

The use of statistical and simulation modelling as decision support tools in veterinary public health



L'utilisation de la modélisation statistique et simulation comme outils de support de décision en santé publique vétérinaire

L. J. Ron-Garrido
Thèse présentée en vue de l'obtention du grade de
Docteur en Sciences Vétérinaires

ANNEE ACADEMIQUE 2015–2016

Wallonia-Europe University Academy
University of Liege-Faculty of Veterinary Medicine
Department of Infectious and Parasitic Diseases
Fundamental and Applied Research for Animal and Health (FARAH)
Research Unit of Epidemiology and Risk Analysis Applied to Veterinary Sciences
(UREAR-ULg)

Institute of Tropical Medicine (Antwerp)
Department of Biomedical Sciences
Unit of Veterinary Epidemiology

Universidad Central del Ecuador (Quito)
Centro Internacional de Zoonosis

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L. J. RON-GARRIDO

Promoter:

Prof. Dr. Claude SAEGERMAN

Research Unit of Epidemiology and Risk Analysis Applied to Veterinary Sciences
(UREAR-ULg)

Fundamental and Applied Research for Animal and Health (FARAH)
Department of Infectious and Parasitic Diseases
Faculty of Veterinary Medicine - University of Liege

Co-promoter:

Prof. Dr. ir. Dirk BERKVENNS

Unit of Veterinary Epidemiology
Department of Biomedical Sciences
Institute of Tropical Medicine (Antwerp)



Académie Universitaire Wallonie-Europe
Université de Liège - Faculté de Médecine Vétérinaire
Département des Maladies Infectieuses et Parasitaires
Fundamental and Applied Research for Animal and Health (FARAH)
Unité de Recherche en Epidémiologie et Analyse de Risques
appliquées aux Sciences Vétérinaires (UREAR-ULg)

Institut de Médecine Tropicale (Anvers)
Département de Sciences Biomédicales
Unité d'Epidémiologie Vétérinaire

Universidad Central del Ecuador (Quito)
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Fundamental and Applied Research for Animal and Health (FARAH)
Département des Maladies Infectieuses et Parasitaires
Faculté de Médecine Vétérinaire - Université de Liège

Co-promoter:

Prof. Dr. ir. Dirk BERKVENNS

Unité d'Epidémiologie Vétérinaire
Département de Sciences Biomédicales
Institut de Médecine Tropicale (Anvers)



Acknowledgements

This study was carried out thanks to the academic support from the University of Liege, Belgium, the Institute of Tropical Medicine in Antwerp, Belgium (ITM), and the Centro Internacional de Zoonosis, Ecuador (CIZ) at the Universidad Central del Ecuador in Quito as well the financial support of the Belgium Cooperation for Development (DGCD) in framework of the institutional collaboration between the ITM and CIZ.

The accomplishment of this document would not have been possible without the participation and guidance of several persons, which I would like to mention:

I would like to express me deep gratitude to Professor Claude Saegerman for your academmic support, your field experience, scientific contribution and guidance to my work, and for encouraging me to finish this manuscript.

I am very grateful to Professor Dirk Berkvens, your field experience and your programming skills really contributed to improve the significance and the understanding of the problems concerned.

I would like to thank to Professor Washington Benitez for his local support during this long journey and for having involved me in these topics. Thank you also to Dr. Jorge Ron-Román and Dr. Marco Coral to share data with me on Brucellosis and Cysticercosis respectively.

I am also grateful to Dr. Emmanuel Abatih for sharing your scientific experience, publication skills, and your friendship.

Additionally, I would like to offer my thanks to the Biometry group in Buenos Aires, Argentina and Professors Norberto Bartoloni and Rodolfo Cantet for advice and for the introduction in the biometry topics when I started my scientific endeavour.

I would like to mention the participation and my sincere acknowledgment to Professor Pierre Dorny, Dr. Sara Gabriel and Professor Niko Speybroeck for their positive criticism en encouragement. Their opinions were fundamental to accomplish some of the scientific publications that are part of this document.

