAPTER 8

GENERAL DISCUSSION

The work presented represents a comprehensive exposure assessment of MRSA acquisition in pet dogs over a 24 hour period. This assessment was an attempt to draw together the studies that are already published in this field of putative importance and to identify priority areas for future research effort on the issue of MRSA in dogs. Formal risk assessment is a relatively recently adopted approach in the field of animal health (MacDiarmid and Pharo, 2003) and is commonly used to quantify the risk of emerging or poorly defined pathogens or disease, in particular in the areas of food safety (Hope *et al.*, 2002; Hald *et al.*, 2004; Parsons *et al.*, 2005; Clough *et al.*, 2006) and live animal or animal product importation (Moutou *et al.*, 2001; de Vos *et al.*, 2004; Jones *et al.*, 2005; Peeler *et al.*, 2006; Martinez-Lopez *et al.*, 2008). However, application of the processes associated with risk assessment to alternative areas of animal health, or indeed human health, is less common (MacDiarmid and Pharo, 2003). The risk assessment presented here was specified in a qualitative and quantitative format. Supporting data-generating studies that facilitated parameterisation of the quantitative model were presented in the form of dedicated data collection exercises and an expert opinion elicitation. The quantitative risk assessment was also extended by the implementation of sensitivity analyses, using three different methods. The results of these studies allowed the aims of the overall thesis: a) to assess the risk of acquisition of MRSA in dogs over a defined time period, b) to identify priority data gaps for future research efforts in this data-sparse area and c) to comment on the usefulness of risk assessment, in a data sparse area and within a field in which it has not previously been used, to be addressed.

The preliminary development of a conceptual model that described the likely pathways of acquisition of MRSA in dogs provided a framework for the qualitative and quantitative risk assessments to follow. Importantly, this framework was structured to enable overall assessment of risk of acquisition of MRSA by consideration of both exposure and transmission (representing consequence) sub-sections within each putative pathway of organism acquisition. The division of the model in this manner allowed for the consideration of dog-dependent variation in exposure and, along with the stratification of the model by community and veterinary clinic routes, also allowed the inclusion of data that predominated the literature, that of prevalence estimates in various populations (Abudu *et al.*, 2001; Cesur and Cokca, 2004; Loeffler *et al.*, 2005; Hanselman *et al.*, 2006b; Hanselman *et al.*, 2008). This framework also aligned the analyses with previously defined risk assessment structures (CAC, 1999; OIE, 2008b), allowing both exposure and

consequence assessments to be conducted. However, structuring the model in this way also necessitated estimation of probabilities of transmission, which proved difficult in the quantitative assessment (discussed subsequently). The qualitative risk assessment was parameterised using categorical estimators and these estimates were combined using a well documented and previously utilised matrix approach (EFSA, 2007). However, this approach was found to be unsuitable for the model as specified. Whilst an alternative specification of categorical estimators relative to the median value within each setting (community or veterinary clinic), rather than as absolute estimates, allowed for intra-setting comparison and greater ability to differentiate between qualitative estimates for a low prevalence event, the combined risk of acquisition from all pathways could not be estimated using this modification. Although some of the limitations that were encountered have previously been described (Cox *et al.*, 2005; Cox, 2008), the application of matrix methods to less biologically complex systems has previously been found to be appropriate and has resulted in the generation of defensible results (Moutou *et al.*, 2001; Clough *et al.*, 2006). A more generalised model that relied on mutually exclusive pathways and that did not differentiate between exposure and transmission may have avoided many of the issues discussed, although specifying the model in this manner would undoubtedly have resulted in a less accurate, or indeed inaccurate, representation of the biological system and would have obviated the use of much of the data as published in the literature. It is postulated that qualitative risk assessment implemented using matrix combination of categorical variables is likely to be inadequate when applied to a biologically complex scenario, and that the findings presented here are unlikely to be unique.

Notwithstanding the limitations of a qualitative assessment as described, the process of assessing the risk using a qualitative format resulted in identification of key areas of uncertainty with respect to the subject. The identification of these areas of uncertainty prompted targeted data-generation studies. Similarly, potential associations and dependencies were also identified that had to be accounted for by representation of these relationships within the quantitative model when specified. The results of the qualitative assessment were that, in absolute terms, the greatest risk was likely to be from humans (owners and staff) in the community and veterinary clinic. In relative terms, within the community it was found that family members were the most important source of MRSA, followed by non-family members and the environment and lastly other dogs. Within the veterinary clinic, the qualitative assessment identified staff to be the most important source, followed by the environment and then dogs at a veterinary clinic.

The greatest strength of risk assessment is often defined as its ability to rank, in order of importance, the effect of multiple inputs on a single output and thus identify high priority data gaps, or priority candidate mitigation targets (Saltelli, 2000; Vose, 2000; Frey and Patil, 2002; MacDiarmid and Pharo, 2003). A quantitative risk assessment, to fulfil this aim and to corroborate (or repudiate) the results of the qualitative risk assessment, was specified. While model structure and validation require high quality data (Law and Kelton, 2000; Vonk Noordegraaf *et al.*, 2003), it is acknowledged that these data are often limited (Vose, 2000; Vonk Noordegraaf *et al.*, 2003). Thus, the development of a model using alternative sources of data, such as expert opinion, in addition to more traditional published sources, is often required and performed (Law and Kelton, 2000; Vose, 2000; Vonk Noordegraaf *et al.*, 2003).

In order to parameterise the quantitative risk assessment, a number of data-generating studies were completed. The first reported prevalence estimates of i) MRSA contamination in the environment of a tertiary referral small animal hospital (1.4%) and ii) MRSA colonisation in the staff within that hospital (3.1%). In this study, genetic profiling provided the inference that the dissemination of environmental MRSA isolates was most likely to have occurred through veterinary staff. This study reported a lower prevalence of MRSA contamination within the environment and of MRSA carriage in the external nares of staff (Heller *et al.*, 2009), than had been reported in a previous study in a tertiary referral hospital within the UK (Loeffler *et al.*, 2005). This finding was of importance in parameterisation of the quantitative model as it mitigated over-estimation of the uncertainty associated with environmental contamination and staff carriage of MRSA within veterinary hospital settings. As a result, the data obtained from the previously published study was down-weighted within the quantitative risk assessment. Whilst this down-weighting was necessary to provide more accurate representation of an 'average' scenario within any veterinary hospital, it must be stressed that this does not reflect reduced confidence in the findings of the previously published study, but simply the lack of representativeness of these findings when applied to any veterinary clinic on any given day. The findings of this study support this down-weighting of the dominant literature at the time of specification of the model, which otherwise would have been a speculative change to the weight of the input data.

A separate data-generating study corroborated the findings of prior studies on dog-dog and dog-human interactions (Westgarth *et al.*, 2007; Westgarth *et al.*, 2008). These findings increased confidence in the use of these data to populate the quantitative risk assessment

and also allowed extrapolation of some of the findings of Westgarth and colleagues (2008) for appropriate parameterisation of the quantitative risk assessment model in the format that it was specified, i.e., enabled estimation of overall exposure and individual contacts on a daily basis. Similarly, the results obtained from the final data-generating study gave confidence in the classification of food (in particular raw meat and pig ear products) as a contributor to the environmental source of MRSA rather than requiring modelling as an independently specified pathway.

The use of an expert opinion elicitation procedure aimed to fill the remaining data gaps, identified by the qualitative assessment, where estimates were required for parameterisation of the quantitative model. The use of a novel application of previously utilised expert opinion techniques (Otway and von Winterfeldt, 1992; Clemen and Winkler, 1999; Bedford and Cooke, 2001; Goossens *et al.*, 2008) enabled the specification of distributions to describe numerous variables associated predominantly with prevalence and transmission of MRSA. The calibration variables that were utilised in this study provided an estimate of the accuracy of the experts, resulting in the exclusion of a single expert from all analyses, along with documentation of confidence in the remaining experts. However, notwithstanding this demonstrated confidence, markedly divergent opinions were obtained with respect to some questions, in particular for estimates of transmission, resulting in wide and minimally-informative combined expert distributions. This finding was also reflected in the difficulty reported by the respondents in completing the questionnaire with respect to estimation of transmission variables. While many of the issues surrounding this reported difficulty are difficult to improve, the application of a spreadsheet-based interactive distribution estimation procedure, as proposed by Vose (2000), in future studies may increase the understanding of the experts with respect to the implications of their overall and confidence estimates. An interactive spreadsheet was devised (Appendix 11) but could not be implemented in the email format that was required in this study. The integration of a method such as this may have given the expert a feeling of greater control with respect to their estimation and perhaps could be considered for inclusion in future email-based techniques, with access via a web-based link.

The quantitative assessment followed the conceptual structure previously outlined and was parameterised using previously published and unpublished data, along with the data obtained through small studies discussed above. The risk assessment was specified as a second order stochastic simulation model that allowed consideration of both uncertainty and variability. While the variability was not entirely independent of uncertainty, the

specification of the model in this manner allowed separate consideration of these two aspects of overall uncertainty. The results of the uncertainty analysis showed that the modelled process was dominated by uncertainty rather than variability and, as such is, in theory, reducible by further data collection (Saltelli, 2000; Vose, 2000; MacDiarmid and Pharo, 2003). The overall outcome of the model showed that the median proportion of dogs that were likely to acquire MRSA on any given day was 1.5%. This estimate was within the 95% binomial confidence intervals surrounding prior estimates of the prevalence of MRSA in dogs within the community (Murphy *et al.*, 2006; Rich and Roberts, 2006; Vengust *et al.*, 2006; Hanselman *et al.*, 2008). Consideration of the temporal component modelled within the risk assessment, inclusion of dogs at higher risk than those in the general community within the model and the knowledge that MRSA is likely to be a transient coloniser or contaminator in dogs (Weese and van Duijkeren, 2009), gives confidence in the overall outcome of the model when corroborated with these prior prevalence estimates. The mean proportion of dogs that were likely to acquire MRSA on any given day, 4.2%, was considerably higher than the median, demonstrating a marked right skew to the uncertainty distribution. Moreover, the uncertainty analysis revealed that some input variables were likely to be highly influential on the outcome of acquisition of MRSA in dogs, contributing to the skewness of this distribution. Sensitivity analyses (discussed below) were subsequently utilised in an attempt to identify these highly influential variables.

The specification of the variables included within the quantitative model required a great deal of careful consideration. Within the quantitative application of the risk assessment, the subsections of exposure and transmission were further divided into numerous contributing inputs. The exposure subsection required estimates of the number of exposures that the at-risk dog experienced to MRSA positive humans, dogs and environments within a given 24 hours. These estimates used uncertain probabilities of, taking as an example exposure to MRSA positive family members: number of people within the family, the probability that each person belonged to a certain pre-defined risk group and the probability that the person was MRSA positive given that he/she belonged to a certain predefined risk group. Within the transmission subsection, estimates of probability of transmission from exposure to a source of MRSA were modelled on a per-contact basis. The probability of transmission per 24 hours was estimated using the probability of transmission per contact, along with the number of (potentially infectious) contacts between an at-risk dog and the putative source of MRSA. The decision to specify the transmission variables in this manner was difficult. The reasoning behind this

categorisation was to allow full use of the literature and to enable the model to be fully transparent and updateable as future data became available. However, no data relating to transmission were available within the literature and the experts found estimation of the transmission variables in this manner to be difficult. Estimation of probability of transmission on a 24 hour, rather than per contact, basis may have been easier for the experts, and some of the comments from the experts suggested this format. However, it was considered that estimation of transmission in this manner would require the implicit estimation or knowledge of average number and type of contacts with putative sources of MRSA per 24 hours, along with other demographic data that were unlikely to be within the expertise of the participants, given the selection criteria. While estimation in this manner may have been appropriate for well-known putative sources such as owners, contact information for alternative sources such as non-family members or other dogs is likely to vary markedly from at-risk dog to dog and day to day. Thus, to enable representation of this variability and uncertainty, it was decided to specify the model to allow the inclusion of this information through consideration of data such as demographic contact information (Westgarth *et al.*, 2008). However, in this area of data sparsity this may have resulted in overcomplication of the model. Notwithstanding this, the specification of the model in this manner does allow for iterative updating as new data are obtained, and also has the potential to give more precise indications of where future research may be required.

As noted above, the results of the simulation model indicated that the system was dominated by uncertainty which, in theory, could be reduced by further study and data collection. Sensitivity analyses were applied to attempt to determine which inputs of the model were most influential on the output, and therefore deserving of greater study resulting in the potential to reduce the surrounding uncertainty. The sensitivity analyses were specified in the form of LR models (with and without interaction terms), OAT analyses and fractional factorial design in the form of P-B analyses. The results that were obtained from these models were, in many ways, ambiguous and difficult to interpret. The effect of the home environment dominated the results of all of the sensitivity analyses. A number of reasons can be postulated for this. First, and in line with the discussion of the previous paragraph, the specification of environmental contact sites to be 10cm x 10cm in size is likely to have resulted in an overrepresentation of this source within the models. Again, the environment was specified in this manner to allow the inclusion of published prevalence estimates of environmental contamination, to enable updating of the model with future data as they emerged and to facilitate quantification of the number of contacts between at-risk dogs and the environment over a 24 hour period, in line with the prescribed

model structure. However, consideration of the environment in this manner represented a marked difference to the consideration of contacts and transmission between at-risk dogs and humans. Thus, while biologically this might be the most appropriate way to consider this variable, it may have resulted in overspecification with respect to its inclusion within the simulation and LR models. Vastly more contacts between dogs and 10cm x 10cm environmental sites are likely in any given day than the number of contacts between dogs and owners. Furthermore, the estimation of transmission from environmental sites of this size is likely to have presented an increased level of difficulty for experts which may have resulted in overestimation of probabilities. Expert estimates of transmission from the environment were likely to have been made 'relative' to those from humans and animals. However, given the size of the environmental sites, estimates may have required modification with respect to transmission from other sources by a factor greater than was considered by the experts, particularly given that all probabilities are likely to be clustered at the lower end of the probability scale. Furthermore, it is possible that the estimation of whole numbers, required by the expert opinion questionnaire, may have limited the adjustment of these overall estimates of transmission to an order of magnitude that is not sufficiently small to reflect reality. As no data exist to corroborate or compare with these estimates, this discussion will remain speculative, although these points should be considered if any future risk analyses and expert opinion exercises are undertaken in an area comparable to this.

In addition to the issues surrounding specification of environmental variables, it is difficult to consider the contribution of the environment as important in the absence of a human or animal source of MRSA as, indeed, environmental contamination is not believed to be an independent phenomenon. This was modelled explicitly in the simulation model and was also reflected in the results of the LR models that were fitted to account for interactions between the environment and other sources of MRSA. However, whilst these issues are taken into account and while it is noted that the environment does not provide an independent source of MRSA, its contribution as a potential reservoir in the immediate absence of an alternative source of the organism is deserving of greater quantification. As this model is specified over a 24 hour period, the potential for the existence of environmental reservoirs is possible and plausible. How to increase quantification of the environmental contribution to MRSA acquisition in dogs poses a separate and independent question. A more thorough documentation of the existence of environmental areas most likely to be contaminated with MRSA, as has been done in human hospitals may be an initial step, within the home and veterinary hospitals. Similarly, a thorough observational

study of the nature of contact between dogs and environmental sites within the home and at veterinary clinics would allow a more accurate definition of the number of 10cm x 10cm sites and the types of contact that occur over a given time frame, potentially resulting in refinement of the uncertainty that is currently included in the model.

In the absence of the effect of the environment, the sensitivity analyses provided no consistent indication of the rankings of alternative sources of MRSA. While family members were arguably identified as highly important within the sensitivity analysis models, their indisputable ranking as the most important or vastly more important than all other variables was not possible. While direction towards further elucidation of the effect of the environment and further study into quantification of the potential for transmission from family members may be appropriate, it cannot be definitively proposed that all other variables are markedly less worthy of further study and research. In short, the work presented has revealed the difficulty in determining priority areas for future research in the absence of suitable data inputs upon which to base a complex model. The inclusion of marked uncertainty in the inputs of models such as this, combined with complexity within the model structure makes prioritisation of inputs through sensitivity analyses difficult.

It is instructive to consider that, had a single sensitivity analysis been applied to the quantitative risk assessment, the overall results and conclusion may have been vastly different, with a single result and, in the case of the application of the OAT analysis, a logical and to some degree expected, conclusion. However, given the results of the other sensitivity analyses that were applied, it is now known that this would have been incorrect. Ultimately, a combination of: i) the application of a quantitative risk assessment to a data sparse area that encompassed marked uncertainty, ii) a non-linear relationship along with numerous modelled dependencies and iii) a low prevalence event, resulted in the variation in the results of the different sensitivity analyses and the inability to reach consistent and uncomplicated conclusions.

The application of LR models to the input/output data from a quantitative risk assessment model is a novel application of variance-based sensitivity analysis in the area of veterinary medicine. While a previous study has applied this statistical method to the output of a fractional factorial design (Vonk Noordegraaf *et al.*, 2003), explicit *de novo* application of LR has not previously been reported. The limitations of an approach such as this have been discussed but in general, this method appears to be easily applicable and interpretable in the absence of categorical interaction terms as were used within this research. The

specification of the models required two separate LR models to be applied to each of the simulation model scenarios. Fitting of the LR models in this manner was required to avoid collinearity and to enable differentiation between exposure and transmission. Similarly, re-running of the simulation model for all dogs enabled ranking of within-veterinary exposures and transmission, which otherwise would have been overshadowed by the higher prevalence within-community factors.

Finally, the use of the computer programme R (R Development Core Team, 2008) provided marked advantages and limitations, in equal measure. The ability to specify the simulation model in R allowed far more complex dependencies and conditional links to be included than would have been possible in a spreadsheet-based programme such as @RISK (Pallisade Corporation; Middlesex, UK). It also allowed a large number of iterations to be implemented, which was imperative in a low prevalence event as specified. However, the R programming code is far less user-friendly than the menu-based interface that is present in alternative simulation and statistical programmes. The code-based programming resulted in a marked amount of time spent in bug-fixing and code-checking, not to mention the time invested learning the language and writing the code in the first instance. It is acknowledged that the time spent completing these activities is likely to decrease as experience increases, and that the capacity of this programme far outweighs the capacity of others used for the same purpose. However, within the analyses undertaken a number of problems were encountered that could have befallen a programmer of any level of experience and were only identified through painstaking code checking. Two such problems are outlined: First, the omission of a single letter defining a variable in one set of code resulted in the LR models for the second specification of the simulation model (dogs that attended veterinary clinics only) being run against the outcome of the first specification of the simulation model (all dogs). Whilst it is acknowledged that this fault was entirely programmer-based, the ability for small but important errors such as this to occur, and go undetected, using this programme is of great importance and care should be taken in selecting variable names to enable ease of subsequent error checking. Second, the specification of all input matrices with '0' prior to writing the data to these matrices resulted in difficulty in identification of incorrect code. Within a low prevalence event, the specification of all initial input matrices with entries as 'NA' instead of '0' should be routine, so that outputs do not appear plausible if the code for a variable does not work correctly within the code loop (which is ostensibly impossible to check without breaking the loop). In short, while R is a very powerful and useful programme, painstaking bug checking and adherence to simple but important rules, which are not specified in any

literature but obtained through user experience, should be exhaustively practised by all programmers.

Mathematical models have previously been defined and parameterised to explore the transmission of MRSA within closed human populations (Cooper and Lipsitch, 2004; Kajita *et al.*, 2007). However, the use of mathematical, or other forms of risk assessment models have not previously been applied to MRSA acquisition outwith a contained environment such as this, and have not explicitly included animal sources of the pathogen. Similarly, the use of risk assessment for pathogen transmission studies in general in the veterinary literature is limited, allthough numerous mathematical models relating to pathogen transmission exist. While the nomenclature relating to risk assessment is ambiguous and a number of these mathematical models may address the same aim as a risk assessment without explicitly being defined as such, dedicated risk assessments are generally limited to exposure assessments within the field of animal health (de Vos *et al.*, 2004; Peeler and Thrush, 2004; Jones *et al.*, 2005). Thus, the study presented here is a new approach to prioritising research in an emerging area of animal health.

In conclusion, qualitative and quantitative risk assessment processes have been used to identify a median risk of acquisition of MRSA in pet dogs over any given 24 hours as 1.5% (5^{th} – 95^{th} percentile range 0 – 19%). While extensive sensitivity analyses were conducted, no clear prioritisation of influential inputs could be obtained. However, community and veterinary hospital routes were both found to be important in acquisition of MRSA in dogs and, while it cannot be concluded that other sources of MRSA are unimportant, the role of the environment and of family members, were found to be deserving of further study. The identification of further studies that may be useful in improving the specification of a model such as defined were themselves difficult, given the marked and in some cases intractable uncertainty surrounding estimates of transmission. The usefulness of a risk assessment approach in this data sparse area was unconvincing as difficulties were encountered in accurate specification of the model and interpretation of the results of both the qualitative and quantitative assessments, despite thorough and extensively researched methods implementation. While results were easily obtained for qualitative and quantitative assessments, along with sensitivity analyses, the consideration of the uncertainty surrounding these results reduced the confidence in these outputs. It is postulated that the limitations encountered within this work are likely to be encountered when applying formal risk assessment methods to any other biologically complex phenomenon that is not represented by a closed or modular system, is non-sequential and

non-linear in nature, includes dependencies and is characterised by sparse data. Consequently, whilst the subject of this research relates to a single biological system, the use of risk assessment processes should not be considered an easy or appropriate approach to the initial assessment of any system with the above properties.

APPENDICES

APPENDIX 1

Questionnaire used to investigate interactions between dogs, other animals and humans

Questionnaire administered to Dog Owners:

Dog Owner

Interactions with YOUR pet

1) What pets do you own?

a) Dogs ☐ Number: _____

b) Cats ☐ Number: _____

c) Other ☐ Type: _____ Number: _____

2) a) Does your dog have physical contact with any / all of these?