I am pleased to acknowledge the Student Service staff of ITM and Danielle Debois in the Department of Biomedical Sciences for their support during my stay in Antwerp. Equally, I really appreciate the collaboration of CIZ staff and my colleagues of the Central University in Quito.

Last but not least, I really wish to thank Miriam and my daughter Daniela Lucia for their unconditional love and unfailing support. Also I am grateful to my mother Lucia, my father Fernando, my brother Rommel and to my sister Yadira for their trust on me and for their encouragement.

List of Abbreviations

| | |
|-------|--|
| AI | Artificial Insemination |
| BLUP | Best Linear Unbiased Prediction |
| CIZ | Centro Internacional de Zoonosis, Universidad Central Del Ecuador, Quito |
| DALY | The disability-adjusted life year |
| FAO | United Nations Food and Agriculture Organisation |
| GBD | Global Burden of Disease |
| GIS | Geographical Information System |
| IBD | Genes Identical by Decendence |
| ICD | International Classification of Diseases |
| ICONZ | Integrated Control of Neglected Zoonoses |
| IHC | Incidence of hospitalized cases |
| N_e | Effective Population Size |
| NB | Negative Binomial |
| NCC | Neurocysticercosis |
| NZD | Neglected Zoonotic Disease |
| OIE | World Organization for Animal Health |
| Se | Test sensitivity |
| Sp | Test specificity |
| VPH | Veterinary Public Health |
| WHO | World Health Organisation |
| ZINB | Zero Inflated Negative Binomial model |
| ZIP | Zero Inflated Poisson Model |

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Abstract

The Ecuadorian case of two livestock-related neglected zoonoses (brucellosis and cysticercosis) is treated. We tried to find and evaluate their relationships with components of food security, such as livestock populations, adequate sanitation and biological variability within livestock species. Generally, in extensive and small-holder livestock systems in developing countries, there is a carelessness when it comes to the evaluation and assessment of risks related to problems in animal husbandry and as such in the prevention of health problems in human and livestock populations. Data provided by a passive surveillance system, generated in Ecuadorian hospitals were used for the analysis and non-specific epilepsies were used additionally to support neuro-cysticercosis data. We state that this kind of surveillance provides critical information for monitoring community and livestock health statuses with relation to those zoonoses in a relatively cheap way. This strategy may help to cover larger areas, to gain insights in zoonotic diseases and to prioritise areas of intervention. In the case of human brucellosis several clusters were found in time and space, and later on several of our hypotheses were verified in the Northern and central parts of the country and in some regions in the coastal part of the country. For farmers, the rationale for the elimination of this disease is often founded in benefit-cost calculations providing them with the incentives for prevention and eradication of the disease. A mathematical model for the transmission dynamics of brucellosis in cattle was developed, allowing estimation of losses in cattle farms due to brucellosis. Losses were estimated in terms of abortions and milk yield losses that result from a reduction in the cattle farm population or from delays in the production due to abortions. It was estimated that, in the absence of control measures, brucellosis in cattle is an economically catastrophic problem because of noticeable lack of female replacements and as a consequence and inefficient production systems. The model predicts that brucellosis in a dairy herd is an eradicable disease. Strict vaccination of female calves and biannual testing half the population and culling the seropositive animals can reduce the true prevalence in cattle to negligible levels with minimum economic losses. In neuro-cysticercosis (NCC), also traditional and previously recognised endemic zones were identified, but recent, new clusters of epilepsy have appeared. Overall, an increasing trend for the incidence of hospitalised cases (IHC) of epilepsy and a decreasing trend for the IHC of NCC were observed over time; oddly enough, within municipalities a significant positive linear relationship between both disorders was confirmed. Risk models demonstrated that rates of epilepsy and NCC in hospitals were related to the presence of systems for eliminating human excreta. On the other hand, but in the same vein, the increasing demand for food of animal origin has tended to favour international high-output breeds over local breeds. Consequently, there has been a worldwide tendency to reduce the effective size of populations under selection. Animal genetic diversity is a critical factor for food security, and it may also reduce the risk of disease threats in livestock. In this document, a methodology to estimate the effective population size is given. It can be applied when partial information of family sizes is known in a beef cattle population. Additionally, this genetic structure of livestock populations must be taken into account when statistical data analyses are performed for features with genetic backgrounds, because of genetic resemblance. The use of statistical and modelling tools in the epidemiology of livestock-related neglected zoonoses still plays an important role when advising decision makers in public health.