☐ Yes

☐ No

b) Which ones:

_____

3) a) Does your dog have close contact with any other animals?

YES ☐ **NO** ☐

b) Please describe: _____

_____

_____

4) a) How many times in a day or week do you walk your dog?

b) How long (in minutes) is the average walk?

5) On an average walk:

a) How many people does your dog interact with?

b) What type of interaction is it?

☐ Patting / Clapping
☐ Throwing a ball
☐ Licking
☐ Other: _____

c) How many other dogs does your dog interact with?

d) Besides on a walk, how many people does your dog come into contact with in a week?

e) What type of interaction is it?

☐ Patting / Clapping
☐ Throwing a ball
☐ Licking
☐ Other: _____

_____

_____

6) Is there anywhere within the house that is restricted to the dog?

_____

_____

7) What king of things do you do with your dog:

- ☐ Patting
- ☐ Playing
- ☐ Cuddling
- ☐ Feed it treats
- ☐ You don't really touch it at all

8) Does your dog:

- ☐ Sit on the sofa
- ☐ Lick your hands
- ☐ Lick your face
- ☐ Eat from household plates
- ☐ Sleep on the bed

9) a) Does your dog eat raw meat?

YES ☐ NO ☐

b) If yes – what proportion of the diet is raw?

10) a) In the last year, how many times has your dog been to the vet?

b) Were they hospitalized?

YES ☐ NO ☐

i) How many times?

ii) For how long?

iii) What types of illness did your dog suffer from?

11) a) Is your dog wormed?

- ☐ Yes
- ☐ No
- ☐ Not as often as you should

b) How often? _____

Interactions with OTHER dogs

12) *N/A – non dog question*

13) Do you have contact with other:

- ☐ Dogs
- ☐ Cats
- ☐ Both

14) a) On average, how many times a week do you interact with someone elses dog?

b) Where is this most likely to occur?

- ☐ In your house
- ☐ Someone elses house
- ☐ Outside

c) Who do the dogs belong to?

- ☐ Family
- ☐ Friend
- ☐ Someone you don't know

d) What kind of things would you do with the dog?

- ☐ Patting
- ☐ Playing
- ☐ Cuddling
- ☐ Feed it treats
- ☐ You don't really touch it at all

Hygiene

15) Would you generally wash your hands after the following?

- ☐ Public transport
- ☐ Gardening
- ☐ Petting a dog
- ☐ Taking out the bin
- ☐ Handling money
- ☐ Going to the GP
- ☐ Handling raw meat
- ☐ Blowing nose/sneezing
- ☐ Going to the toilet
- ☐ Handling cooked meat
- ☐ Before eating
- ☐ Picking up dog poo

16) a) Do you have other dogs visiting your home?

YES ☐ **NO** ☐

b) If yes – does it affect your cleaning habits?

YES ☐ **NO** ☐

17) If you pet a dog in the park, would you wash your hands when you got home?

YES ☐ **NO** ☐

18) *NA – non dog question*

19) *NA – non dog question*

Knowledge of Disease

20) a) Do your recognize any of these diseases?

- ☐ Roundworms
- ☐ Ringworm
- ☐ Tapeworms
- ☐ Distemper
- ☐ Toxoplasma Infection
- ☐ Superbug (MRSA)
- ☐ Parvovirus
- ☐ Cat-scratch fever
- ☐ Campylobacter
- ☐ Salmonella

b) Which do you think can be passed back and forth between you and pets?

- ☐ Roundworms
- ☐ Tapeworms
- ☐ Distemper
- ☐ Toxoplasma Infection
- ☐ Superbug (MRSA)
- ☐ Ringworm
- ☐ Parvovirus
- ☐ Cat-scratch fever
- ☐ Campylobacter
- ☐ Salmonella

Demographics

21) Do you live in the:

- ☐ City
- ☐ Country

22) a) Number of people in the household? ☐

b) Children 0-2: _____ Children 6-10_____

Children 3-5: _____ Children 11-16_____

23) ☐ Male ☐ Female

24) Age

- ☐ 19 or under
- ☐ 20's
- ☐ 30's
- ☐ 40's
- ☐ 50's
- ☐ 60 +

25) Education Level

- ☐ Secondary
- ☐ College or Trade School
- ☐ University
- ☐ Post Graduate

Questionnaire administered to non-Dog Owners:

Non-Dog Owner

1) What pets do you own?

a) Dogs ☐ Number: _____

b) Cats ☐ Number: _____

c) Other ☐ Type: _____ Number: _____

12) Are there any specific reasons for not owning a dog?

- ☐ Money
- ☐ Hygiene/disease concerns
- ☐ Time commitment
- ☐ Allergies
- ☐ Don't care for them
- ☐ No specific reason

13) Do you have contact with:

- ☐ Dogs
- ☐ Cats
- ☐ Both

14) a) On average, how many times a week do you interact with a dog? ☐

b) Where does this occur?

- ☐ In your house
- ☐ Someone elses house
- ☐ Outside

c) Who do the dogs belong to?

- ☐ Family
- ☐ Friend
- ☐ Someone you don't know

d) What kind of things would you do with the dog?

- ☐ Patting
- ☐ Playing
- ☐ Cuddling
- ☐ Feed it treats
- ☐ You don't really touch it at all

Hygiene

15) Would you generally wash your hands after the following? (tick if yes)

- ☐ Public transport
- ☐ Gardening
- ☐ Petting a dog
- ☐ Taking out the bin
- ☐ Handling money
- ☐ Going to the GP
- ☐ Handling raw meat
- ☐ Blowing nose/sneezing
- ☐ Going to the toilet
- ☐ Handling cooked meat
- ☐ Before eating
- ☐ Picking up dog poo

16) a) Do you have dogs visiting your home?

YES ☐ **NO** ☐

b) If yes – does it affect your cleaning habits?

YES ☐ **NO** ☐

17) If you pet a dog in the park, would you wash your hands when you got home?

YES ☐ **NO** ☐

18) If you had a dog, would you allow it to:

- ☐ Sit on the sofa
- ☐ Lick your hands
- ☐ Lick your face
- ☐ Eat from household plates
- ☐ Sleep on the bed

19) If you had a dog, would you allow it to eat raw meat?

YES ☐ **NO** ☐

Knowledge of Disease

20) a) Do your recognize any of these diseases? (tick if yes)

- ☐ Roundworms
- ☐ Tapeworms
- ☐ Distemper
- ☐ Toxoplasma Infection
- ☐ Superbug (MRSA)
- ☐ Ringworm
- ☐ Parvovirus
- ☐ Cat-scratch fever
- ☐ Campylobacter
- ☐ Salmonella

b) Which do you think can be passed back and forth between you and pets?

- ☐ Roundworms
- ☐ Tapeworms
- ☐ Distemper
- ☐ Toxoplasma Infection
- ☐ Superbug (MRSA)
- ☐ Ringworm
- ☐ Parvovirus
- ☐ Cat-scratch fever
- ☐ Campylobacter
- ☐ Salmonella

Demographics

21) Do you live in the:

- ☐ City
- ☐ Country

22) a) Number of people in the household? ☐

b) Children 0-2: _____ Children 6-10_____

Children 3-5: _____ Children 11-16_____

23) ☐ Male ☐ Female

24) Age

- ☐ 19 or under
- ☐ 20's
- ☐ 30's
- ☐ 40's
- ☐ 50's
- ☐ 60 +

25) Education Level

- ☐ Secondary
- ☐ College or Trade School
- ☐ University
- ☐ Post Graduate

APPENDIX 2

Questionnaire for expert opinion elicitation in the area of MRSA in pet dogs

University of Glasgow | Faculty of Veterinary Medicine

Expert Opinion Elicitation

MRSA in dogs

Introduction

The following questions are designed to elicit **expert opinion.** This exercise is undertaken to augment sparse data and will be used to inform a simulation model.

There are **no 'correct' or 'incorrect'** responses.

The questionnaire consists of 17 questions and will take approximately 10-20 minutes to complete.

Please note the format of the questions – in most cases responses will require estimation of numbers out of 1,000

- Please use decimals if required – e.g. 1.5 people
- For ease of reference;
  0.1% of 1,000 = 1
  1% of 1,000 = 10
  10% of 1,000 = 100

For most questions you will be asked to estimate the **minimum**, **maximum** and **most likely** amount. You will also be asked to enter the level of 'certainty' that you have around the most likely estimate (i.e. how confident you are in your estimate).

- Lack of confidence may relate to personal uncertainty or lack of current knowledge in the area (these do not require separation)
- Confidence is graded between 1 (very unsure) and 10 (absolutely certain)
- A level of certainty of 4 will be ascribed if left blank

Please note:

3. The term **positive** will be used to account for any bodily carriage of the organism (nasal, skin, perineal …). **It does not attempt to distinguish between colonisation** (intermittent or consistent) **and temporary carriage**. This term will also encompass **any infection** with MRSA.

4. Please answer all questions for the average situation expected **within the UK**.

Prevalence of MRSA

Question 1:

Consider **1,000 people** selected at random from the general population.

How many of these people would you expect, on average, to be **positive for MRSA**?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 2:

Consider **1,000 human health care workers** selected at random from the general population.

How many of these health care workers would you expect, on average, to be **positive for MRSA**?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 3:

Consider **1,000 veterinarians (large and small animal)** selected at random from the general population.

How many of these veterinarians would you expect, on average, to be **positive for MRSA**?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 4:

Place an '**x**' on the line next to any groups that you consider to have an **increased risk** of carriage of MRSA in comparison to the general population:

a) Health Care Workers _____

b) Veterinarians : - large animal _____
- small animal _____

c) Veterinary nurses and other veterinary staff : - large animal _____
- small animal _____

d) Farmers _____

e) Immunosuppressed _____

f) Hospitalised within the last year _____

g) Other, please state _____
_____
_____

Question 5:

Consider **1,000 dogs** selected at random from the general population.

How many of these dogs would you expect, on average, to be **positive for MRSA**?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 6:

Consider **1,000 hospitalised dogs** selected at random at veterinary clinics.

How many of these dogs would you expect, on average, to be **positive for MRSA**?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Environmental contamination

Question 7:

Consider **1,000 houses** selected at random.

How many of these houses would you expect, on average, to be positive for the presence of any **environmental contamination with MRSA anywhere in the house** on a single day?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 7a:

Consider **one average house** that is **positive** for environmental contamination with MRSA.

If **1,000 sites** (approximately 10cm x 10cm) are sampled randomly within that house, how many of these sites would you expect, on average, to be positive for MRSA?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 8:

Consider **1,000 houses** selected at random that each have **at least one member of the household that is positive for MRSA**.

How many of these houses would you expect, on average, to be positive for the presence of any **environmental contamination with MRSA** on a single day?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 8a:

Consider one of the houses in Q8, with **at least one member of the household positive for MRSA,** that is also **positive for environmental contamination with MRSA**.

If **1,000 sites** are sampled randomly within that house, how many of these sites would you expect, on average, to be positive for MRSA?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 9:

Consider **1,000 small animal veterinary clinics** selected at random.

How many of these clinics would you expect, on average, to be positive for the presence of any **environmental contamination with MRSA** on a single day?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 9a:

Consider one of these small animal veterinary clinics (Q9) that is positive for environmental contamination with MRSA.

If **1,000 sites** are sampled randomly within that veterinary clinic, how many of these sites would you expect, on average, to be positive for MRSA?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 10:

Consider **1,000 small animal veterinary clinics** selected at random that each have at least one member of staff that is positive for MRSA.

How many of these clinics would you expect, on average, to be positive for the presence of any **environmental contamination with MRSA** on a single day?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 10a:

Consider one of these small animal veterinary clinics (Q10) that is positive for environmental contamination with MRSA.

If **1,000 sites** are sampled randomly within that veterinary clinic, how many of these sites would you expect, on average, to be positive for MRSA?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 11:

Consider **1,000 small animal veterinary clinics** selected at random that each have **at least one canine patient** that is positive for MRSA.

How many of these clinics would you expect, on average, to be positive for the presence of any **environmental contamination with MRSA** on a single day?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 11a:

Consider one of these small animal veterinary clinics (Q11) that is positive for environmental contamination with MRSA.

If **1,000 sites** are sampled randomly within that veterinary clinic, how many of these sites would you expect, on average, to be positive for MRSA?

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Transmission of MRSA

Question 12:

Consider **1,000 MRSA negative dogs**.

Estimate the **number of dogs**, on average, that will become **positive for MRSA after a single average contact** with the following:

a) An MRSA positive owner

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

b) Another MRSA positive human (not their owner)

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

c) An MRSA positive dog within their household

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

d) An MRSA positive non-household dog

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

e) An MRSA positive veterinarian

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

f) MRSA positive veterinary staff-member (non-veterinarian)

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

g) An MRSA positive household environmental site (10cm x 10cm)

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

h) An MRSA positive veterinary hospital environmental site (10cm x 10cm)

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 13:

Consider **1,000 MRSA negative humans, selected from the general population.**

Estimate the **number of people**, on average, that will become **positive for MRSA after a single average contact with** the following:

a) An MRSA positive spouse or live-in partner

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

b) An MRSA positive non-co-habiting partner

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

c) An MRSA positive (non-spousal) household member <15 years old

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

d) An MRSA positive (non-spousal) household member >15 years old

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

e) An MRSA positive non-household member

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

f) An MRSA positive household environment (10cm x 10cm)

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

g) An MRSA positive dog

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

h) MRSA in food

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 14:

Consider **1,000 MRSA negative hospitalised humans.**

Estimate the **number of people**, on average, that will become **positive for MRSA after a single average contact with** the following (all of whom are positive for MRSA at the time of contact):

a) An MRSA positive patient

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

b) An MRSA positive doctor

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

c) An MRSA positive (non-doctor) health-care worker

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

d) An MRSA positive visitor

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

e) An MRSA hospital environment (10cm x 10cm)

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

f) An MRSA positive dog (therapy animal)

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 15:

What percentage (0 to 100%) of transmission of **MRSA to humans** would you estimate takes place by:

a) Direct contact (humans or animals)

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

b) Aerosolisation

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

c) Environmental contact

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

d) Food

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 16:

What percentage (0 to 100%) of transmission of MRSA **to dogs** would you estimate takes place by:

a) Direct contact (humans or animals)

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

b) Aerosolisation

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

c) Environmental contact

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

e) Food

Minimum	Maximum	Most Likely

Confidence in estimate (1-10): _____

Question 17:

Please rate your level of specialisation or expertise in the following areas:

Please place a **'x'** in the relevant box for each category. A rating scale of 1-10 is used where 1 represents the equivalent knowledge to a member of the general public and 10 represents the knowledge of a specialist at the top of the particular field

	1	2	3	4	5	6	7	8	9	10
a) Veterinary microbiology										
b) Human microbiology										
c) Small animal veterinary medicine										
d) Large animal veterinary medicine										
e) Human medicine										
f) MRSA										
g) Public health										
h) Zoonotic disease										
i) Epidemiology										
j) Food safety										
k) Communicable disease control										
l) Other – please state and rate below										

Your participation in this questionnaire is greatly appreciated.

Any additional comments: _____

APPENDIX 3

Covering letter emails for the questionnaire for expert opinion elicitation in the area of MRSA in pet dogs

Accompanying the initial emailed mailing:

Dear _____,

I am writing to ask if you would be willing to participate in an exercise to obtain expert opinion regarding MRSA in dogs and the potential for transfer of this organism between dogs and humans (file attached).

As you know, MRSA is classed as an emerging pathogen in small animals and its potential for zoonotic transfer is currently unquantified. Recent calls have been made to assess the overall risk of MRSA in animals and humans using a formal risk assessment approach (FVE conference on MRSA, Bussels, 2008).

I have been working on a quantitative risk assessment of MRSA acquisition in dogs and transfer to humans as part of an epidemiological PhD and DEFRA research fellowship.

At the final stage of this risk assessment, it is imperative that the model that has been developed is parameterised appropriately. As this area is data sparse, expert opinion is of great importance in obtaining parameter estimates.

The use of expert opinion within the model will entail the combination of the opinions of a number of experts to result in an overall distribution of estimates for important parameters.

Please be assured that all responses will be confidential and anonymised. The overall results from the questionnaire will be fed back to the group of experts.

If you are willing to participate, I would be very grateful if you would reply with a completed questionnaire (attached). I am happy to receive printed questionnaires (mailed to the address below) if preferred.

With many thanks,

Jane Heller BSc, BVSc, DipVetClinStud, MVetClinStud, MRCVS
Research Fellow (DEFRA VTRI)

Comparative Epidemiology and Informatics
Institute of Comparative Medicine
Faculty of Veterinary Medicine
University of Glasgow
Bearsden
G61 1QH
UK

Ph: 0141 330 3437
email: J.Heller@vet.gla.ac.uk

Accompanying the follow-up emailed mailing:

Dear _____,

A few weeks ago, I wrote to ask if you would be willing to participate in an exercise to obtain expert opinion regarding MRSA in dogs and the potential for transfer of this organism between dogs and humans (file attached).

To date, I have received responses from approximately half of the experts that I contacted, which have been very interesting and informative.

The experts that have been contacted were targeted based on publications and involvement in the MRSA field and it was hoped that a broad representation of fields, facilities and countries would limit bias that might otherwise occur. As such, I am still hoping to obtain some more responses.

It has been brought to my attention on a number of occasions that this questionnaire is uncomfortable to fill in. I totally agree and, whilst I apologise for the difficulty that may be encountered, I must reassure you that the purpose of the questionnaire is not to replace data that are likely to be available within the next years or decades, but to allow initial modeling of the problem at hand with the aim of identification of important data gaps and crucial areas for future research.

The elicitation of expert opinion is a recognised technique within the field of risk assessment and is often undertaken to enable an initial analysis where data are sparse.

The results of these questionnaires will not be used or published on their own, but combined to parameterise inputs for a stochastic model and go some way to an initial estimation of the variability and uncertainty that we are likely to encounter in this area of research.

Again, please be assured that all responses will be confidential and anonymised.

I thank you for taking the time to read this and please do not hesitate to contact me if any further information is required.

If you are willing to participate, I would be very grateful if you would reply with a completed questionnaire (attached). I am happy to receive printed questionnaires (mailed to the address below) if preferred.

With many thanks,

Jane Heller BSc, BVSc, DipVetClinStud, MVetClinStud, MRCVS
Research Fellow (DEFRA VTRI)

Comparative Epidemiology and Informatics
Institute of Comparative Medicine

Faculty of Veterinary Medicine
University of Glasgow
Bearsden
G61 1QH
UK

Ph: 0141 330 3437
email: J.Heller@vet.gla.ac.uk

APPENDIX 4

User-defined R code for modified beta distributions (beta-PERT and modified beta-PERT)

1) Function to model a "beta-PERT" distribution. Written by G.T. Innocent (g.innocent@vet.gla.ac.uk) (Adapted from formulae published by Vose (2000), pp170,171)

- Parameter 1 = number of samples required
- Parameter 2 = minimum
- Parameter 3 = mode
- Parameter 4 = maximum

- Parameters 2-4 can be vectors and will be recycled if necessary
- Alternatively parameter 2 can be the vector c(min,mode,max)

```
rbetapert<-function(n=1,min,Mode,max){

if(Mode==(min+max)/2){Mode<-Mode+0.001}
if((2*Mode-min-max)==0){Mode<-Mode+0.001}

mean<-(min+4*Mode+max)/6

if((Mode-mean)==0){Mode <- Mode+0.001}
if((mean-min)==0){mean <- mean+0.001}
if((max-mean)==0){max <- max+0.001}

alpha<-(mean-min)*(2*Mode-min-max)/((Mode-mean)*(max-min))
beta<-alpha*(max-mean)/(mean-min)
return(rbeta(n,alpha,beta)*(max-min)+min)

}
```

2) Function to model a "modified beta-PERT" distribution Written by J. Heller (j.heller@vet.gla.ac.uk) and G.T. Innocent (g.innocent@vet.gla.ac.uk). (Adapted from formulae published by Vose (2000), pp170,171)

- Parameter 1 = number of samples required
- Parameter 2 = minimum
- Parameter 3 = mode
- Parameter 4 = maximum

- Parameters 2-4 can be vectors and will be recycled if necessary
- Alternatively parameter 2 can be the vector c(min,mode,max)

```
rmodbetapert<-function(n=1,min,Mode,max,gamma){

  if(Mode==(min+max)/2){Mode<-Mode+0.001}
  if((2*Mode-min-max)==0){Mode<-Mode+0.001}

  mean<-(min+gamma*Mode+max)/(gamma+2)

  if((Mode-mean)==0){Mode <- Mode+0.001}
  if((mean-min)==0){mean <- mean+0.001}

  beta.part <- (max-mean)/(mean-min)

  if(beta.part==0){beta.part<-0.001}

  alpha<-((mean-min)*(2*Mode-min-max))/((Mode-mean)*(max-min))
  beta<-alpha*beta.part
  value <- (rbeta(n,alpha,beta)*(max-min)+min)

}
```

APPENDIX 5

Concerns and comments noted by experts in association with the expert opinion elicitation exercise

1. I found it hard to estimate risk based on single contacts (as opposed to cumulative risk over a given time period). I may have overestimated risk from single contact, because I think any type of single contact results is a low risk of transmission.

2. I felt very uncomfortable filling it in as there is so little one can refer to and so much guess work so I left some blank. I still wanted to give it a try in order to help you with your data collection but with MRSA being such a media-friendly area, this approach seems very vulnerable to abuse?

3. a) I found the questionnaire VERY uncomfortable to fill in. I tried to do my best but my feeling is that very few questions can be answered scientifically on the basis of the current knowledge

   b) The questionnaire is long and many questions are extremely difficult to answer on a scientific basis due to the lack of published data. It takes much more than 10-20 minutes to complete it…

4. Found it difficult to determine what an average contact between people and other people and people and dogs would be.

5. a) I think the answers would be significantly different if the definition of positive did not include all types of positive.

   b) The main problem for dogs is that they are like large dusters in hospital and veterinary environments and they also tend to lie on the floor. So they are perfectly placed to transiently carry MRSA on their coats, picking it up from environmental dust.

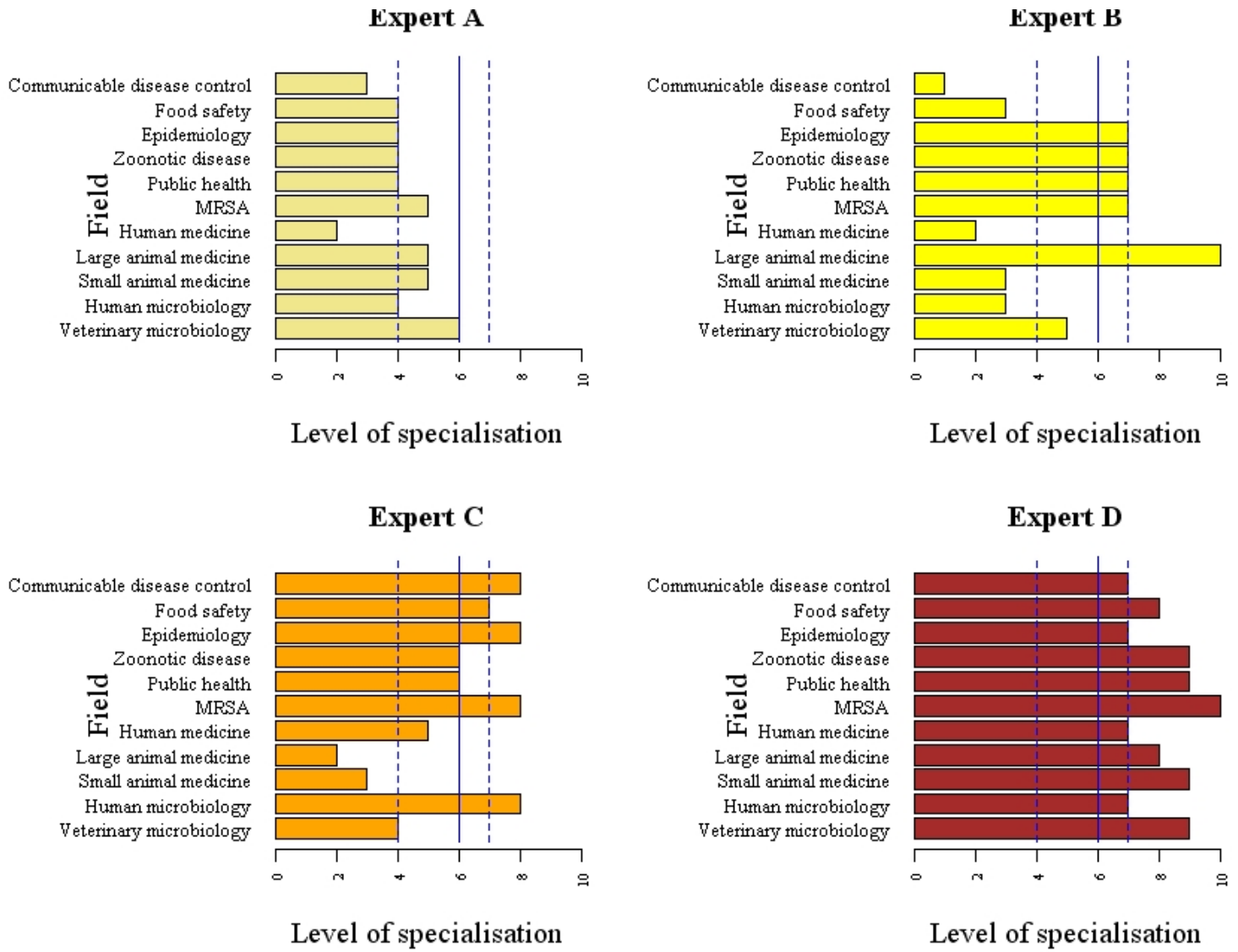
   c) I would urge that the results are presented with care, bearing in mind that some members of the public might throw their dogs into the local canal if they perceive them to be a human health risk. The questionnaire defines positive as any carriage, so if we are commenting on transfer between humans/ dogs, that might only be transient skin/ coat colonisation of either species.

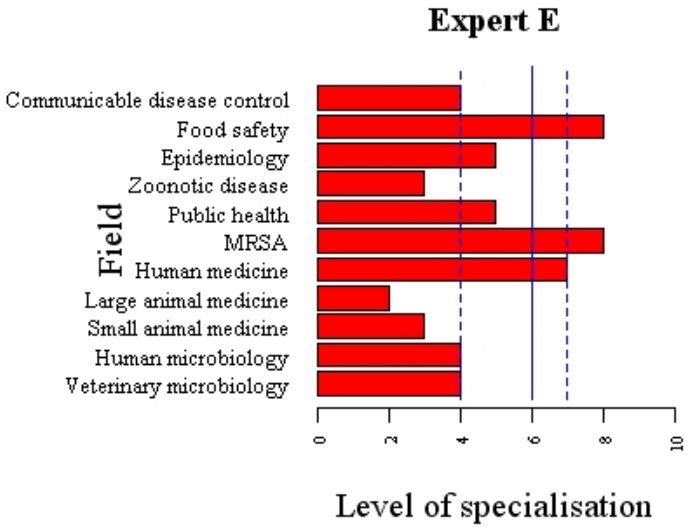
6. Guessing for a different setting(UK) = double guess Not sure how helpful that can be

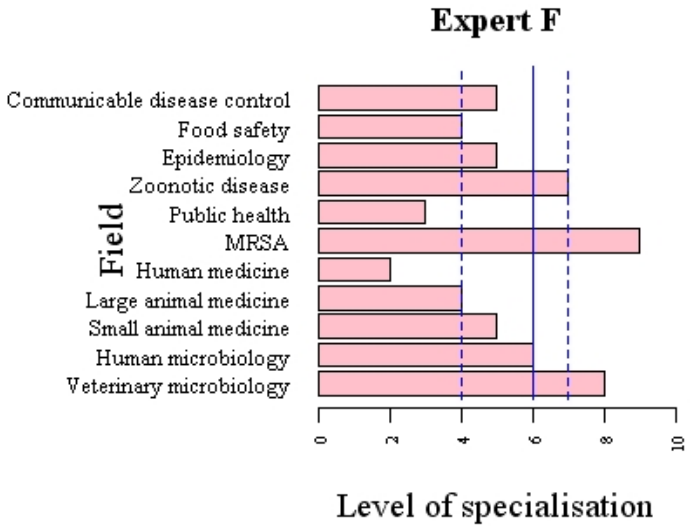
APPENDIX 6

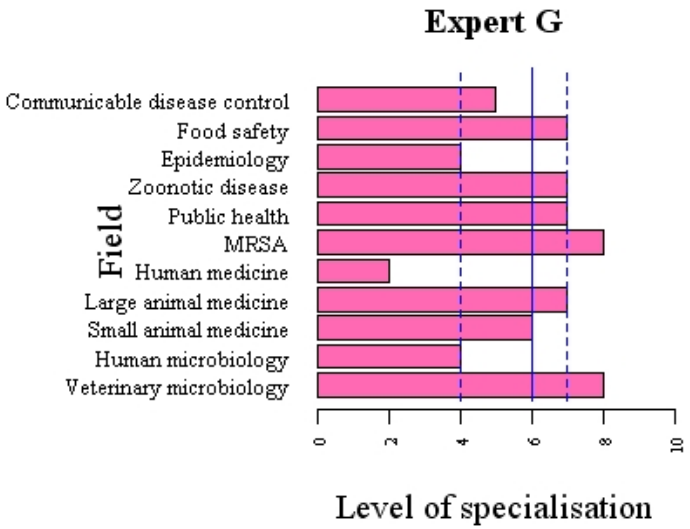
Expert profiles of ‘expertness’

Graphs of individual 'expertness'. The solid line represents the median and dashed lines represent 25th and 75th percentiles for all experts:




Expert E
Communicable disease control
Food safety
Epidemiology
Zoonotic disease
Public health
MRSA
Human medicine
Large animal medicine
Small animal medicine
Human microbiology
Veterinary microbiology
Field
0
2
4
6
8
10
Level of specialisation


Expert F
Communicable disease control
Food safety
Epidemiology
Zoonotic disease
Public health
MRSA
Human medicine
Large animal medicine
Small animal medicine
Human microbiology
Veterinary microbiology
Field
0
2
4
6
8
10
Level of specialisation


Expert G
Communicable disease control
Food safety
Epidemiology
Zoonotic disease
Public health
MRSA
Human medicine
Large animal medicine
Small animal medicine
Human microbiology
Veterinary microbiology
Field
0
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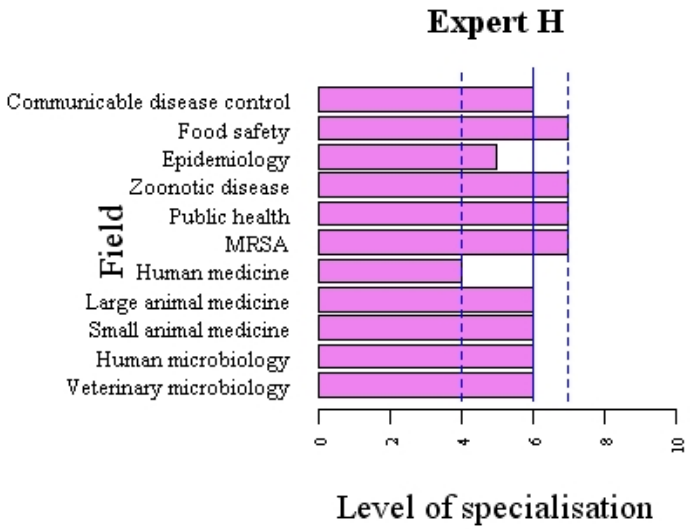
4
6
8
10
Level of specialisation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Communicable disease control
Food safety
Epidemiology
Zoonotic disease
Public health
MRSA
Human medicine
Large animal medicine
Small animal medicine
Human microbiology
Veterinary microbiology
Field
0
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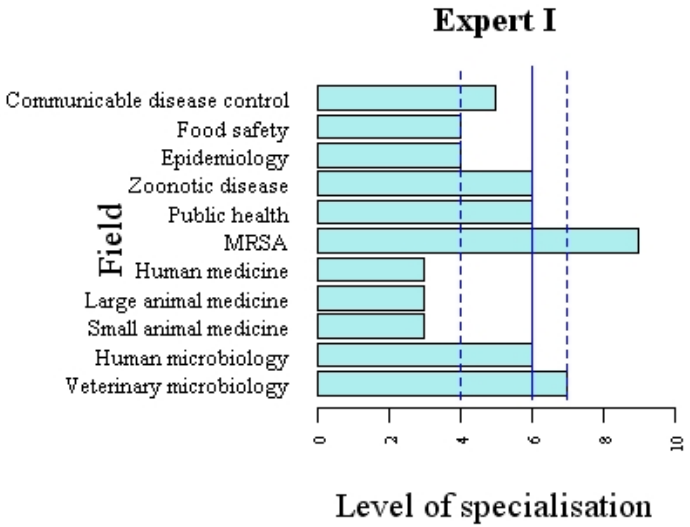
6
8
10
Level of specialisation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Communicable disease control
Food safety
Epidemiology
Zoonotic disease
Public health
MRSA
Human medicine
Large animal medicine
Small animal medicine
Human microbiology
Veterinary microbiology
Field
0
2
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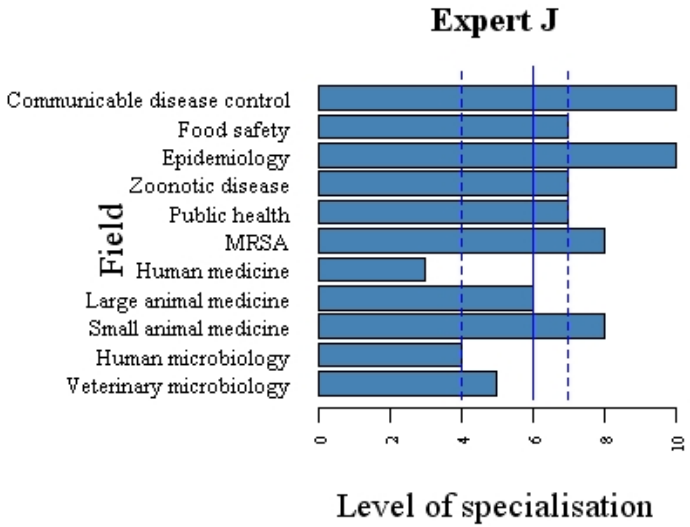
6
8
10
Level of specialisation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Epidemiology
Zoonotic disease
Public health
MRSA
Human medicine
Large animal medicine
Small animal medicine
Human microbiology
Veterinary microbiology
Field
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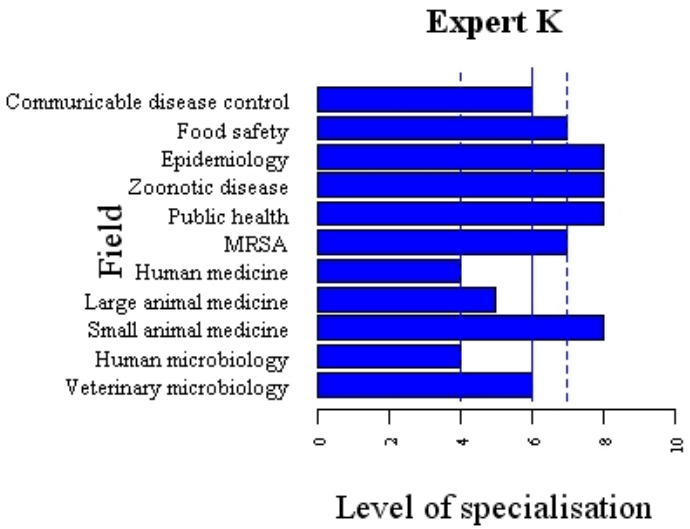
6
8
10
Level of specialisation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Communicable disease control
Food safety
Epidemiology
Zoonotic disease
Public health
MRSA
Human medicine
Large animal medicine
Small animal medicine
Human microbiology
Veterinary microbiology
Field
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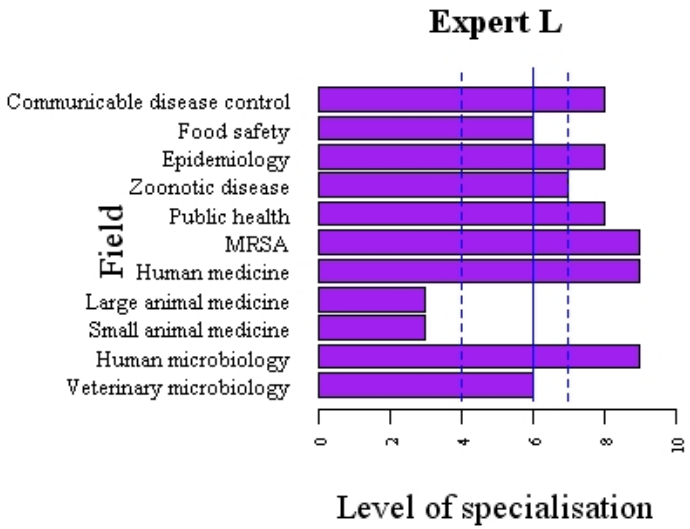
Level of specialisation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Epidemiology
Zoonotic disease
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Human medicine
Large animal medicine
Small animal medicine
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Field
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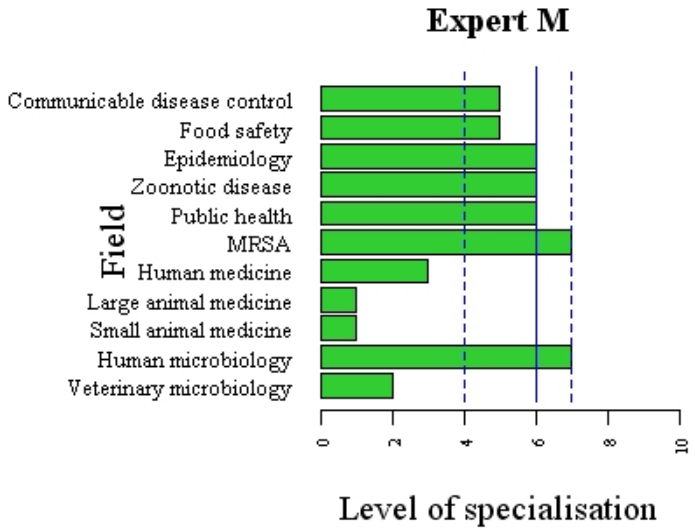
4
6
8
10
Level of specialisation