Resumen

Este documento trata sobre la epidemiología de dos enfermedades zoonóticas relacionadas a la cría de animales en Ecuador. Los objetivos fueron tratar de encontrar y evaluar las relaciones de la presencia e incidencia de estas enfermedades con componentes de la seguridad alimentaria tales como la presencia de poblaciones de ganado, adecuado saneamiento y la diversidad biológica dentro de las especies. Tanto, en los sistemas extensivos de cría animal como de pequeños productores en los países en desarrollo, hay una carencia en la evaluación y valoración de los riesgos de salud relacionados a la cría de animales y por lo tanto en la prevención de esos problemas para las poblaciones humana y animal. Datos provistos por los sistemas de vigilancia pasiva, generados en el sistema de hospitales del Ecuador fueron usados para este análisis. Adicionalmente el uso de datos de vigilancia pasiva de epilepsias en hospitales también fueron usados para dar soporte a la información en el caso neurocisticercosis. Se establece que este tipo de vigilancia provee información crítica para el monitoreo de los estados de salud de la comunidad y los animales de cría en relación con esas zoonosis a relativo bajo costo en comparación a otros sistemas de vigilancia. Esta estrategia puede ayudar a cubrir mayores áreas de vigilancia, a ganar mayor percepción sobre estas zoonosis y para priorizar áreas de intervención. En el caso de brucelosis humana en Ecuador, varios agrupamientos de zonas de alta incidencia fueron reconocidos a través del tiempo y del área de estudio. Estos mismos resultados fueron comprobados posteriormente en la zonas norte, central y algunas partes de la región costera del país con datos de campo. Para los productores de animales, la racionalidad para la eliminación de esta enfermedad se fundamenta frecuentemente en el cálculo del costo-beneficio, mismo que debe proveer incentivos para la prevención y erradicación de la enfermedad. Un modelo matemático para la dinámica de transmisión de brucelosis bovina fue desarrollado. El modelo permite estimar las pérdidas que se generan en las ganaderías afectadas por brucelosis bovina. Las pérdidas fueron estimadas en términos de número de abortos y pérdidas en la producción de leche, mismas que resultan en el decrecimiento de la población bovina del hato y/o en retrasos en la producción de leche debido a los abortos. En ausencia de medidas de control, la brucelosis bovina puede convertirse en un problema económico catastrófico debido a la notoria falta de hembras para reemplazos y como consecuencia los sistemas productivos bovinos se vuelven ineficientes. Las simulaciones permitieron predecir que para un hato ganadero la brucelosis es una enfermedad erradicable. Para esto, estricta vacunación de las terneras y diagnóstico serológico bianual con descarte-sacrificio de al menos la mitad de los animales seropositivos del hato sería suficiente para reducir la prevalencia de la enfermedad a niveles indetectables con mínimas pérdidas económicas. En Neurocisticercosis (NCC), zonas endémicas tradicionales en Ecuador previamente identificadas en otros estudios fueron reconocidas como zonas de mayor riesgo; sin embargo, nuevas zonas con mayor riesgo de incidencia para epilepsia han aparecido más recientemente. En global, una creciente tendencia para la incidencia de casos hospitalizados de epilepsia y una decreciente tendencia para la incidencia de casos hospitalizados de Neurocisticercosis fueron observados a través del tiempo; pero curiosamente, al contrario, dentro de las municipalidades una relación lineal positiva significativa entre los casos de epilepsia y NCC fue confirmada. Los modelos utilizados para valorar el riesgo demostraron que las tasas de epilepsia y NCC en hospitales fueron relacionadas a la presencia de sistemas para la eliminación de excrementos humanos en la municipalidades. Por otro lado, pero en el mismo tema, la creciente demanda de alimentos de origen animal ha tendido a favorecer a razas internacionales con altos rendimientos. Consecuentemente, ha existido una tendencia global a