Expert M
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Food safety
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Zoonotic disease
Public health
MRSA
Human medicine
Large animal medicine
Small animal medicine
Human microbiology
Veterinary microbiology
Field
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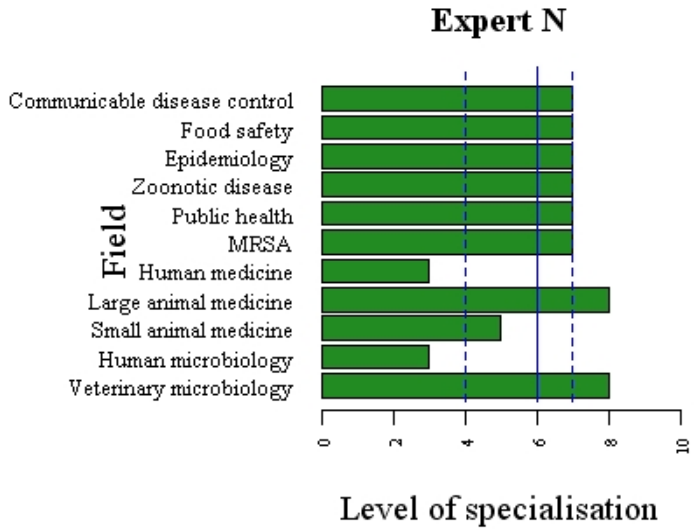
10
Level of specialisation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Zoonotic disease
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Small animal medicine
Human microbiology
Veterinary microbiology
Field
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4
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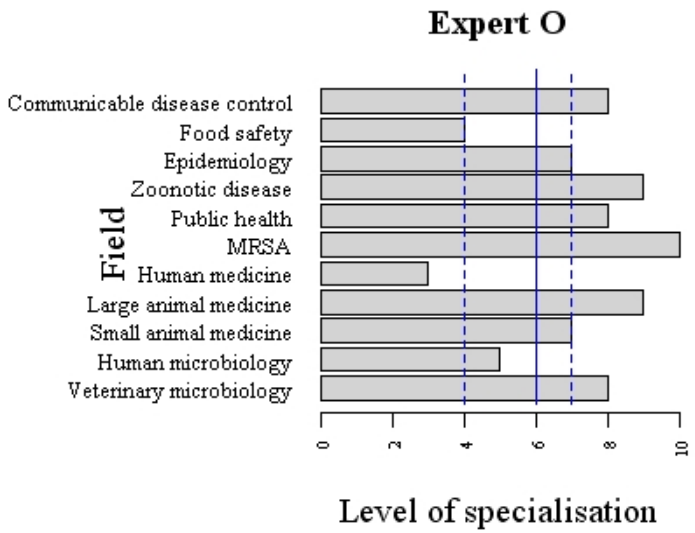
8
10
Level of specialisation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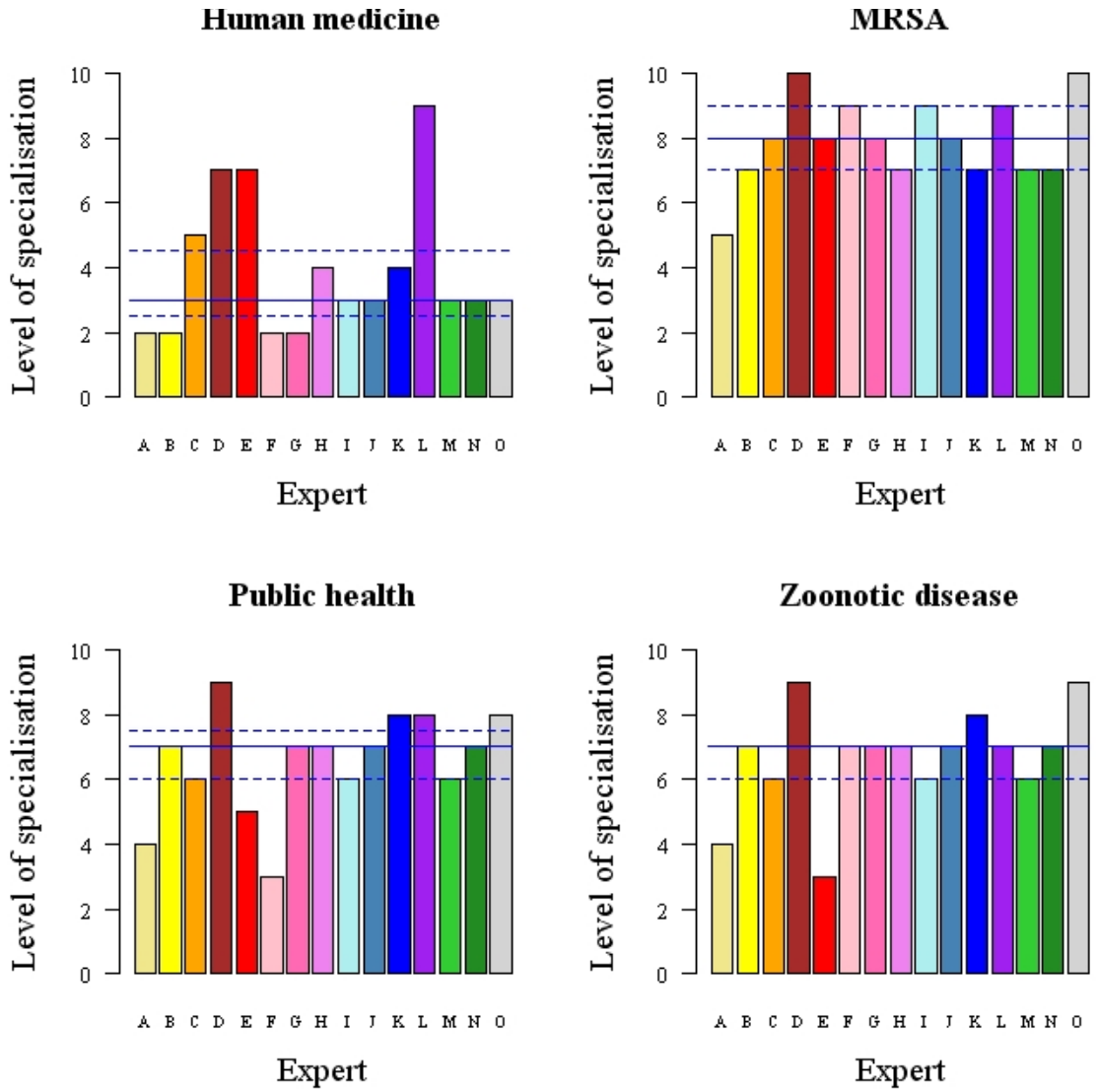
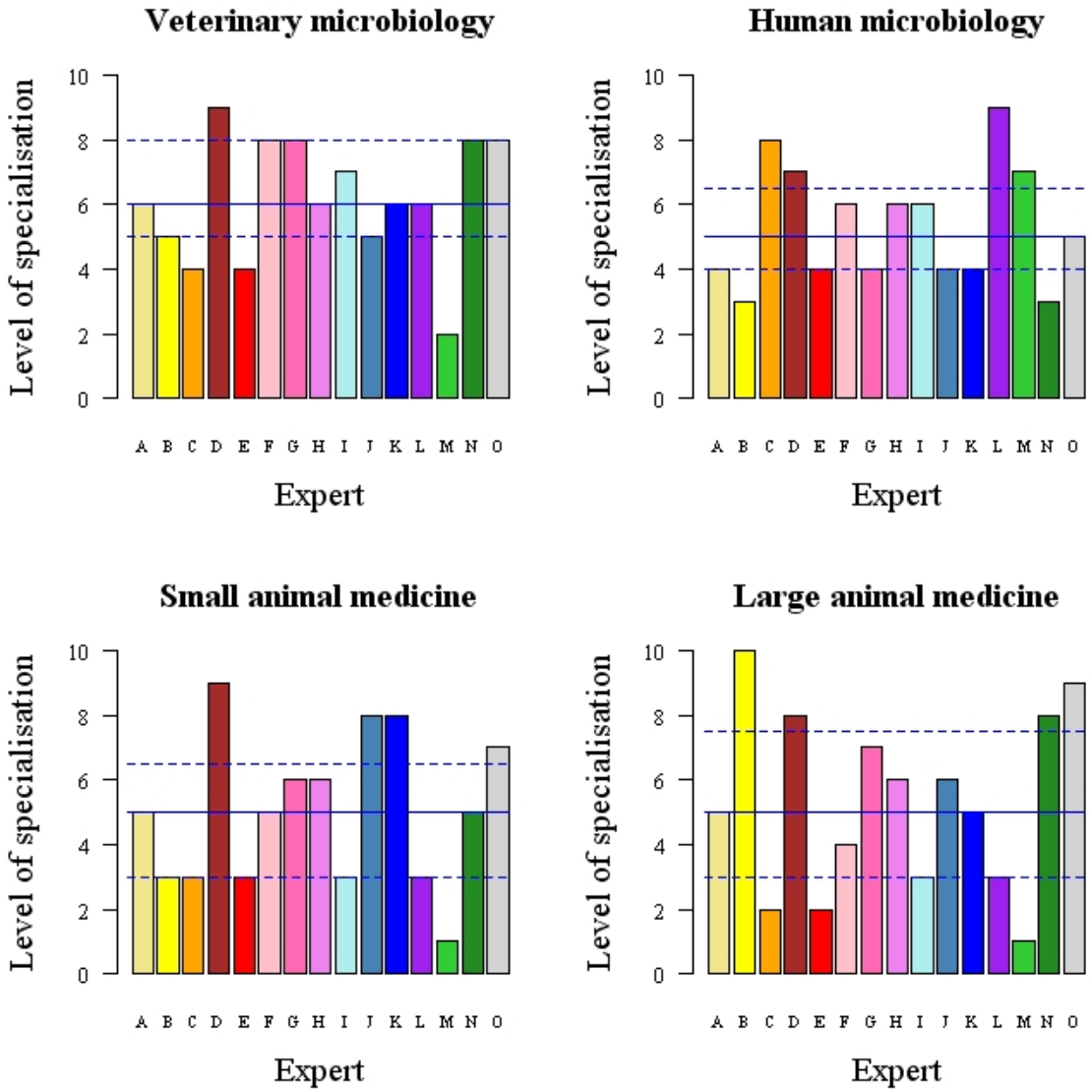
Epidemiology
Zoonotic disease
Public health
MRSA
Human medicine
Large animal medicine
Small animal medicine
Human microbiology
Veterinary microbiology
Field
0
2
4
6
8
10
Level of specialisation

Graphs of 'expertness' grouped for specialisation. The solid line represents the median and dashed lines represent 25th and 75th percentiles across specialisation:

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Human microbiology
Small animal medicine
Large animal medicine
Level of specialisation
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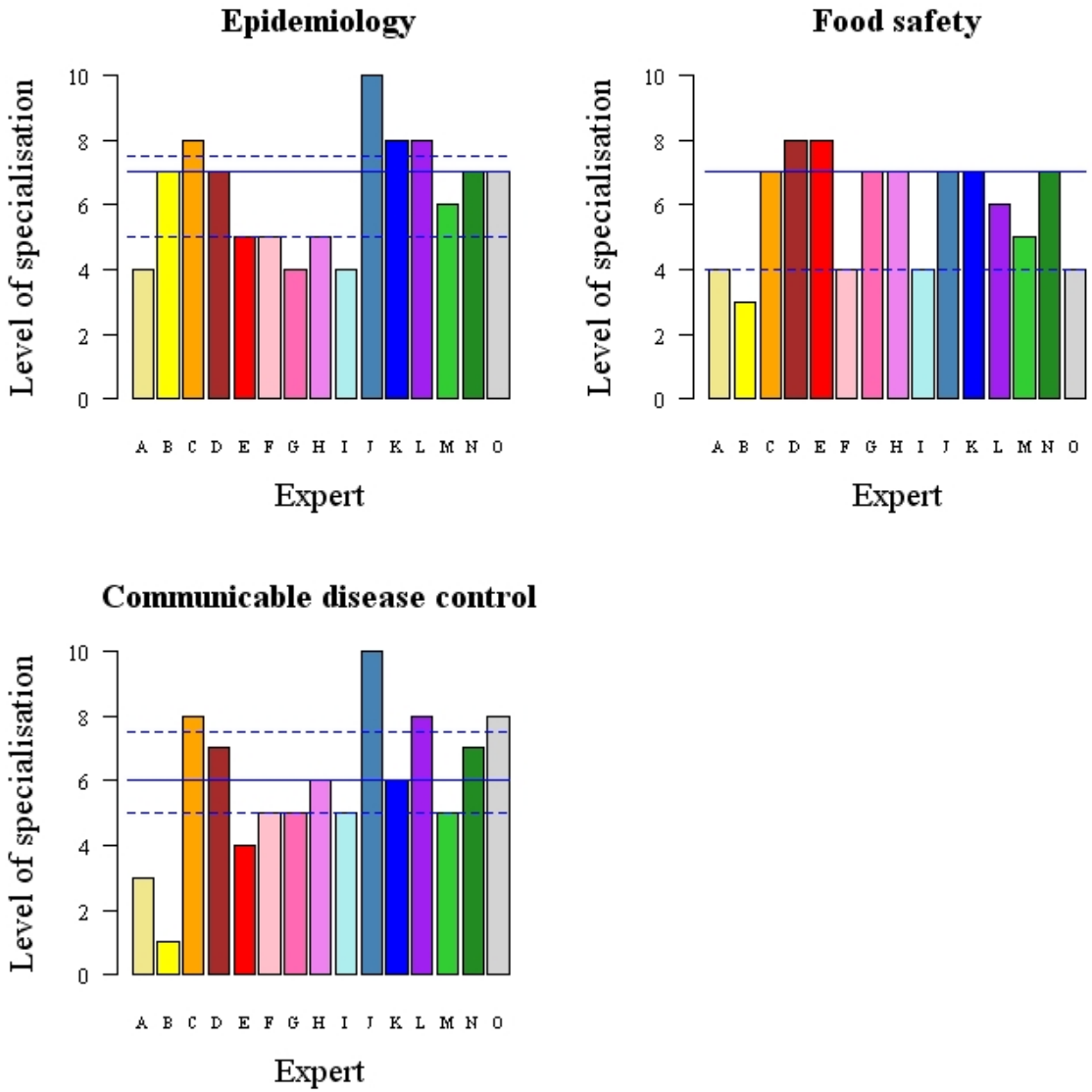
2
4
6
8
10
A B C D E F G H I J K L M N O
Expert


Epidemiology
Level of specialisation
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4
6
8
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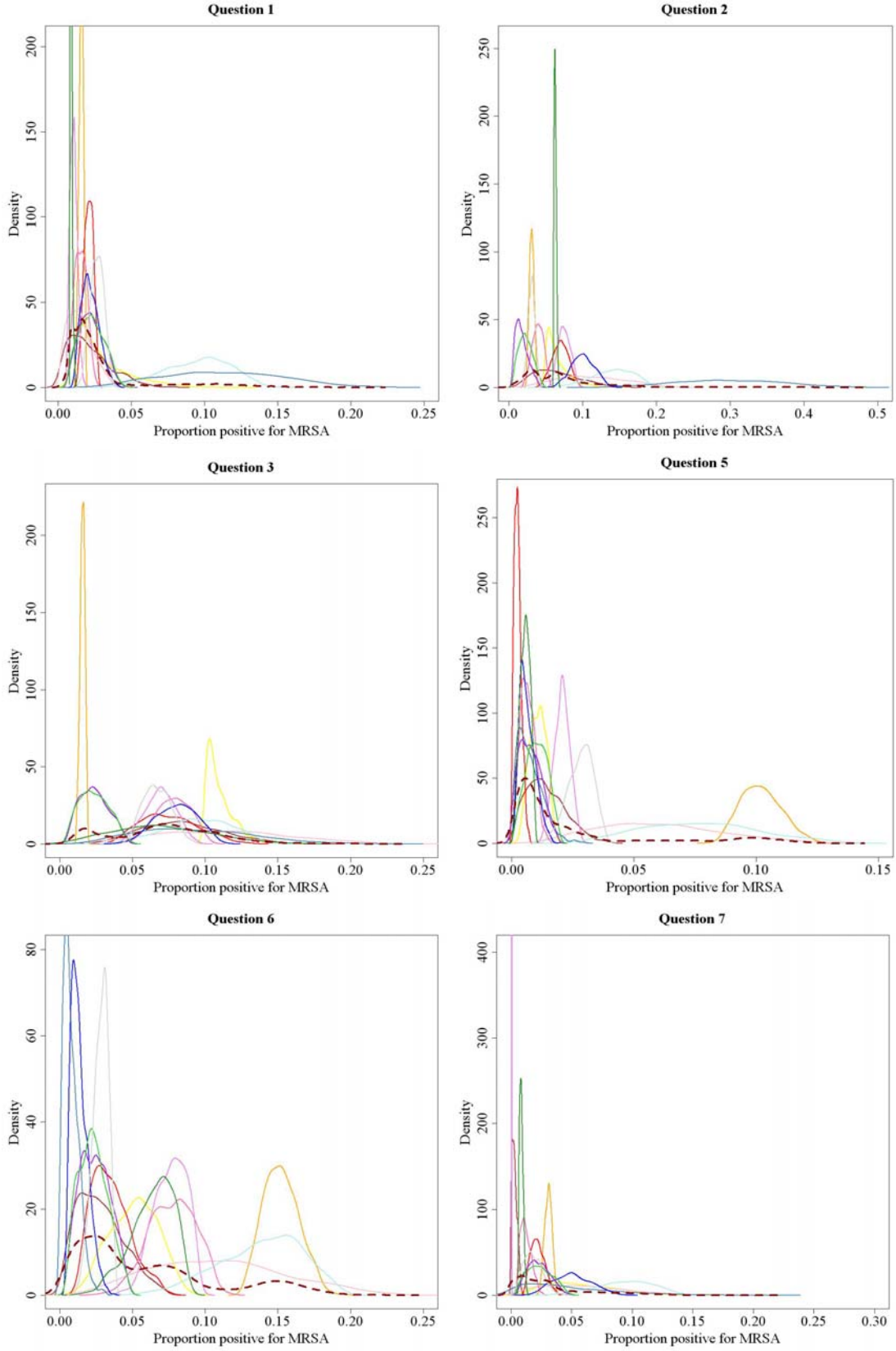
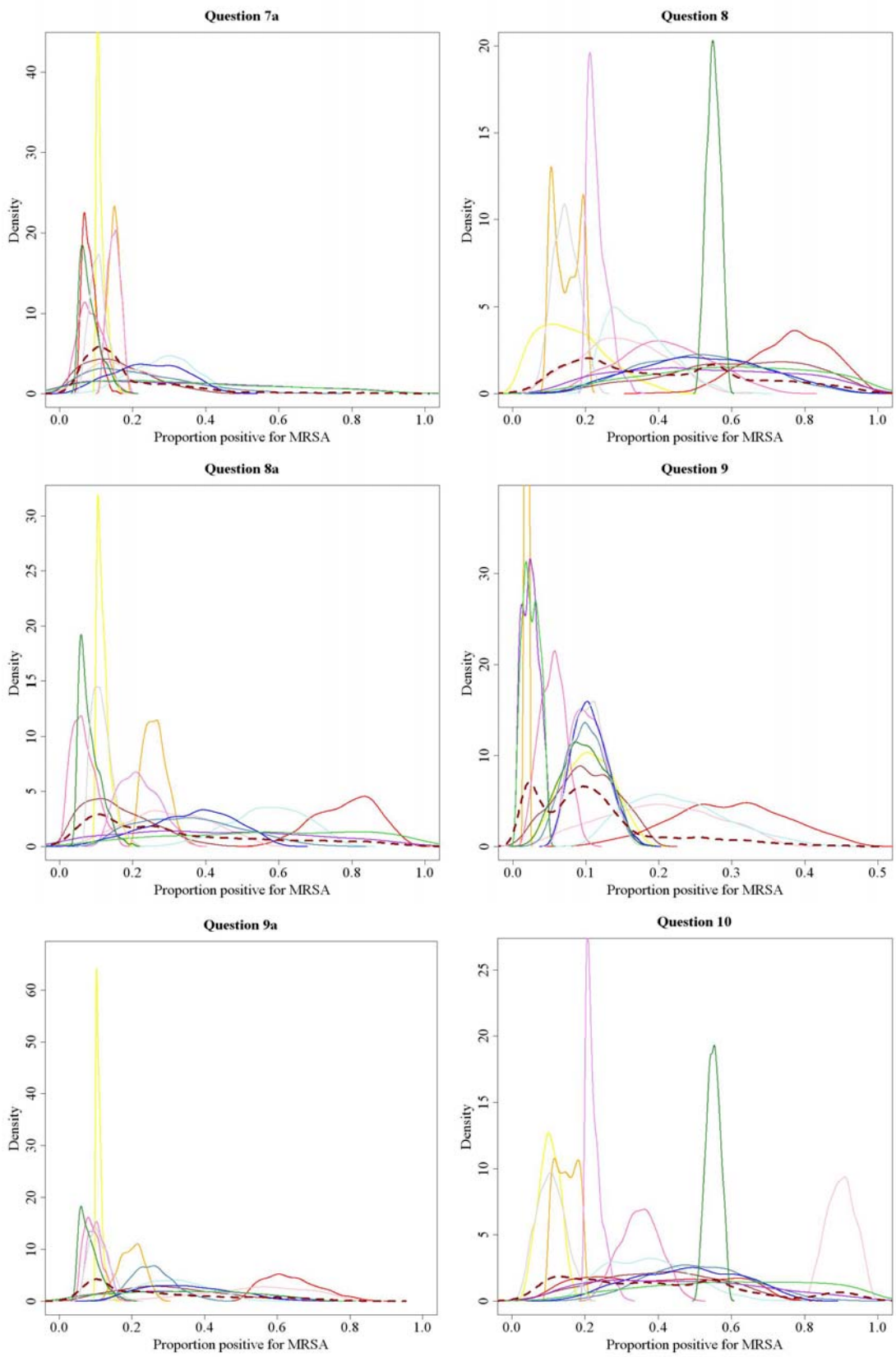
A B C D E F G H I J K L M N O
Expert
Food safety
Level of specialisation
0
2
4
6
8
10
A B C D E F G H I J K L M N O
Expert
Communicable disease control
Level of specialisation
0
2
4
6
8
10
A B C D E F G H I J K L M N O
Expert