reducir el Tamaño Efectivo de las poblaciones bajo selección (N_e). En este sentido, la diversidad genética de los animales, es un factor crítico para la seguridad alimentaria, tanto como esta puede reducir el riesgo debido a las amenazas que representan las enfermedades en ganadería. En este documento, una metodología para estimar en tamaño efectivo de una población es dado. El mismo puede ser aplicado cuando información parcial de los tamaños de familia de los individuos es conocida, por ejemplo en poblaciones de ganado de carne. Adicionalmente, la estructura genética de la poblaciones de los animales de cría debe ser tomada en cuenta a la hora de realizar análisis estadísticos con estos individuos en características con cierta base genética debido a la respuesta similar con origen genético. El uso de herramientas estadísticas y de modelamiento en la epidemiología de zoonosis relacionadas con animales de cría conserva aún un importante rol a la hora dar soporte a los tomadores de decisiones en salud pública.

Chapter 1

INTRODUCTION

1.1 General introduction

Animal husbandry is the management and care of farm animals by humans for profit (Jarman et al., 1976). The products and services that livestock provide include meat, milk, eggs, fibre, skins, transport, draught power, manure for fertilizer, fuel, and are a source of capital accumulation especially for small farmers in developing countries (Sansucy, 1995). In many parts of the world, livestock production is undergoing a process of rapid intensification carried by industrialised systems, although in low- and middle-income countries, small-scale production with smallholders and extensive systems persist (Liverani et al., 2013; Sansucy, 1995; Graham et al., 2008). Furthermore, in recent years there has been an increase in the production and consumption of animal-source foods and products (Nabarro and Wannous, 2014); for instance, the production of animal meat and milk in developing countries has risen by 5% and 4% per year respectively. Intensive systems are characterised by concentration of large numbers of animals in housing units, use of concentrate feed, reduced genetic diversity, vertical integration, sustained use of antibiotics, and frequent movement of livestock (Liverani et al., 2013). Intensive systems usually have norms of biosecurity because they are usually more isolated from the external environment and they sometimes require sustained veterinary control and management procedures. By contrast, smallholder systems tend to use small-scale free-range production in which production tends to use a few inputs and low levels of biosecurity (Nabarro and Wannous, 2014; Otte et al., 2005; Liverani et al., 2013). Production at small scale (and extensive systems) often have limited access to comprehensive veterinary care for their herds which makes it difficult to protect their livestock from disease agents. In extensive and small-scale systems, farmers tend to invest in animal health up to the point beyond which further investment would no longer be profitable (FAO, 2015). Thus,

for small farmers, investments in animal husbandry, whether in nutrition, animal genetics or housing, may become profitable provided the risk of high-impact livestock disease has first been contained.