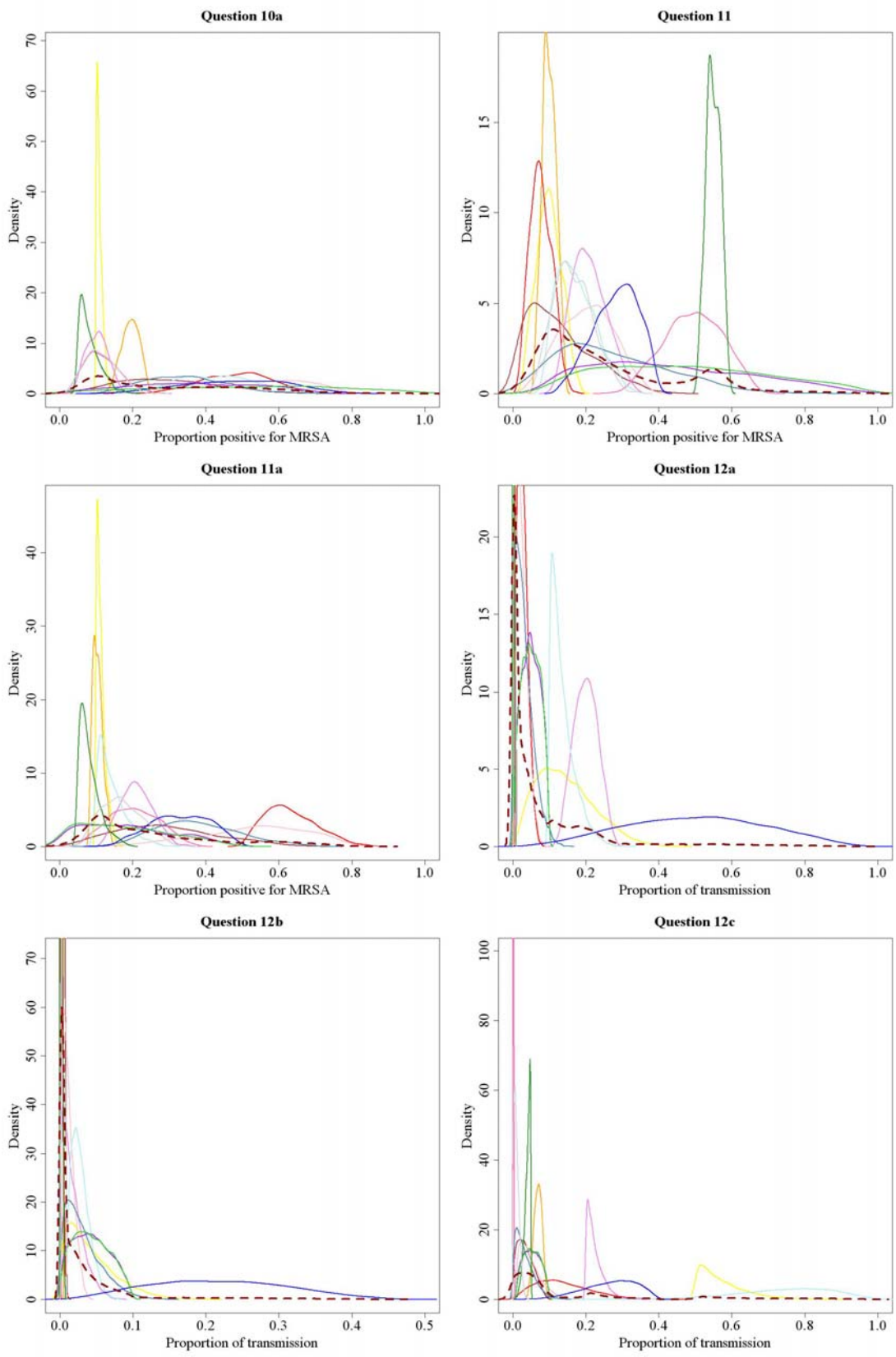
APPENDIX 7

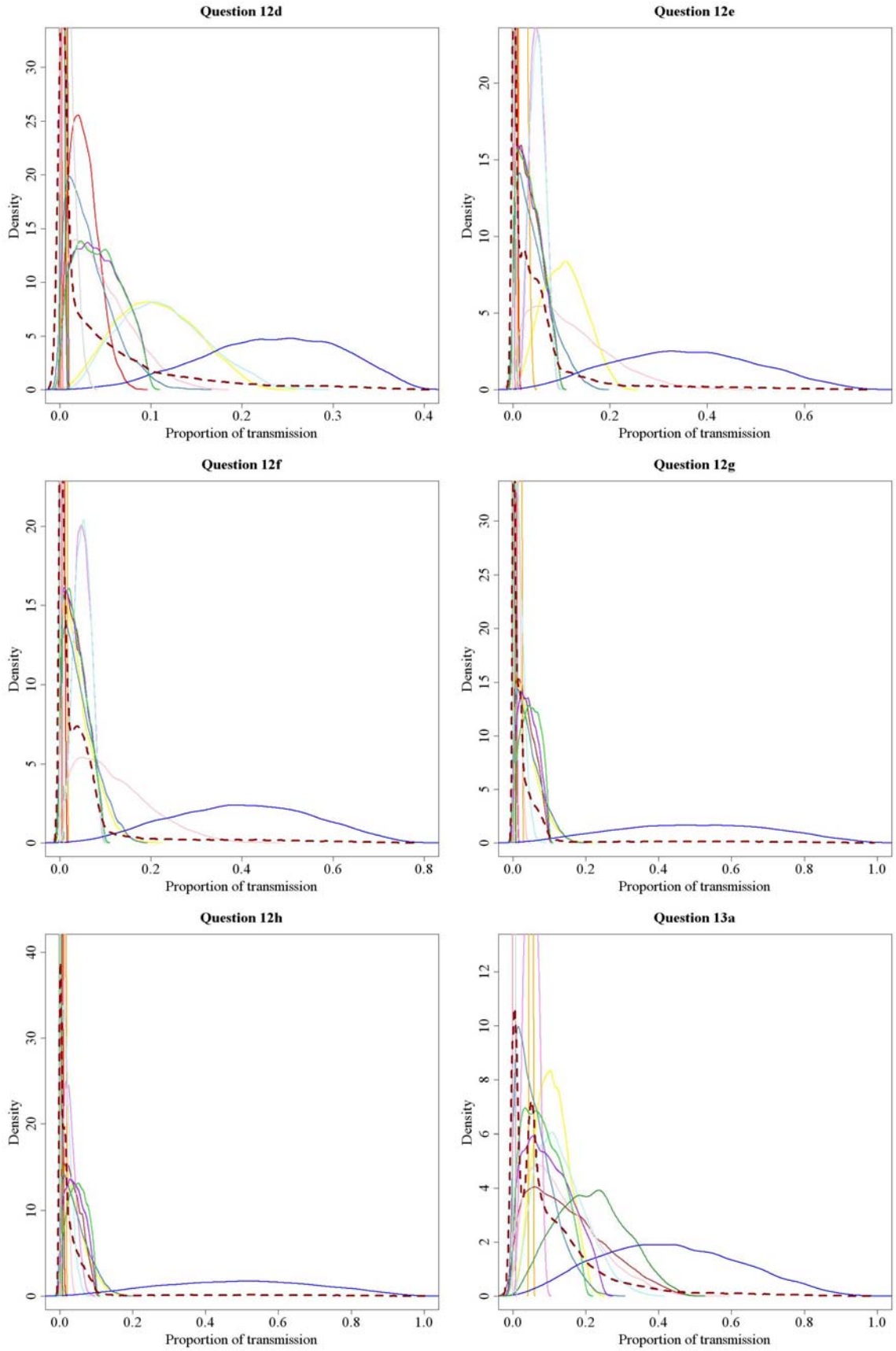
Expert uncertainty distributions

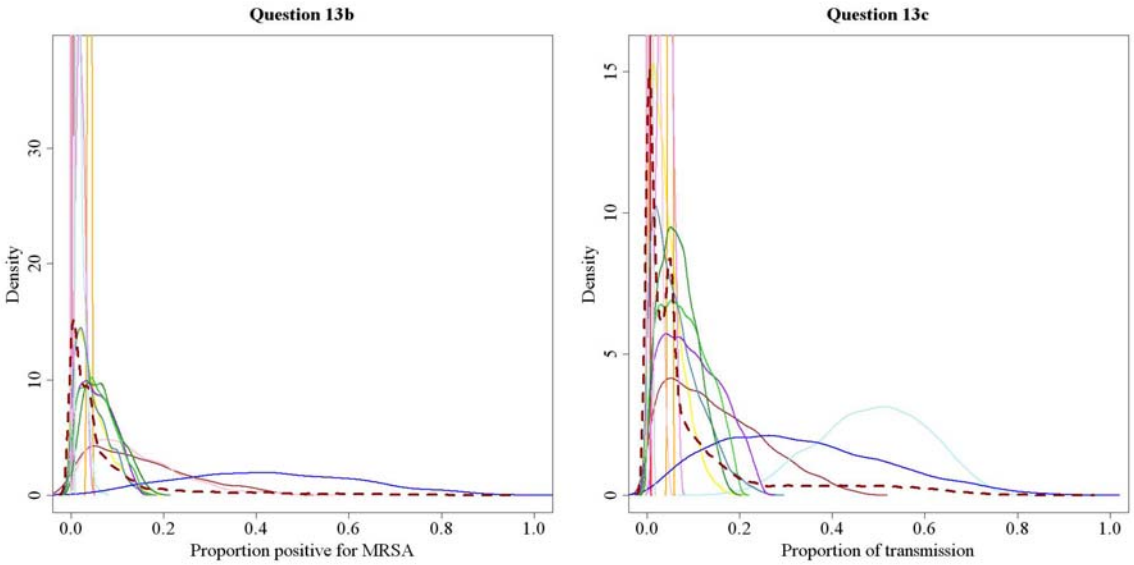
Red dashed lines represent combined (composite) expert distributions:



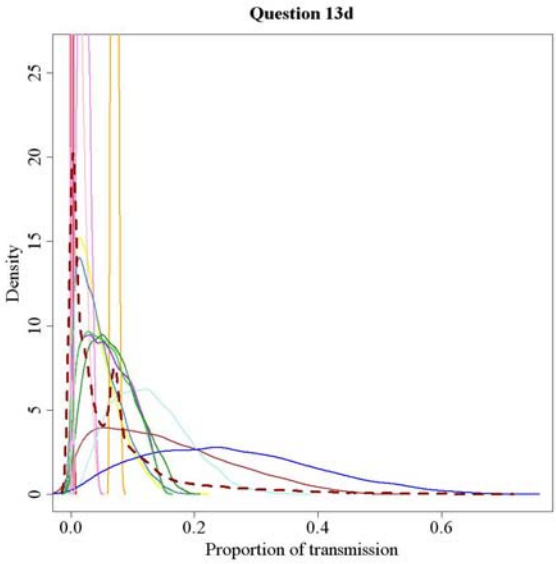

Question 7a
Density
Proportion positive for MRSA
Question 8
Density
Proportion positive for MRSA
Question 8a
Density
Proportion positive for MRSA
Question 9
Density
Proportion positive for MRSA
Question 9a
Density
Proportion positive for MRSA
Question 10
Density
Proportion positive for MRSA

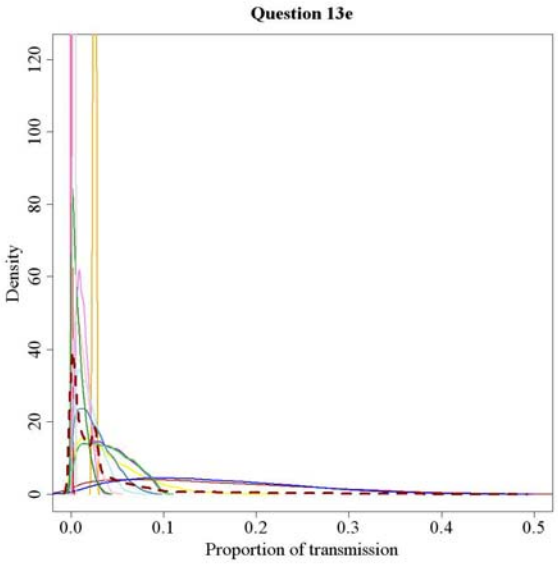

Question 10a
Density
Proportion positive for MRSA
Question 11
Density
Proportion positive for MRSA
Question 11a
Density
Proportion positive for MRSA
Question 12a
Density
Proportion of transmission
Question 12b
Density
Proportion of transmission
Question 12c
Density
Proportion of transmission

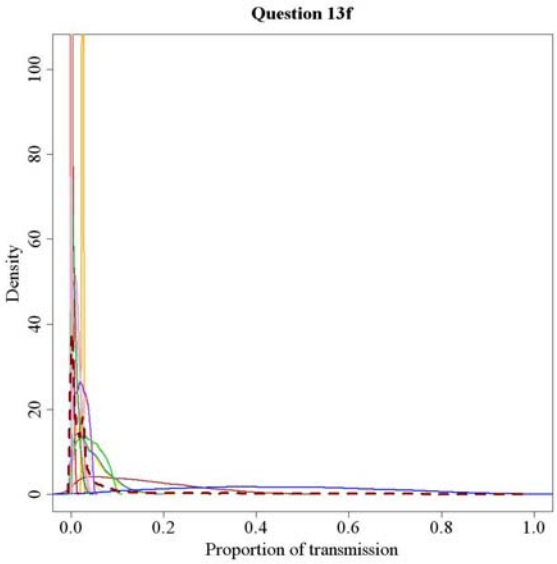

Question 12d
Density
Proportion of transmission
Question 12e
Density
Proportion of transmission
Question 12f
Density
Proportion of transmission
Question 12g
Density
Proportion of transmission
Question 12h
Density
Proportion of transmission
Question 13a
Density
Proportion of transmission

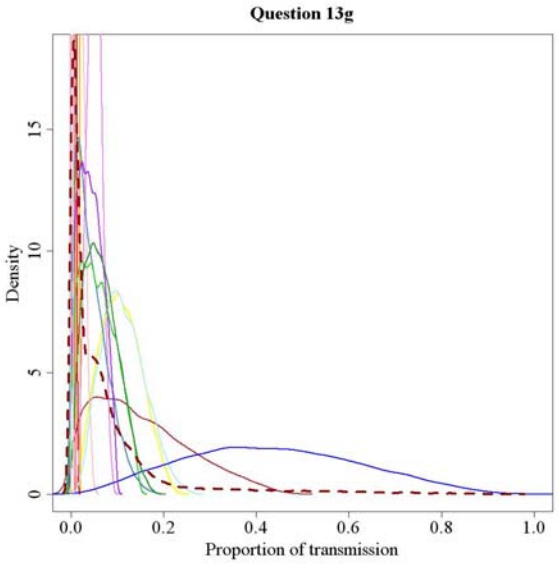

Question 13b
Density
Proportion positive for MRSA

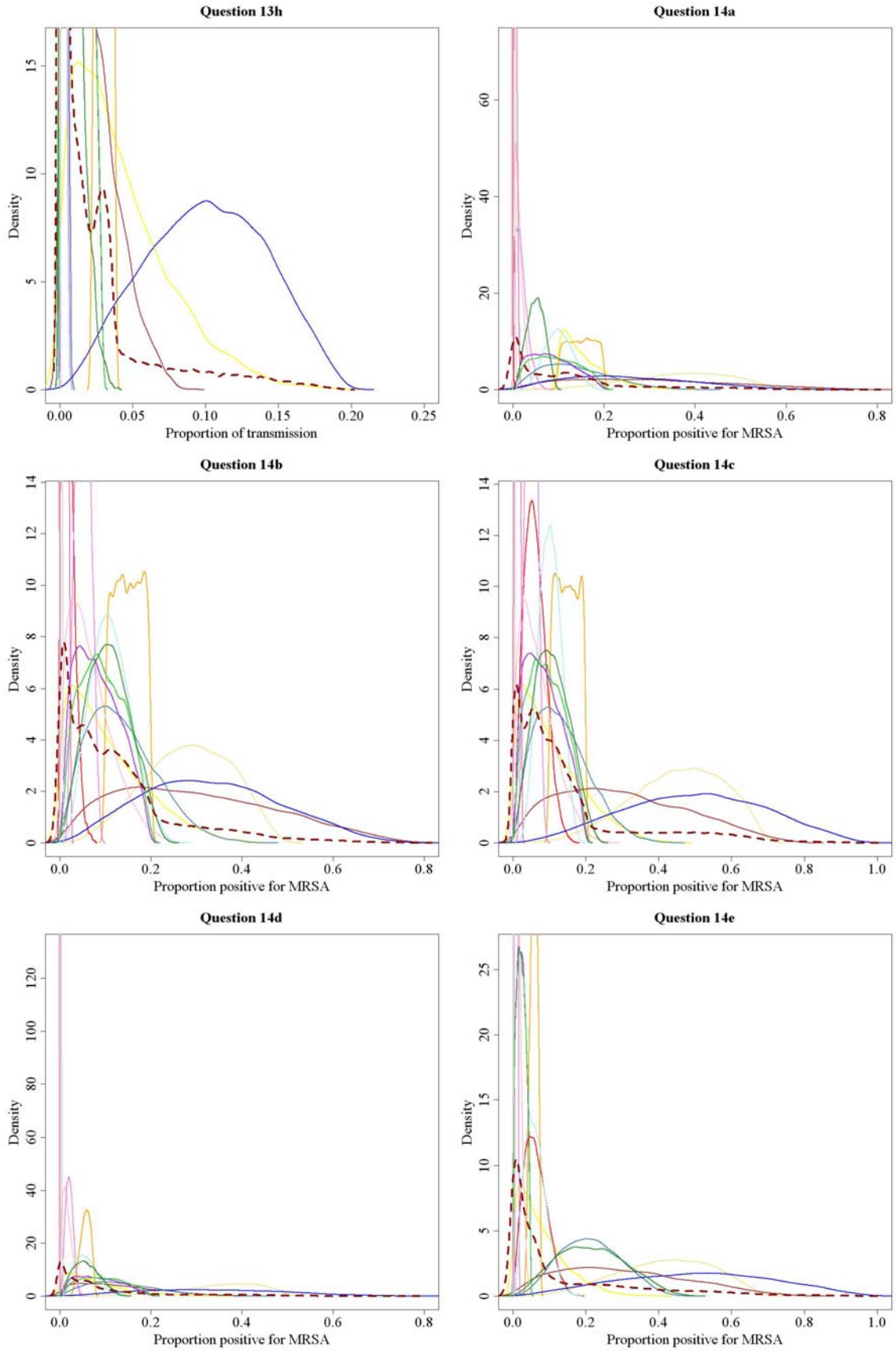
Question 13c
Density
Proportion of transmission

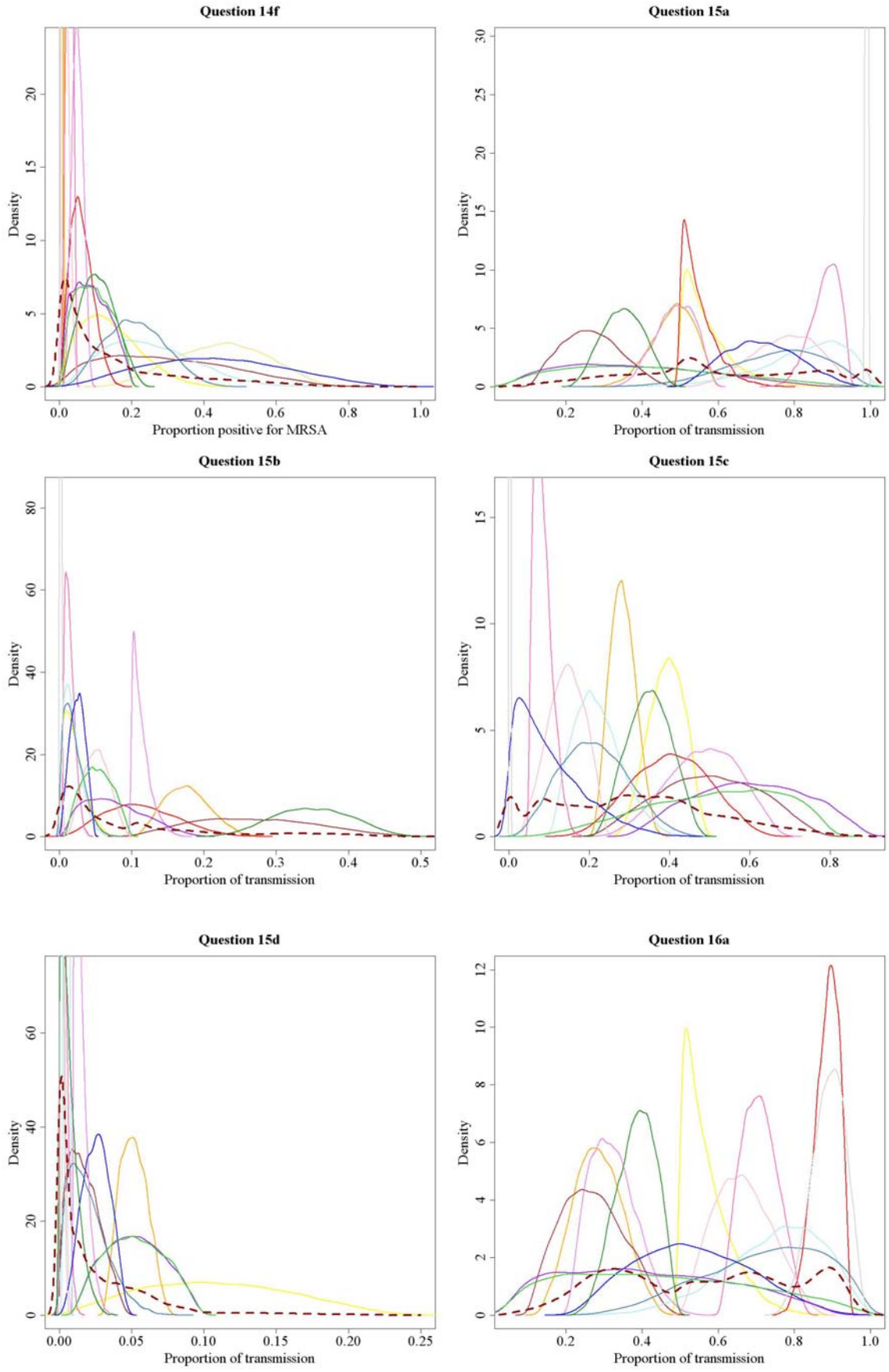

Question 13d
Density
Proportion of transmission

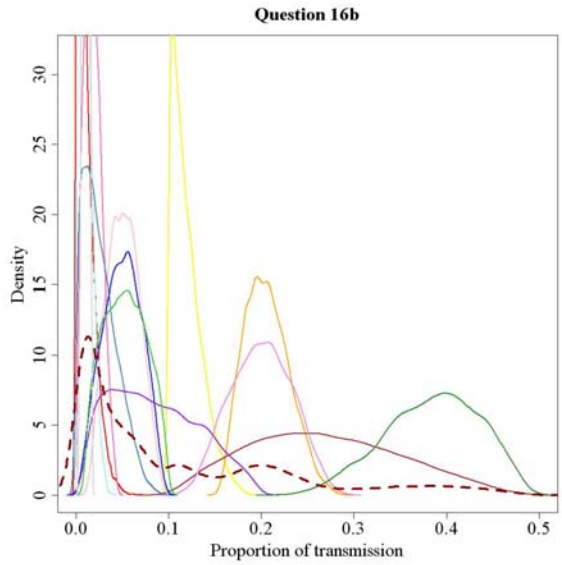

Question 13e
Density
Proportion of transmission

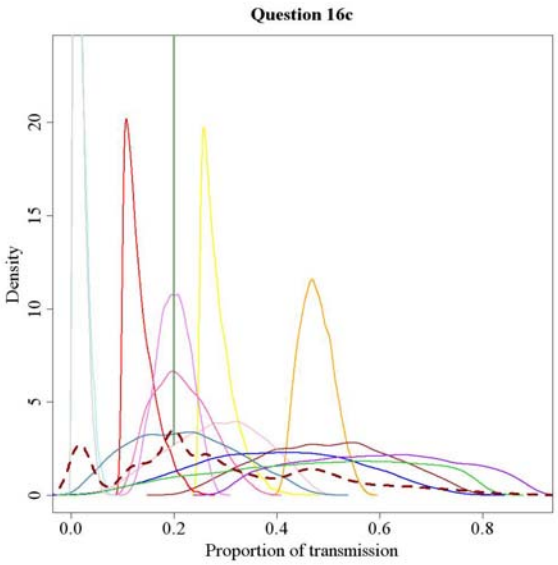

Question 13f
Density
Proportion of transmission

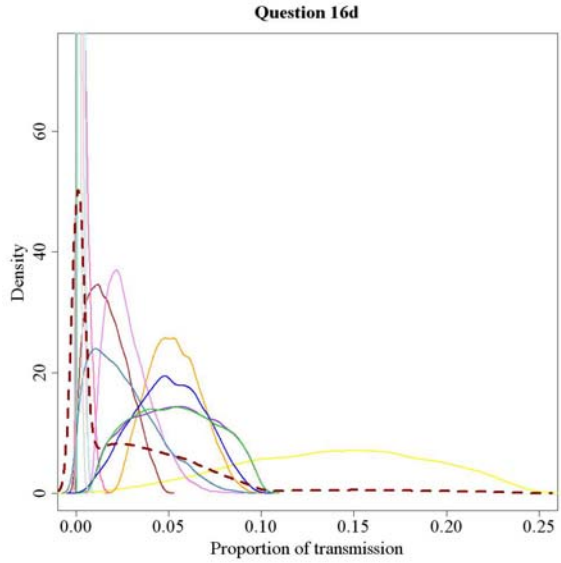

Question 13g
Density
Proportion of transmission


Question 13h
Density
Proportion of transmission
Question 14a
Density
Proportion positive for MRSA
Question 14b
Density
Proportion positive for MRSA
Question 14c
Density
Proportion positive for MRSA
Question 14d
Density
Proportion positive for MRSA
Question 14e
Density
Proportion positive for MRSA


Question 14f
Density
Proportion positive for MRSA
Question 15a
Density
Proportion of transmission
Question 15b
Density
Proportion of transmission
Question 15c
Density
Proportion of transmission
Question 15d
Density
Proportion of transmission
Question 16a
Density
Proportion of transmission


Question 16b
Density
0
5
10
15
20
25
30
0.0
0.1
0.2
0.3
0.4
0.5
Proportion of transmission


Question 16c
Density
0
5
10
15
20
0.0
0.2
0.4
0.6
0.8
Proportion of transmission


Question 16d
Density
0
20
40
60
0.00
0.05
0.10
0.15
0.20
0.25
Proportion of transmission

APPENDIX 8

R code for simulation model

```
library(epicalc)
library(MCMCpack)
library(odesolve)
library(survival)
library(coda)
library(RWinEdt)
library(sensitivity)
library(sm)
library(stats)
library(MASS)
library(colorspace)
library(vcd)
library(RGtk2)
library(rggobi)
library(lhs)

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

r.repeats <- 50
n.dogs <- 200
iterations <- 1000
expert.iterations <- 5000

#############################################
#FOR DISTRIBUTIONS FOR ALL EXPERT OPINION QUESTIONS FOR INPUT TO THE MODEL...

qnum <- read.csv("H://My Documents//Work//PhD//Expert opinion//question.numbers.csv")
qnum <- qnum[,1]

n.experts <- 15
n.sample <- expert.iterations
combination.data <- matrix(nrow=n.sample, ncol=length(qnum))

for(i in 1:length(qnum)){
filename <- paste("H://My Documents//Work//PhD//Expert opinion//question",qnum[i],".csv",sep="")
data <- read.csv(filename)
opinion <- numeric(n.experts*n.sample)
dim(opinion) <- c(n.experts,n.sample)
for(expert in (1:n.experts)){
        if(i>37){
        opinion[expert,] <-
        rmodbetapert(n.sample,data[expert,2]/100,data[expert,3]/100,data[expert,4]/100,data[expert,5])}
        else{
        opinion[expert,] <-
        rmodbetapert(n.sample,data[expert,2]/1000,data[expert,3]/1000,data[expert,4]/1000,data[expert,5])
        }
}
combination.data[,i] <- sample(opinion,n.sample,replace=TRUE, prob=rep(c(0,1,1,1,1,1,1,1,1,1,1,1,1,1,1),n.sample))
}

###################################MODEL BELOW HERE:

simulation <- function(n.dogs,iterations){

##uncertainty
#iterations = number of uncertainty samples


  p.dog.goes.to.vet.1yr <- numeric(iterations)
  p.dog.goes.to.vet.24h <- numeric(iterations)
  p.owner.is.HCW <- numeric(iterations)
  p.owner.is.vet <- numeric(iterations)
  p.owner.is.other.risk <- numeric(iterations)
  p.owner.no.risk <- numeric(iterations)
  p.col.HCW <- numeric(iterations)
  p.col.vet <- numeric(iterations)
  p.col.oth.risk <- numeric(iterations)
```

```
  p.col.gen.pop <- numeric(iterations)
  max.in.family <- 7
  dirichlet.1.mat <- matrix(0,ncol=max.in.family,nrow=iterations)
  p.exposure.to.other.dogs <- numeric(iterations)
  p.house.contam.family.col <- numeric(iterations)
  p.house.contam.family.not.col <- numeric(iterations)
  p.environ.contam <- numeric(iterations)
  p.community.dog.colonised.1 <- numeric(iterations)
  p.community.dog.colonised.2 <- numeric(iterations)
  p.community.dog.colonised.3 <- numeric(iterations)
  p.community.dog.colonised <- numeric(iterations)
  p.vet.dog.col.1 <- numeric(iterations)
  p.vet.dog.col.2 <- numeric(iterations)
  p.vet.dog.col.3 <- numeric(iterations)
  p.vet.dog.col.4 <- numeric(iterations)
  p.vet.dog.col.sample <- numeric(iterations)
  p.vet.dog.colonised <- numeric(iterations)
  p.vet.environ.contam.1 <- numeric(iterations)
  p.vet.environ.contam.2 <- numeric(iterations)
  p.vet.environ.contam <- numeric(iterations)
  p.vet.col.1 <- numeric(iterations)
  p.vet.col.2 <- numeric(iterations)
  p.vet.col.3 <- numeric(iterations)
  p.vet.col.4 <- numeric(iterations)
  p.transmission.owner.per.contact <- numeric(iterations)
  p.transmission.non.owner.per.contact <- numeric(iterations)
  p.transmission.community.dog.per.contact <- numeric(iterations)
  p.transmission.community.environ.house.per.contact <- numeric(iterations)
  p.transmission.vet.dog.per.contact <- numeric(iterations)
  p.transmission.vet.environ.per.contact <- numeric(iterations)
  p.transmission.vet.per.contact <- numeric(iterations)

##data

s.dog.to.vet <- 234
n.dog.to.vet <- 279
s.hcw <- 89
n.general.pop <- 2118
s.vet <- 15671
n.general.pop.1 <- 37804500
min.other.risk <- 0.01
ml.other.risk <- 0.02
max.other.risk <- 0.05
min.O.oth.risk <- 0.015
ml.O.oth.risk <- 0.05
max.O.oth.risk <- 0.15
s.gen.pop <- 4
n.gen.pop <- 274

s.exposure.to.other.dogs <- 246
n.exposure.to.other.dogs <- 272
s.community.dog.colonised.1 <- 1
n.community.dog.colonised.1 <- 255
s.community.dog.colonised.2 <- 0
n.community.dog.colonised.2 <- 188
s.community.dog.colonised.3 <- 2
n.community.dog.colonised.3 <- 203
s.vet.dog.col.1 <- 4
n.vet.dog.col.1 <- 200
s.vet.dog.col.2 <- 3
n.vet.dog.col.2 <- 287
s.vet.dog.col.3 <- 7
n.vet.dog.col.3 <- 230
s.vet.dog.col.4 <- 4
n.vet.dog.col.4 <- 45
s.vet.environ.contam.1 <- 3
n.vet.environ.contam.1 <- 27
```

```
s.vet.environ.contam.2 <- 2
n.vet.environ.contam.2 <- 158
s.vet.col.1 <- 12
n.vet.col.1 <- 271
s.vet.col.2 <- 5
n.vet.col.2 <- 51
s.vet.col.3 <- 14
n.vet.col.3 <- 78
s.vet.col.4 <- 2
n.vet.col.4 <- 64

##for variability

  does.dog.go.to.vet <- array(0,c(n.dogs,iterations))
  owner.category <- array(0,c(n.dogs,iterations))
  owner.colonised <- array(0,c(n.dogs,iterations))
  others.in.family <-array(0,c(n.dogs,iterations))
  family.members <- array(0,c(n.dogs,iterations,max.in.family))
  family.members.colonised <- array(0,c(n.dogs,iterations,max.in.family))
  temp.family.members.colonised <- numeric(length=max.in.family*n.dogs)
  n.family.non.owner.colonised <- array(0,c(n.dogs,iterations))
  others.not.in.family <- array(0,c(n.dogs,iterations))
  max.others.not.in.family <- 20
  non.family.members <- array(0,c(n.dogs,iterations,max.others.not.in.family+1))
  temp.non.family.members.colonised <- numeric(length=(max.others.not.in.family+1)*n.dogs)
  non.family.members.colonised <- array(0,c(n.dogs,iterations,(max.others.not.in.family+1)))
  n.non.family.members.colonised <- array(0,c(n.dogs,iterations))
  total.humans.colonised <- array(0,c(n.dogs,iterations))
  exposure.to.other.dogs <- array(0,c(n.dogs,iterations))
  dog.contacts <- array(0,c(n.dogs,iterations))
  n.colonised.community.dogs <- array(0,c(n.dogs,iterations))
  house.contam <-  array(0,c(n.dogs,iterations))
  outside.contam <-  array(0,c(n.dogs,iterations))
  environ.contam <-  array(0,c(n.dogs,iterations))
  no.environ.sites <- array(0,c(n.dogs,iterations))
  no.contam.environ.sites <- array(0,c(n.dogs,iterations))
  no.dogs.contacted.at.vet <-  array(0,c(n.dogs,iterations))
  no.col.dogs.contacted.at.vet <- array(0,c(n.dogs,iterations))
  p.vet.environ.contam <- numeric(iterations)
  no.vet.environ.sites <- array(0,c(n.dogs,iterations))
  no.contam.vet.environ.sites <- array(0,c(n.dogs,iterations))
  no.vets <- array(0,c(n.dogs,iterations))
  which.vet.p <- numeric(iterations)
  no.col.vet <- array(0,c(n.dogs,iterations))
  transmission.col.O <- array(0,c(n.dogs,iterations,r.repeats))
  transmission.col.non.O.fam <- array(0,c(n.dogs,iterations,r.repeats))
  transmission.col.non.O <- array(0,c(n.dogs,iterations,r.repeats))
  transmission.col.community.dog <- array(0,c(n.dogs,iterations,r.repeats))
  transmission.col.house.environ <- array(0,c(n.dogs,iterations,r.repeats))
  transmission.col.vet.dog <- array(0,c(n.dogs,iterations,r.repeats))
  transmission.col.vet.environ <- array(0,c(n.dogs,iterations,r.repeats))
  transmission.col.vet <- array(0,c(n.dogs,iterations,r.repeats))
  n.sources <- 9
  MRSA.positive <- array(0,c(n.dogs,iterations,r.repeats))
  MRSA.positive.1 <- array(0,c(n.dogs,iterations,r.repeats))

##data

human.risk.category <- c("hcw","vet","other.risk","non.risk")
min.non.fam.exposure <- 0
ml.non.fam.exposure <- 4
max.non.fam.exposure <- 15
min.dog.exposure <- 1
ml.dog.exposure <- 2
max.dog.exposure <- 15
which.environ.estimate <- c(0.1,1)
min.environ.site.exposure <- 1
```

```
ml.environ.site.exposure <- 20
max.environ.site.exposure <- 200
min.vet.dog.exposure <- 0
ml.vet.dog.exposure <- 1
max.vet.dog.exposure <- 10
min.vet.environ.site.exposure <- 1
ml.vet.environ.site.exposure <- 10
max.vet.environ.site.exposure <- 100
min.vet.exposure <- 1
ml.vet.exposure <- 2
max.vet.exposure <-5

#contact data per 24h - contact between at-risk-dog and source:
min.O.contacts <- 0
ml.O.contacts <- 5
max.O.contacts <- 20
min.non.O.contacts <- 0
ml.non.O.contacts <- 1
max.non.O.contacts <- 10
min.dog.contacts <- 0
ml.dog.contacts <- 1
max.dog.contacts <- 10
min.environ.contacts <- 0
ml.environ.contacts <- 1
max.environ.contacts <- 100
min.vet.dog.contacts <- 0
ml.vet.dog.contacts <- 1
max.vet.dog.contacts <- 10
min.vet.environ.contacts <- 1
ml.vet.environ.contacts <-10
max.vet.environ.contacts <- 25

min.vet.contacts <- 0
ml.vet.contacts <- 3
max.vet.contacts <- 20
```

```
##uncertainty and variabiliy for contact information between at-risk-dog and source:

O.contacts.per.24h <- matrix(round(rbetapert(iterations*n.dogs,min.O.contacts,ml.O.contacts,max.O.contacts)), nrow=n.dogs, ncol=iterations, byrow=TRUE)
non.O.contacts.per.24h <- matrix(round(rbetapert(iterations*n.dogs,min.non.O.contacts,ml.non.O.contacts,max.non.O.contacts)),nrow=n.dogs, ncol=iterations, byrow=TRUE)
dog.contacts.per.24h <- matrix(round(rbetapert(iterations*n.dogs,min.dog.contacts,ml.dog.contacts,max.dog.contacts)),nrow=n.dogs, ncol=iterations, byrow=TRUE)
environ.contacts.per.24h <- matrix(round(rbetapert(iterations*n.dogs,min.environ.contacts,ml.environ.contacts,max.environ.contacts)),nrow=n.dogs, ncol=iterations, byrow=TRUE)
vet.dog.contacts.per.24h <- matrix(round(rbetapert(iterations*n.dogs,min.vet.dog.contacts,ml.vet.dog.contacts,max.vet.dog.contacts)),nrow=n.dogs, ncol=iterations, byrow=TRUE)
vet.environ.contacts.per.24h <- matrix(round(rbetapert(iterations*n.dogs,min.vet.environ.contacts,ml.vet.environ.contacts,max.vet.environ.contacts)),nrow=n.dogs, ncol=iterations, byrow=TRUE)
vet.contacts.per.24h <- matrix(round(rbetapert(iterations*n.dogs,min.vet.contacts,ml.vet.contacts,max.vet.contacts)),nrow=n.dogs, ncol=iterations, byrow=TRUE)

##########  MODEL:

lhs.1 <- randomLHS(iterations,1)                ##for latin hypercube sampling

p.dog.goes.to.vet.1yr <- rbeta(iterations,s.dog.to.vet+1,n.dog.to.vet-s.dog.to.vet+1)
p.dog.goes.to.vet.24h <- 1-(1-p.dog.goes.to.vet.1yr)^(1/365)

#p.dog.goes.to.vet.1yr <- rep(1,iterations)    #THESE FOR RUNNING L-R MODEL FOR ALL DOGS ATTENDING VET ONLY
#p.dog.goes.to.vet.24h <- rep(1,iterations)

p.owner.is.HCW <- rbeta(iterations,s.hcw+1,n.general.pop-s.hcw+1)
p.owner.is.vet <- rbeta(iterations,s.vet+1, n.general.pop.1-s.vet+1)
p.owner.is.other.risk <- rbetapert(iterations,min.other.risk,ml.other.risk,max.other.risk)
p.owner.no.risk <- 1-(p.owner.is.HCW + p.owner.is.vet + p.owner.is.other.risk)

p.col.HCW <- quantile(combination.data[,2],lhs.1)
names(p.col.HCW) <- NULL
p.col.vet <- quantile(combination.data[,3],lhs.1)   #n.b. this is for ALL vets (large and small animal) NOT for estimates whiile at small animal vet clinic
names(p.col.vet) <- NULL
p.col.oth.risk <- rbetapert(iterations,min.O.oth.risk,ml.O.oth.risk,max.O.oth.risk)
p.col.gen.pop <- rbeta(iterations,s.gen.pop+1,n.gen.pop-s.gen.pop+1)

###to model dependency between owner status and status of other family members:
min.p.col.contact.col.O <- 0.02
ml.p.col.contact.col.O <- 0.1
max.p.col.contact.col.O <- 0.5
p.col.contact.col.O <- rbetapert(iterations, min.p.col.contact.col.O, ml.p.col.contact.col.O, max.p.col.contact.col.O)    ## this is P(household member is colonised given that they have contact with an
owner that is colonised)
```

```
dirichlet.1.mat <-  rdirichlet(iterations,c(39,86,51,57,27,4,3))  # data from excel sheet 1
p.exposure.to.other.dogs <- rbeta(iterations,s.exposure.to.other.dogs+1,n.exposure.to.other.dogs-s.exposure.to.other.dogs+1)

p.community.dog.colonised.1 <- rbeta(iterations,s.community.dog.colonised.1+1,n.community.dog.colonised.1-s.community.dog.colonised.1+1)
p.community.dog.colonised.2 <- rbeta(iterations,s.community.dog.colonised.2+1,n.community.dog.colonised.2-s.community.dog.colonised.2+1)
p.community.dog.colonised.3 <- rbeta(iterations,s.community.dog.colonised.3+1,n.community.dog.colonised.3-s.community.dog.colonised.3+1)
p.community.dog.colonised <- sample(c(p.community.dog.colonised.1,p.community.dog.colonised.2,p.community.dog.colonised.3),iterations,replace=TRUE)

p.house.contam.family.col <- quantile(combination.data[,8],lhs.1)
names(p.house.contam.family.col) <- NULL
p.house.contam.family.not.col <- quantile(combination.data[,6],lhs.1)
names(p.house.contam.family.not.col) <- NULL

p.vet.dog.col.1 <- rbeta(iterations, s.vet.dog.col.1+1, n.vet.dog.col.1-s.vet.dog.col.1+1)
p.vet.dog.col.2 <- rbeta(iterations, s.vet.dog.col.2+1, n.vet.dog.col.2-s.vet.dog.col.2+1)
p.vet.dog.col.3 <- rbeta(iterations, s.vet.dog.col.3+1, n.vet.dog.col.3-s.vet.dog.col.3+1)
p.vet.dog.col.4 <- rbeta(iterations, s.vet.dog.col.4+1, n.vet.dog.col.4-s.vet.dog.col.4+1)
p.vet.dog.colonised <- sample(c(p.vet.dog.col.1,p.vet.dog.col.2,p.vet.dog.col.3,p.vet.dog.col.4),iterations,replace=TRUE)

p.vet.environ.contam.1 <- rbeta(iterations, s.vet.environ.contam.1+1,n.vet.environ.contam.1-s.vet.environ.contam.1+1)
p.vet.environ.contam.2 <- rbeta(iterations, s.vet.environ.contam.2+1,n.vet.environ.contam.2-s.vet.environ.contam.2+1)

p.vet.col.1 <- rbeta(iterations, s.vet.col.1+1,n.vet.col.1-s.vet.col.1+1)
p.vet.col.2 <- rbeta(iterations, s.vet.col.2+1,n.vet.col.2-s.vet.col.2+1)
p.vet.col.3 <- rbeta(iterations, s.vet.col.3+1,n.vet.col.3-s.vet.col.3+1)
p.vet.col.4 <- rbeta(iterations, s.vet.col.4+1,n.vet.col.4-s.vet.col.4+1)
p.vet.col <- sample(c(p.vet.col.1,p.vet.col.2,p.vet.col.3,p.vet.col.4),iterations,replace=TRUE, c(rep(1,iterations),rep(1,iterations),rep(0.1,iterations),rep(1,iterations)))

p.transmission.owner.per.contact.1 <- quantile(combination.data[,16],lhs.1)              #these transmission parameters estimated PER AVERAGE CONTACT
names(p.transmission.owner.per.contact.1) <- NULL
p.transmission.owner.per.contact <- matrix(rep(p.transmission.owner.per.contact.1,n.dogs),nrow=n.dogs, ncol=iterations,byrow=TRUE)
p.transmission.owner.24h <- 1-((1-p.transmission.owner.per.contact)^O.contacts.per.24h)

p.transmission.non.owner.per.contact.1 <- quantile(combination.data[,17],lhs.1)
names(p.transmission.non.owner.per.contact.1) <- NULL
p.transmission.non.owner.per.contact <- matrix(rep(p.transmission.non.owner.per.contact.1,n.dogs),nrow=n.dogs, ncol=iterations,byrow=TRUE)
p.transmission.non.owner.24h <- 1-((1-p.transmission.non.owner.per.contact)^non.O.contacts.per.24h)
```

```
p.transmission.community.dog.per.contact.1 <- quantile(combination.data[,19],lhs.1)
names(p.transmission.community.dog.per.contact.1) <- NULL
p.transmission.community.dog.per.contact <- matrix(rep(p.transmission.community.dog.per.contact.1,n.dogs),nrow=n.dogs, ncol=iterations,byrow=TRUE)
p.transmission.community.dog.24h <- 1-((1-p.transmission.community.dog.per.contact)^dog.contacts.per.24h)
p.transmission.community.environ.house.per.contact.1 <- quantile(combination.data[,22],lhs.1)
names(p.transmission.community.environ.house.per.contact.1) <- NULL
p.transmission.community.environ.house.per.contact <- matrix(rep(p.transmission.community.environ.house.per.contact.1,n.dogs),nrow=n.dogs, ncol=iterations,byrow=TRUE)
p.transmission.community.environ.house.24h <- 1-((1-p.transmission.community.environ.house.per.contact)^environ.contacts.per.24h)

p.transmission.vet.dog.per.contact.1 <- quantile(combination.data[,18],lhs.1)
names(p.transmission.vet.dog.per.contact.1) <- NULL
p.transmission.vet.dog.per.contact <- matrix(rep(p.transmission.vet.dog.per.contact.1,n.dogs),nrow=n.dogs, ncol=iterations,byrow=TRUE)
p.transmission.vet.dog.24h <- 1-((1-p.transmission.vet.dog.per.contact)^vet.dog.contacts.per.24h)

p.transmission.vet.environ.per.contact.1 <- quantile(combination.data[,23],lhs.1)
names(p.transmission.vet.environ.per.contact.1) <- NULL
p.transmission.vet.environ.per.contact <- matrix(rep(p.transmission.vet.environ.per.contact.1,n.dogs),nrow=n.dogs, ncol=iterations,byrow=TRUE)
p.transmission.vet.environ.24h <- 1-((1-p.transmission.vet.environ.per.contact)^vet.environ.contacts.per.24h)

p.transmission.vet.per.contact.1 <- quantile(combination.data[,20],lhs.1)
names(p.transmission.vet.per.contact.1) <- NULL
p.transmission.vet.per.contact <- matrix(rep(p.transmission.vet.per.contact.1 ,n.dogs),nrow=n.dogs, ncol=iterations,byrow=TRUE)
p.transmission.vet.24h <- 1-((1-p.transmission.vet.per.contact)^vet.contacts.per.24h)


##### variability below here:

if(.Platform$OS.type == "windows"){
dyn.load("resetarray.dll")
}else{
if(Sys.info()$machine=="i386") dyn.load("resetarray_i386.so") else dyn.load("resetarray_ppc.so")
}


for(iter in 1:iterations){

temp.family.members.colonised[] <- NA   #to make sure that we know that the vector isn't resampled
temp.non.family.members.colonised[] <- NA   #as above
```

```
#veterinary attendance (y/n)

does.dog.go.to.vet[,iter] <- rbinom(n.dogs,1,p.dog.goes.to.vet.24h[iter]) #STEP 1 ***************

##COMMUNITY FACTORS:

#owner risk category
owner.category[,iter] <- sample(human.risk.category,n.dogs,replace=TRUE,c(p.owner.is.HCW[iter],p.owner.is.vet[iter],p.owner.is.other.risk[iter],p.owner.no.risk[iter]))

   #owner colonisation status (y/n)
   owner.colonised[owner.category[,iter]=="hcw",iter] <- rbinom(sum(owner.category[,iter]=="hcw"), 1, p.col.HCW[iter])
   owner.colonised[owner.category[,iter]=="vet",iter] <- rbinom(sum(owner.category[,iter]=="vet"), 1, p.col.vet[iter])
   owner.colonised[owner.category[,iter]=="other.risk",iter] <- rbinom(sum(owner.category[,iter]=="other.risk"), 1, p.col.oth.risk[iter])
   owner.colonised[owner.category[,iter]=="non.risk",iter] <- rbinom(sum(owner.category[,iter]=="non.risk"), 1, p.col.gen.pop[iter])

#number of others in the family
others.in.family[,iter] <- sample(c(0,1,2,3,4,5,6),n.dogs,replace=TRUE,c(dirichlet.1.mat[iter,]))

#risk category of others in the family
family.members[,iter,] <- sample(human.risk.category, length(family.members[,iter,]),replace=TRUE,c(p.owner.is.HCW[iter],p.owner.is.vet[iter],p.owner.is.other.risk[iter],p.owner.no.risk[iter]))

##below models a dependency of colonisation on colonisation status of owner (owner acts as proxy for single MRSA positive person in family)
#colonisation status of others in family - needs correlation with owner status
temp.family.members.colonised[family.members[,iter,]=="hcw" & owner.colonised[,iter]==TRUE] <- rbinom(sum(family.members[,iter,]=="hcw" & owner.colonised[,iter]==TRUE),1,(1-((1-
p.col.HCW[iter])*(1-p.col.contact.col.O))))
temp.family.members.colonised[family.members[,iter,]=="hcw" & owner.colonised[,iter]==FALSE] <- rbinom(sum(family.members[,iter,]=="hcw" &
owner.colonised[,iter]==FALSE),1,p.col.HCW[iter])

temp.family.members.colonised[family.members[,iter,]=="vet"& owner.colonised[,iter]==TRUE] <- rbinom(sum(family.members[,iter,]=="vet" & owner.colonised[,iter]==TRUE),1,(1-((1-
p.col.vet[iter])*(1-p.col.contact.col.O))))
temp.family.members.colonised[family.members[,iter,]=="vet"& owner.colonised[,iter]==FALSE] <- rbinom(sum(family.members[,iter,]=="vet" & owner.colonised[,iter]==FALSE),1,p.col.vet[iter])

temp.family.members.colonised[family.members[,iter,]=="other.risk" & owner.colonised[,iter]==TRUE] <- rbinom(sum(family.members[,iter,]=="other.risk" & owner.colonised[,iter]==TRUE),1,(1-
((1-p.col.oth.risk[iter])*(1-p.col.contact.col.O))))
temp.family.members.colonised[family.members[,iter,]=="other.risk" & owner.colonised[,iter]==FALSE] <- rbinom(sum(family.members[,iter,]=="other.risk" &
owner.colonised[,iter]==FALSE),1,p.col.oth.risk[iter])

temp.family.members.colonised[family.members[,iter,]=="non.risk" & owner.colonised[,iter]==TRUE] <- rbinom(sum(family.members[,iter,]=="non.risk" & owner.colonised[,iter]==TRUE),1,(1-((1-
p.col.gen.pop[iter])*(1-p.col.contact.col.O))))
```

```
temp.family.members.colonised[family.members[,iter,]=="non.risk" & owner.colonised[,iter]==FALSE] <- rbinom(sum(family.members[,iter,]=="non.risk" &
owner.colonised[,iter]==FALSE),1,p.col.gen.pop[iter])

family.members.colonised[,iter,] <- temp.family.members.colonised

family.members.colonised[,iter,1] <- 0

resetarrayoutput <- .C('resetarray', data=matrix(as.integer(family.members.colonised[,iter,])), keep=as.integer(others.in.family[,iter]), ncol=as.integer(ncol(family.members.colonised[,iter,])),
nrow=as.integer(nrow(family.members.colonised[,iter,])), replace=as.integer(0))

family.members.colonised[,iter,] <- matrix(resetarrayoutput$data, ncol=ncol(resetarrayoutput$data))

   #number of colonised family members (non-owner) contacted per day
   n.family.non.owner.colonised <- apply(family.members.colonised,c(1,2),sum)

#number of others not in family contacted per day
others.not.in.family[,iter] <- round(rbetapert(n.dogs,min.non.fam.exposure,ml.non.fam.exposure,max.non.fam.exposure))

#risk category of those not in family
non.family.members[,iter,] <-
sample(human.risk.category,length(non.family.members[,iter,]),replace=TRUE,c(p.owner.is.HCW[iter],p.owner.is.vet[iter],p.owner.is.other.risk[iter],p.owner.no.risk[iter]))

temp.non.family.members.colonised[non.family.members[,iter,]=="hcw"] <- rbinom(sum(non.family.members[,iter,]=="hcw"),1,p.col.HCW[iter])
temp.non.family.members.colonised[non.family.members[,iter,]=="vet"] <- rbinom(sum(non.family.members[,iter,]=="vet"),1,p.col.vet[iter])
temp.non.family.members.colonised[non.family.members[,iter,]=="other.risk"] <- rbinom(sum(non.family.members[,iter,]=="other.risk"),1,p.col.oth.risk[iter])
temp.non.family.members.colonised[non.family.members[,iter,]=="non.risk"] <- rbinom(sum(non.family.members[,iter,]=="non.risk"),1,p.col.gen.pop[iter])

non.family.members.colonised[,iter,] <- temp.non.family.members.colonised

non.family.members.colonised[,iter,1] <- 0

resetarrayoutput <- .C('resetarray', data=matrix(as.integer(non.family.members.colonised[,iter,])), keep=as.integer(others.not.in.family[,iter]),
ncol=as.integer(ncol(non.family.members.colonised[,iter,])), nrow=as.integer(nrow(non.family.members.colonised[,iter,])), replace=as.integer(0))

non.family.members.colonised[,iter,] <- matrix(resetarrayoutput$data, ncol=ncol(resetarrayoutput$data))

##STEP Ac**************************
   #number of colonised non-family members contacted
   n.non.family.members.colonised <- apply(non.family.members.colonised,c(1,2),sum)
```

```
##STEP Bc**************************
   #total number of non-owner (family and non-family combined) members that are colonised
   total.humans.colonised[,iter] <- apply(matrix(c(n.family.non.owner.colonised[,iter], n.non.family.members.colonised[,iter]), ncol=2), 1, sum)  # Your comment:  # not sure if I want this as
transmission dynamics are going to be different for family and non-family

#does the dog have any contact with other dogs (y/n)
exposure.to.other.dogs[,iter] <- rbinom(n.dogs,1,p.exposure.to.other.dogs[iter])

#Number of contacts with other dogs
dog.contacts[,iter] <- exposure.to.other.dogs[,iter] * round(rbetapert(n.dogs,min.dog.exposure,ml.dog.exposure,max.dog.exposure))

##STEP Cc*******************
   #number of colonised community dogs contacted
   n.colonised.community.dogs[,iter] <- rbinom(n.dogs,dog.contacts[,iter],p.community.dog.colonised[iter])

#is house contaminated (y/n) (depending on if a member of the family is colonised
house.contam[((owner.colonised[,iter]+n.family.non.owner.colonised[,iter])=="0"),iter] <-
rbinom(length(house.contam[((owner.colonised[,iter]+n.family.non.owner.colonised[,iter])=="0"),iter]),1,p.house.contam.family.not.col[iter])
house.contam[((owner.colonised[,iter]+n.family.non.owner.colonised[,iter])!="0"),iter] <-
rbinom(length(house.contam[((owner.colonised[,iter]+n.family.non.owner.colonised[,iter])!="0"),iter]),1,p.house.contam.family.col[iter])

#how many contam sites per house?
no.environ.sites[,iter] <- round(rbetapert(n.dogs, min.environ.site.exposure, ml.environ.site.exposure, max.environ.site.exposure))*house.contam[,iter]

p.environ.contam[((owner.colonised[,iter]+n.family.non.owner.colonised[,iter])=="0")&(house.contam[,iter]==1)] <- combination.data[iter,7]  #probability of contamination of environmental sites
p.environ.contam[((owner.colonised[,iter]+n.family.non.owner.colonised[,iter])!="0")&(house.contam[,iter]==1)] <- combination.data[iter,9]

   no.contam.environ.sites[,iter] <- rbinom(n.dogs,no.environ.sites[,iter],p.environ.contam[iter])

##STEP Dc**********************
   environ.contam[,iter] <- no.contam.environ.sites[,iter]

######VETERINARY FACTORS:
no.dogs.contacted.at.vet[,iter] <- does.dog.go.to.vet[,iter] * round(rbetapert(n.dogs,min.vet.dog.exposure, ml.vet.dog.exposure, max.vet.dog.exposure))

##STEP Ec**********************
   #number of colonised dogs contacted at vet in 24h
   no.col.dogs.contacted.at.vet[,iter] <- rbinom(n.dogs,no.dogs.contacted.at.vet[,iter],p.vet.dog.colonised[iter])
```

```
p.vet.environ.contam[iter] <- sample(c(p.vet.environ.contam.1[iter], p.vet.environ.contam.2[iter]),1, replace=T, which.environ.estimate)
no.vet.environ.sites[,iter] <- does.dog.go.to.vet[,iter] * round(rbetapert(n.dogs, min.vet.environ.site.exposure, ml.vet.environ.site.exposure, max.vet.environ.site.exposure))
##STEP Fc**********************
   #number of contaminated environmental sites contacted at vet in 24h
   no.contam.vet.environ.sites[,iter] <- does.dog.go.to.vet[,iter] * rbinom(n.dogs,no.vet.environ.sites[,iter],p.vet.environ.contam[iter])

no.vets[,iter] <- does.dog.go.to.vet[,iter] * round(rbetapert(n.dogs,min.vet.exposure,ml.vet.exposure,max.vet.exposure))

##STEP Gc**********************
   #number of colonised vets contacted in 24h
    no.col.vet[,iter] <- rbinom(n.dogs,no.vets[,iter],p.vet.col[iter])
}

###TRANSMISSION DYNAMICS:

#Changing transmission from per contact into per 24h:

for(r in 1:r.repeats){
##STEP A***********
transmission.col.O[,,r] <- rbinom(n.dogs*iterations,owner.colonised,p.transmission.owner.24h)
##STEP B***********
transmission.col.non.O.fam[,,r] <- rbinom(n.dogs*iterations,n.family.non.owner.colonised,p.transmission.owner.24h)
transmission.col.non.O[,,r] <- rbinom(n.dogs*iterations,n.non.family.members.colonised,p.transmission.non.owner.24h)
##STEP C***********
transmission.col.community.dog[,,r] <- rbinom(n.dogs*iterations,n.colonised.community.dogs,p.transmission.community.dog.24h)
##STEP D***********
transmission.col.house.environ[,,r] <- rbinom(n.dogs*iterations,house.contam,p.transmission.community.environ.house.24h)
##STEP E***********
transmission.col.vet.dog[,,r] <- rbinom(n.dogs*iterations,no.col.dogs.contacted.at.vet,p.transmission.vet.dog.24h)
##STEP F***********
transmission.col.vet.environ[,,r] <- rbinom(n.dogs*iterations,no.contam.vet.environ.sites,p.transmission.vet.environ.24h)
##STEP G***********
transmission.col.vet[,,r] <- rbinom(n.dogs*iterations,no.col.vet,p.transmission.vet.24h)
}
```

```
##OUTPUT:

#MRSA.positive[,iter,r] <- apply(matrix(c(transmission.col.O[,iter,r], transmission.col.non.O.fam[,iter,r], transmission.col.non.O[,iter,r], transmission.col.community.dog[,iter,r],
transmission.col.house.environ[,iter,r],transmission.col.vet.dog[,iter,r], transmission.col.vet.environ[,iter,r], transmission.col.vet[,iter,r]), nrow=n.dogs), 1, sum)
MRSA.positive <-
transmission.col.O+transmission.col.non.O.fam+transmission.col.non.O+transmission.col.community.dog+transmission.col.house.environ+transmission.col.vet.dog+transmission.col.vet.environ+tra
nsmission.col.vet
##OUTPUT AS Y/N - ie dog can only become MRSA positive once in 24h:
MRSA.positive.1[,,] <- MRSA.positive[,,]>0

#assigning all the inputs to a global environment:
assign("does.dog.go.to.vet",does.dog.go.to.vet,pos=".GlobalEnv")
assign("p.dog.goes.to.vet.24h",p.dog.goes.to.vet.24h,pos=".GlobalEnv")
#stepA:
assign("owner.colonised",owner.colonised,pos=".GlobalEnv")
assign("transmission.col.O",transmission.col.O,pos=".GlobalEnv")
assign("owner.category",owner.category,pos=".GlobalEnv")
assign("O.contacts.per.24h",O.contacts.per.24h,pos=".GlobalEnv")
assign("p.transmission.owner.per.contact",p.transmission.owner.per.contact,pos=".GlobalEnv")
assign("p.transmission.owner.24h",p.transmission.owner.24h,pos=".GlobalEnv")
assign("p.owner.is.HCW",p.owner.is.HCW,pos=".GlobalEnv")
assign("p.owner.is.vet",p.owner.is.vet,pos=".GlobalEnv")
assign("p.owner.is.other.risk",p.owner.is.other.risk,pos=".GlobalEnv")
assign("p.owner.no.risk",p.owner.no.risk,pos=".GlobalEnv")
#stepB:
assign("n.family.non.owner.colonised",n.family.non.owner.colonised,pos=".GlobalEnv")
assign("n.non.family.members.colonised",n.non.family.members.colonised,pos=".GlobalEnv")
assign("transmission.col.non.O.fam",transmission.col.non.O.fam,pos=".GlobalEnv")
assign("transmission.col.non.O",transmission.col.non.O,pos=".GlobalEnv")
assign("others.in.family",others.in.family,pos=".GlobalEnv")
assign("n.family.non.owner.colonised",n.family.non.owner.colonised,pos=".GlobalEnv")
assign("others.not.in.family",others.not.in.family,pos=".GlobalEnv")
assign("total.humans.colonised",total.humans.colonised,pos=".GlobalEnv")
assign("non.O.contacts.per.24h",non.O.contacts.per.24h,pos=".GlobalEnv")
assign("p.transmission.non.owner.per.contact",p.transmission.non.owner.per.contact,pos=".GlobalEnv")
assign("p.transmission.non.owner.24h",p.transmission.non.owner.24h,pos=".GlobalEnv")
#stepC:
assign("n.colonised.community.dogs",n.colonised.community.dogs,pos=".GlobalEnv")
assign("transmission.col.community.dog",transmission.col.community.dog,pos=".GlobalEnv")
```

```
assign("exposure.to.other.dogs",exposure.to.other.dogs,pos=".GlobalEnv")
assign("dog.contacts.per.24h",dog.contacts.per.24h,pos=".GlobalEnv")
assign("p.community.dog.colonised",p.community.dog.colonised,pos=".GlobalEnv")
assign("p.transmission.community.dog.per.contact",p.transmission.community.dog.per.contact,pos=".GlobalEnv")
assign("p.transmission.community.dog.24h",p.transmission.community.dog.24h,pos=".GlobalEnv")
#stepD:
assign("environ.contam",environ.contam,pos=".GlobalEnv")
assign("transmission.col.house.environ",transmission.col.house.environ,pos=".GlobalEnv")
assign("p.environ.contam",p.environ.contam,pos=".GlobalEnv")
assign("p.transmission.community.environ.house.per.contact",p.transmission.community.environ.house.per.contact,pos=".GlobalEnv")
assign("p.house.contam.family.col",p.house.contam.family.col,pos=".GlobalEnv")
assign("p.house.contam.family.not.col",p.house.contam.family.not.col,pos=".GlobalEnv")
assign("environ.contacts.per.24h",environ.contacts.per.24h,pos=".GlobalEnv")
assign("p.transmission.community.environ.house.24h",p.transmission.community.environ.house.24h,pos=".GlobalEnv")
#stepE:
assign("no.col.dogs.contacted.at.vet",no.col.dogs.contacted.at.vet,pos=".GlobalEnv")
assign("p.transmission.vet.dog.per.contact",p.transmission.vet.dog.per.contact,pos=".GlobalEnv")
assign("no.dogs.contacted.at.vet",no.dogs.contacted.at.vet,pos=".GlobalEnv")
assign("vet.dog.contacts.per.24h",vet.dog.contacts.per.24h,pos=".GlobalEnv")
assign("p.vet.dog.colonised",p.vet.dog.colonised,pos=".GlobalEnv")
assign("transmission.col.vet.dog",transmission.col.vet.dog,pos=".GlobalEnv")
assign("p.transmission.vet.dog.24h",p.transmission.vet.dog.24h,pos=".GlobalEnv")
#stepF:
assign("no.contam.vet.environ.sites",no.contam.vet.environ.sites,pos=".GlobalEnv")
assign("transmission.col.vet.environ",transmission.col.vet.environ,pos=".GlobalEnv")
assign("vet.environ.contacts.per.24h",vet.environ.contacts.per.24h,pos=".GlobalEnv")
assign("p.vet.environ.contam",p.vet.environ.contam,pos=".GlobalEnv")
assign("p.transmission.vet.environ.per.contact",p.transmission.vet.environ.per.contact,pos=".GlobalEnv")
assign("p.transmission.vet.environ.24h",p.transmission.vet.environ.24h,pos=".GlobalEnv")
#stepG:
assign("no.col.vet",no.col.vet,pos=".GlobalEnv")
assign("transmission.col.vet",transmission.col.vet,pos=".GlobalEnv")
assign("no.vets",no.vets,pos=".GlobalEnv")
assign("vet.contacts.per.24h",vet.contacts.per.24h,pos=".GlobalEnv")
assign("p.transmission.vet.per.contact",p.transmission.vet.per.contact,pos=".GlobalEnv")
assign("p.col.vet",p.col.vet,pos=".GlobalEnv")
assign("p.transmission.vet.24h",p.transmission.vet.24h,pos=".GlobalEnv")
###
assign("family.members",family.members, pos=".GlobalEnv")
```

```
assign("family.members.colonised",family.members.colonised, pos=".GlobalEnv")
assign("temp.family.members.colonised",temp.family.members.colonised, pos=".GlobalEnv")
assign("n.family.non.owner.colonised",n.family.non.owner.colonised, pos=".GlobalEnv")
assign("MRSA.positive.1",MRSA.positive.1, pos=".GlobalEnv")

   return(list(n.dogs=n.dogs,iterations=iterations, MRSA.positive.1=MRSA.positive.1))
}
### calling the function:
model <- simulation(n.dogs,iterations)
```