Diseases affecting livestock can have a devastating impact on animal productivity and production, on trade in live animals, meat, on other animal products, on human health and on economic development (FAO, 2015). Zoonotic diseases are diseases that can be passed from animals to humans (Keeling and Rohani, 2008). Among the very large list of zoonoses, Neglected zoonotic diseases (NZD) are those ones that are commonly associated with poverty. They impact the lives and livelihoods of millions of livestock keepers and people living in peri-urban areas primarily in developing countries, as poverty increases the risk of contracting them (King, 2011; Halliday et al., 2015). For example, anthrax, bovine tuberculosis and brucellosis are primarily occupational diseases, and small livestock producers worldwide are at risk and frequently acquire these infections from their animals. Poor people are more vulnerable to diseases associated with consumption of livestock products and are at risk for zoonotic diseases such as cysticercosis and other parasitic and food-borne illnesses (King, 2011). A feature of these neglected diseases is that they are difficult to diagnose and usually do not have specific symptoms or signs, so that they present a challenge for clinicians in both human and animal health (Maudlin et al., 2009; Halliday et al., 2015). As a consequence, the true burden of endemic zoonoses is largely underestimated. In many developing countries, a lack of laboratory diagnostic capacity makes it difficult to perform reliable diagnosis. For instance, the lack of neurological imaging facilities, such as computerised tomographic scans, hamper the elucidation of the causes of neurological syndromes (Halliday et al., 2015). The poor capacities implemented in developing countries to apply isolation of pathogens and molecular diagnosis are also a source of concern. By contrast, although serological tests are more available, their ability to properly distinguish true positives or negatives is a matter of debate in neglected zoonoses (Vang Johansen et al., 2015; Al Dahouk et al., 2013; Berkvens et al., 2006). Likewise, the poor are less likely to receive proper treatment or even worse, they do not have access to health care in hospitals because they live in remote rural areas and may not be able to afford the costs involved in the diagnosis and treatment remaining neglected from public health registers .

Veterinary Public Health (VPH) is a component of public health activities devoted to the application of veterinary skills and knowledge for the protection and improvement of human and animal health (FAO, 2002). The effective containment and control of epidemic diseases depends on early notification of disease events or outbreaks, and the capacity to forecast the spread of pathogens to new areas (Maudlin et al., 2009). Investigation of disease outbreaks is a prime support service to community-based and central veterinarian planners. Additionally, community-based veterinarians need early confirmation of diagnoses in order to increase their knowledge and competence, so that they can provide better

service to individual producers and national authorities. However, VPH programmes are often absent in rural areas. Thus, given the links between livestock species and zoonoses, it is relevant to elucidate whether the different production systems established at country level might increase or decrease the risk of disease emergence in human populations (FAO, 2002).

Effective prevention of disease spread requires modern techniques of risk management, in which epidemiological skills and knowledge are combined with a structured process of identification of likely disease agents, assessment of the severity and nature of the risks and the areas of the country at highest risk for entry and spread of the disease. At this point, spatial analysis is making an increasingly important contribution to disease control measures due to its ability to provide immediate visualisation of the distribution of the phenomena under study within a territory (D’Orazi et al., 2007; Kulldorff, 1997). Rapid detection of emerging geographic clusters due to unexpectedly occurring risk factors can be of great importance for public health (Kulldorff, 1997). Additionally, the effectiveness of control activities in the control of diseases lies in the development of surveillance systems that guarantee the greatest payoff in detecting disease and demonstrating freedom from specific diseases. The use of spatial analysis and visualisation may provide a broad assessment of the disease status in a country and answer specific questions about the national disease status (FAO, 2002; King, 2011; Kulldorff, 1997). As shown in recent epidemics (e.g. foot-and-mouth disease in UK), geographical information systems (GIS) have become an indispensable tool in public and veterinary epidemiological surveillance (Pfeiffer, 2004) (Pfeiffer, 2004). Decision supports systems furthermore require the integration of many data sources to generate disease intelligence information and visualisation by means of GIS aids to make sense of these large databases (Pfeiffer, 2004), GIS can also be used to develop statistically validated proxy indicator variables that can be measured in place of the variable of interest (King, 2011). Thus, incorporating GIS systems may allow the development of specific control strategies in the veterinary field at low cost.

Decision and risk tools to work in control campaigns of NZD have not been well implemented as lack of information persists in developing countries. At the same time, there are questions about the impacts of neglected, endemic zoonotic diseases and surveillance plays a critical role in generating data to properly evaluate the true burden of these diseases for public health priority-setting. Thus, a proper implementation of GIS in neglected zoonoses, where such lack of data might really help to implement control campaigns in high risk places. For instance in Ecuador very few attention has been put in the surveillance of neglected zoonoses such as cattle-brucellosis and and swine cystecircosis.