APPENDIX 9

Plackett-Burman fractional factorial designs

Design matrix for a two-level, 19 factor, 20 design point, Plackett-Burman design with foldover:

Design point	Factors																		
	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9	F 10	F 11	F 12	F 13	F 14	F 15	F 16	F 17	F 18	F 19
1	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+
2	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-
3	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+
4	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-
5	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+
6	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+
7	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+
8	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+
9	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-
10	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-
11	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-
12	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-
13	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+	-	-
14	-	-	-	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	+
15	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+	+
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-
18	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-
19	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	-	-	+	+
20	-	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+
21	-	-	-	+	-	+	-	+	+	+	+	-	-	+	-	-	+	+	-
22	-	+	-	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	+
23	+	-	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	+	-
24	+	-	-	+	-	-	+	+	-	-	-	-	+	-	+	-	+	+	+
25	+	-	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-
26	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+	-
27	+	-	+	-	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-
28	-	+	-	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-
29	-	-	+	-	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+
30	+	+	+	-	-	+	-	-	+	+	-	-	-	-	+	-	+	-	+
31	-	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	+	-	+
32	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+	-	-	+	+
33	+	+	-	-	+	-	-	+	+	-	-	-	-	+	-	+	-	+	+
34	+	+	+	+	-	-	+	-	-	+	+	-	-	-	-	+	-	+	-
35	-	+	-	+	-	+	+	+	+	-	-	+	-	-	+	+	-	-	-
36	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37	-	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+
38	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+	-	-	+
39	-	-	+	-	+	-	+	+	+	+	-	-	+	-	-	+	+	-	-
40	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+	-	-

Design matrix for a two-level, 27 factor, 32 design point, Plackett-Burman design with foldover:

40	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-

	Factors																												
Design point	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8	F 9	F 10	F 11	F 12	F 13	F 14	F 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27
1	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+
2	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-
3	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+
4	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-
5	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+
6	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-
7	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-
8	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+
9	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-
10	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+
11	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-
12	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+
13	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-
14	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-
15	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-
16	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+
17	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-
20	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+
21	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+
22	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+
23	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-
24	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+
25	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-
26	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	+	-
27	+	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+
28	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+
29	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-
30	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+
31	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-	-	-	+	+
32	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	+	-	+	-	+	+	+	-	+	+	-
33	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-
34	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+
35	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-
36	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+
37	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-
38	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+
39	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+

41	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+
42	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-
43	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+
44	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-
45	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+
46	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+
47	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+
48	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-
49	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-
50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
51	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+
52	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-
53	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-
54	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-
55	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+
56	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-
57	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+
58	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+
59	-	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-
60	-	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-
61	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+
62	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-
63	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	-
64	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+

APPENDIX 10

Graphs of the relationships between interaction terms considered in the logistic regression models

For models run for all dogs:

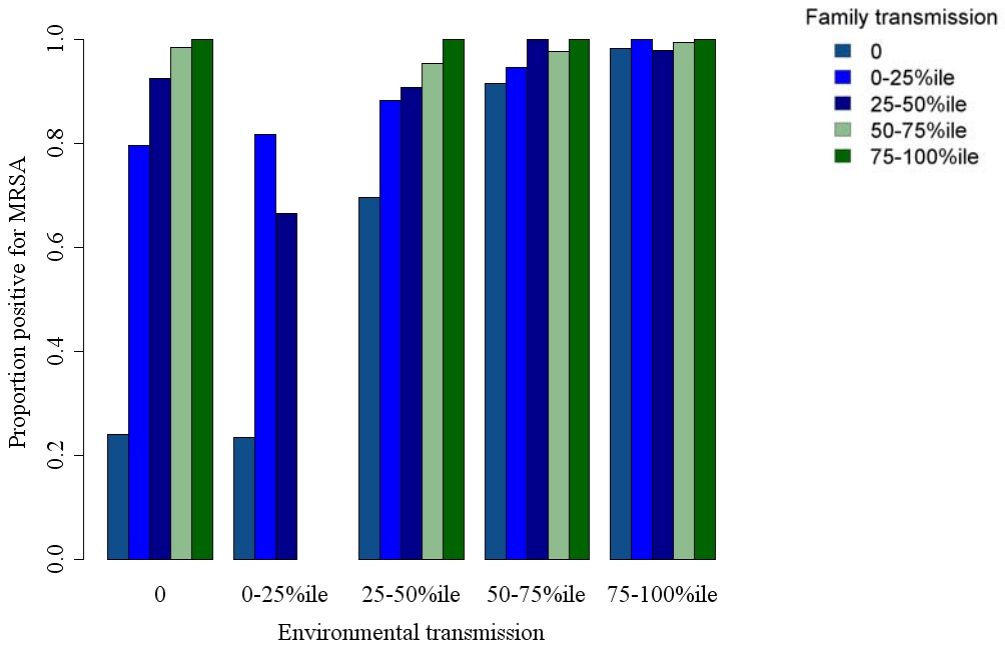


Figure A10.1. Graphical representation of the relationship between the probabilities of transmission from family members and from the environment with regards to MRSA acquisition in a dog.

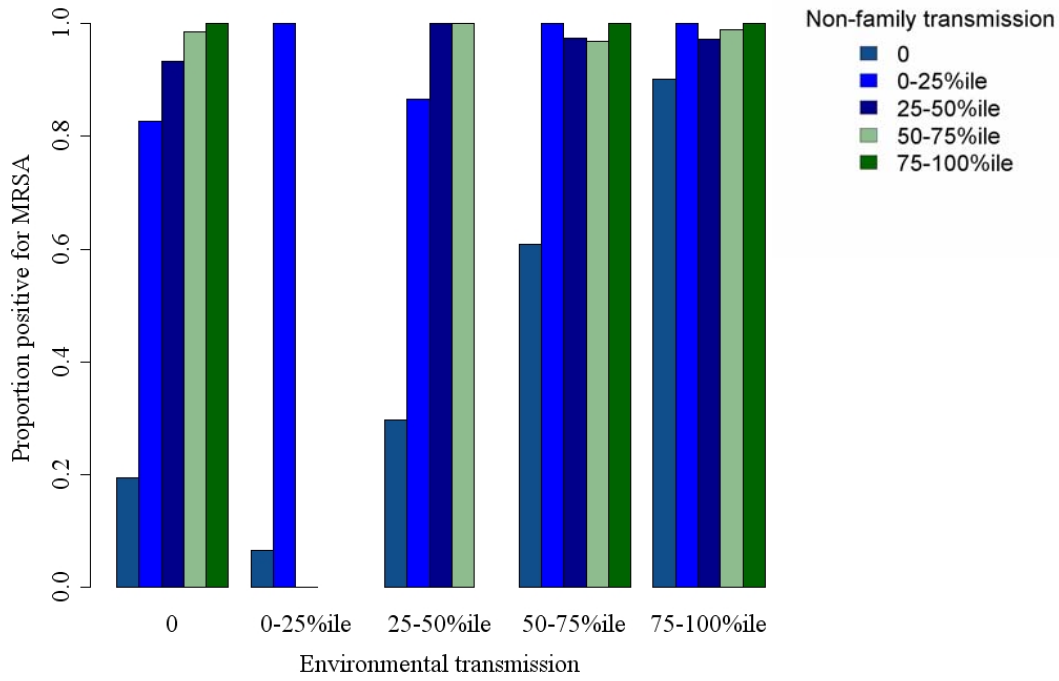


Figure A10.2. Graphical representation of the relationship between the probabilities of transmission from non-family members and from the environment with regards to MRSA acquisition in a dog.

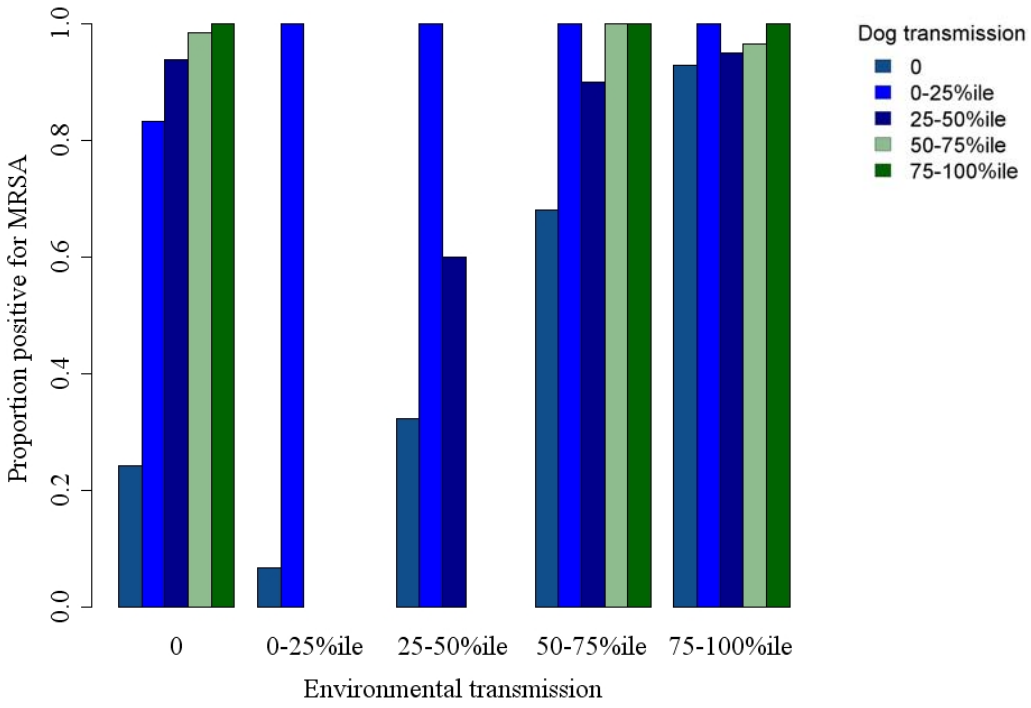


Figure A10.3. Graphical representation of the relationship between the probabilities of transmission from dogs and from the environment with regards to MRSA acquisition in a dog.

For models run for dogs that attended a veterinary clinic only:

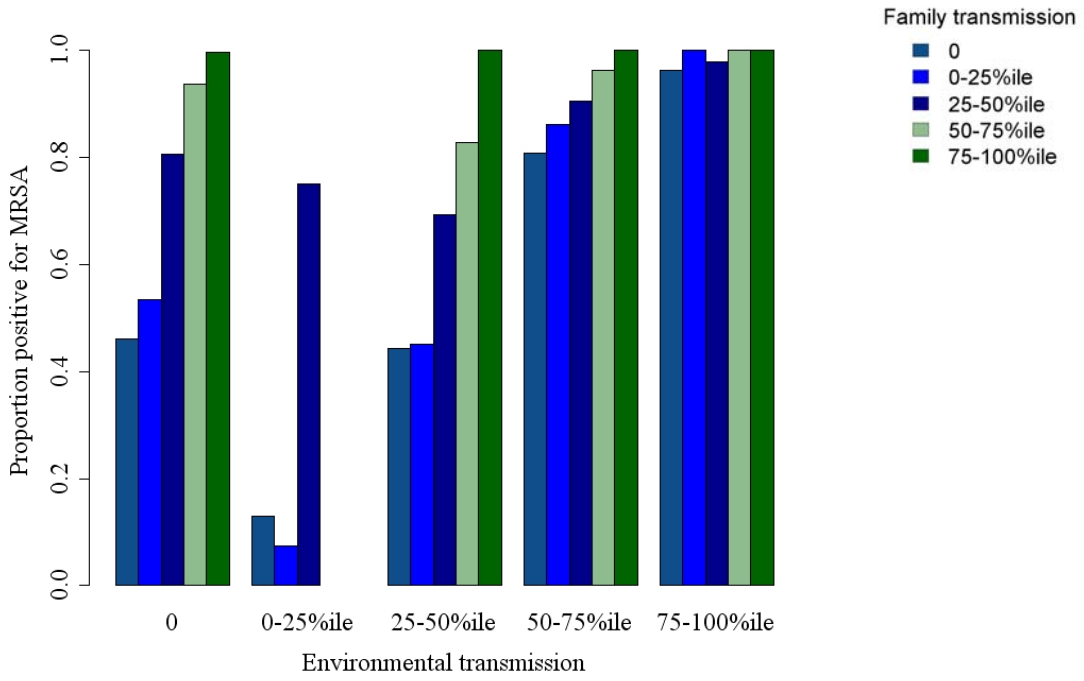


Figure A10.4 Graphical representation of the relationship between the probabilities of transmission from family members and from the environment with regards to MRSA acquisition in a dog that has attended a veterinary clinic.

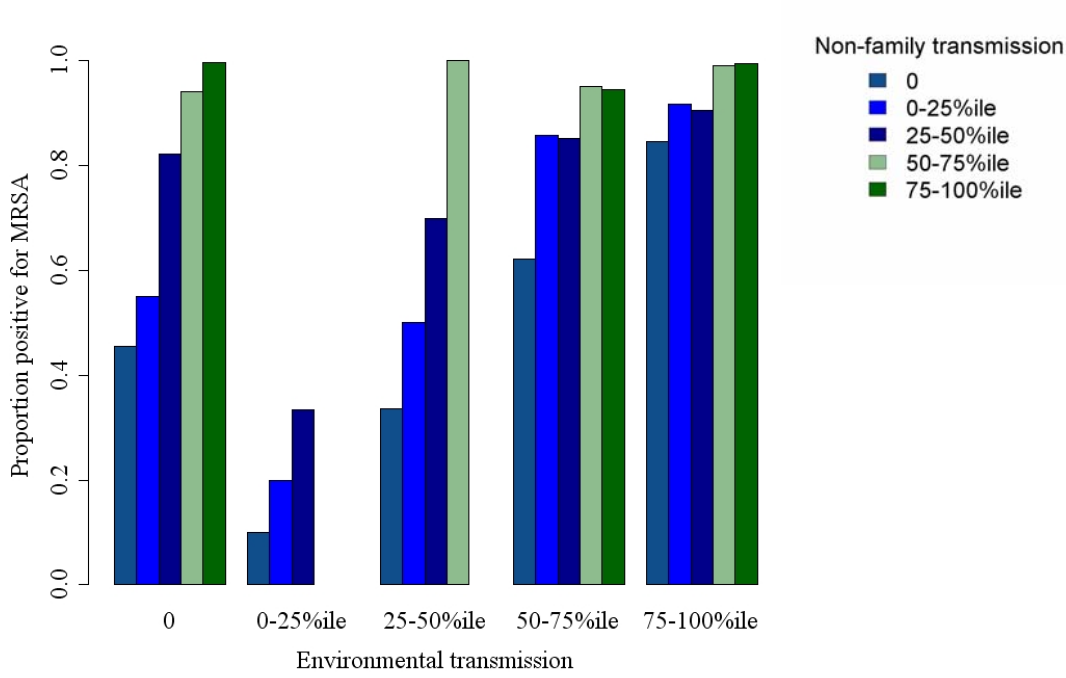


Figure A10.5 Graphical representation of the relationship between the probabilities of transmission from non-family members and from the environment with regards to MRSA acquisition in a dog that has attended a veterinary clinic.

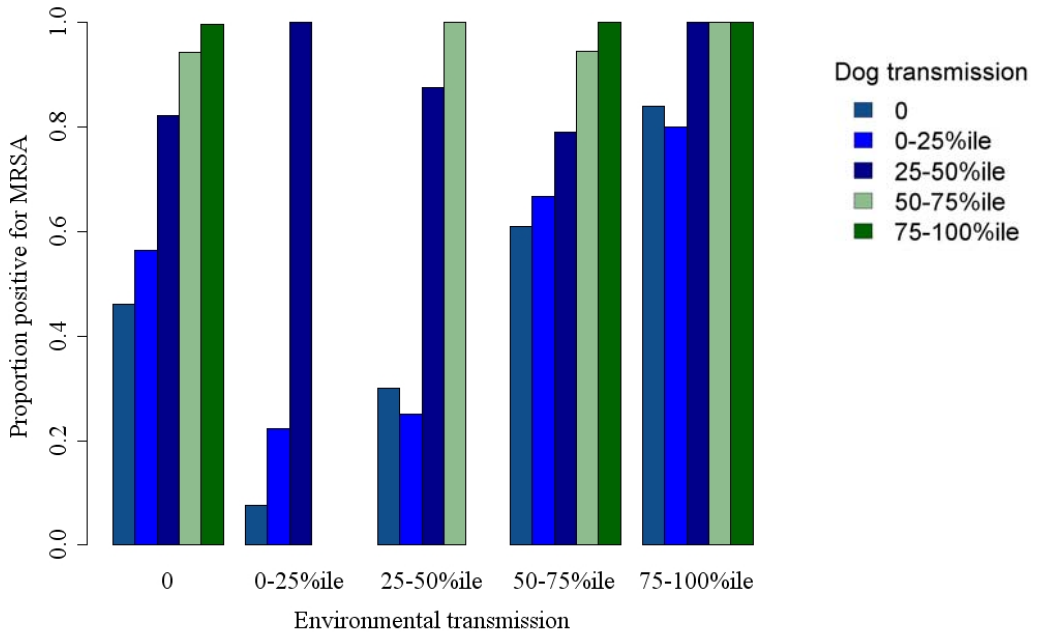


Figure A10.6. Graphical representation of the relationship between the probabilities of transmission from dogs and from the environment with regards to MRSA acquisition in a dog that has attended a veterinary clinic.

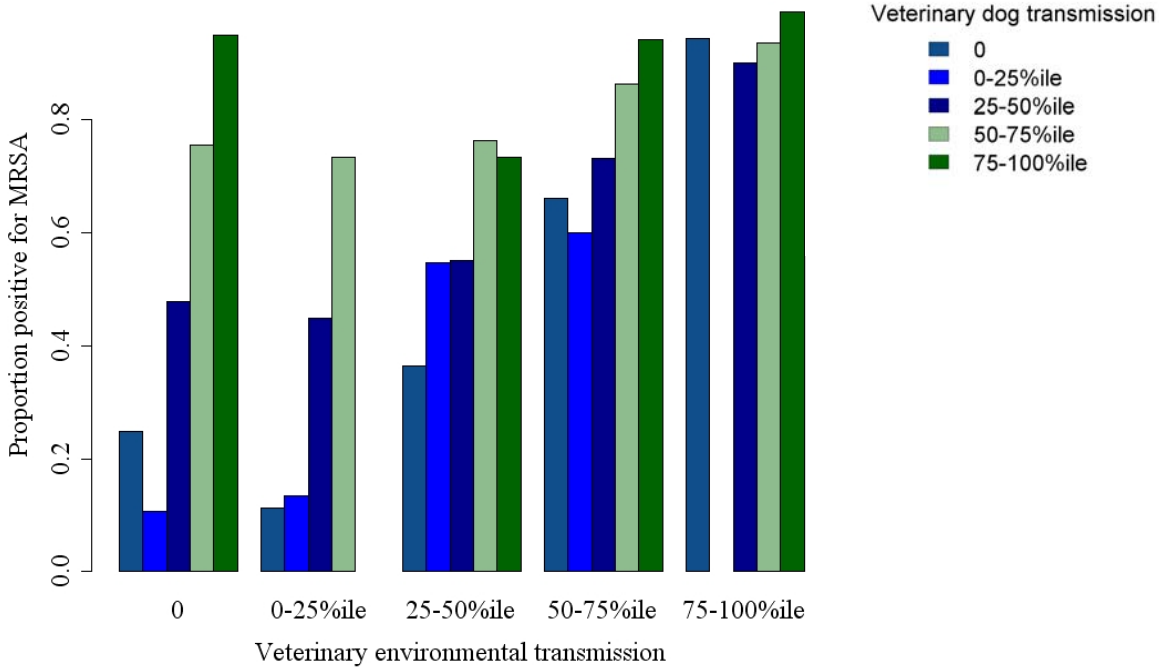


Figure A10.7. Graphical representation of the relationship between the probabilities of transmission from dogs at a veterinary clinic and from the veterinary environment with regards to MRSA acquisition in a dog that has attended a veterinary clinic.

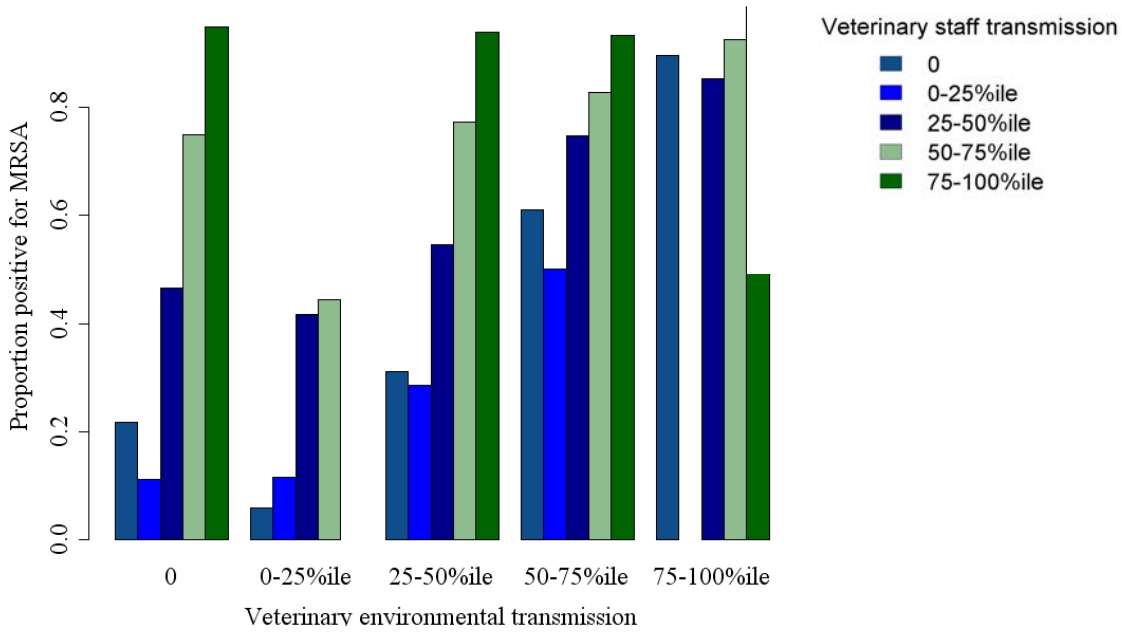


Figure A10.8. Graphical representation of the relationship between the probabilities of transmission from staff at a veterinary clinic and from the veterinary environment with regards to MRSA acquisition in a dog that has attended a veterinary clinic.

APPENDIX 11

Example question from interactive spreadsheet for expert opinion elicitation

Question 1:

How many of 1,000 randomly selected people from the general public would you expect to have nasal carriage of MRSA on a given day?

a) Please fill in the table:

N/A ☐

minimum	most likely	maximum
0	150	300

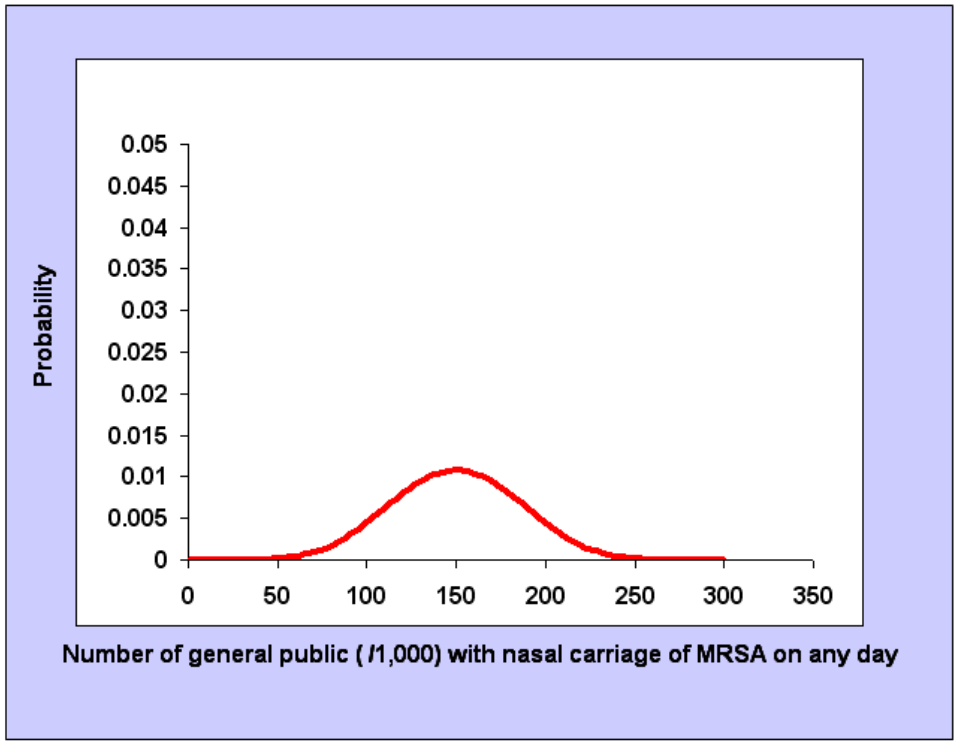
b) Please adjust distribution using the confidence scroll bar:

Confidence scroll bar:

1 < > 15

Confidence = 15

n.b. default confidence = 4



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