The use of hospital-based cases, through the use of ICD-10 codes, may be an effective indicator to monitor general trends of neglected zoonoses (WHO, 2010). In many instances, this is the only information available to estimate (at least part) of the burden of these diseases for the veterinary and public health sectors. Despite the obvious risk of

underreporting, in many cases NZD do require hospitalisation and so leave a trace. Thus, the use of hospitalised cases information is an alternative form of passive surveillance that may give insights in the health status of livestock populations, that may indirectly detect pathogens, that may act as an early warning system and may help to avoid a potential spread in human populations (D’Orazi et al., 2007). The use of hospital-based surveillance has been used to provide guidance and insight in the case of (e.g.) *Clostridium difficile* (Jones et al., 2012), Lyme disease (Nelson et al., 2015) and Hepatitis B (Mahajan et al., 2013). The International Classification of Diseases (ICD) is the standard diagnostic tool for epidemiology, health management and clinical purposes. It is used to monitor the incidence and prevalence of diseases and other health problems, providing a picture of the general health situation among countries, areas, and populations at different times. ICD records also provide the basis for the compilation of national mortality and morbidity statistics by WHO Member States (WHO, 2010). The purpose of the ICD is to permit the systematic recording analysis, interpretation and comparison of mortality and morbidity of data collected. Thus, the use of this information in conjunction with descriptive economic and landscape variables may help to develop statistical models to recognise areas or variables that can be in risk.

On a completely different note, an important aspect, usually not taken into account in the context of animal health and food security, is the importance of the genetic background of the livestock population (Tave, 1999). Most smallholder farmers worry only about animal nutrition, housing, general management, disease control and prevention and ignore the genetic aspects. One of the most important aspects of managing a closed population is the management of the population’s effective breeding number. This number is an indicative measure of the possibilities that a population suffers performance problems because inbreeding and genetic drift. Those phenomena might ruin the genetical improvements reached (Tave, 1999). In fact, the accumulation of inbreeding and genetic drift might reduce growth rate, viability, fecundity, and survival and might make future selective breeding programmes ineffective. Actually, important increases in the rates of inbreeding have recently been observed in dairy cattle populations. Three important factors that have been attributed to this increase are: 1) the tendency to co-select related animals as a result of using BLUP (Best linear unbiased prediction) estimated breeding values, 2) the use of fewer sires and dams facilitated by AI (Artificial insemination) and multiple ovulation and embryo transfer, and 3) selection for only a few traits such as milk yield and type, which are usually positively correlated. For instance, only two sires accounted for nearly one-quarter of the genes of registered american Holstein animals born in 1990 (Kearney et al., 2004). In this area, most of the research has been focused on preservation of rare breeds or maintenance of genetic diversity within closed nucleus breeding schemes (FAO, 2010). However, the apparently large population size of many livestock breeds is misleading, because inbreeding is primarily a function of selection in-

tensity (Weigel, 2001). The use of imported (specialised) animal breeds for farmers is characterised by genetic selection toward increased productivity (FAO, 2010). Although their use has unclear implications for zoonotic risk, they undoubtedly facilitate disease transmission if there exists homogeneity in genetic susceptibility and because of a rapid increase of technologies to improve fertility and increase selection speed, for example the use of artificial insemination (AI) and embryo transfer (FAO, 2010). Risks associated with AI are the dissemination of genetic defects and the spread of infectious diseases at local, national or even international levels (Eaglestone and Garcia, 2000); such the case of Bovine Adhesion Leukocyte Deficiency (BLAD) were one sire with large impact on production traits (Osborndale Ivanhoe) widely helped to disseminate the defective alleles to the world.

Another consequence of the reduced genetic variability, possibly even more important in the context of data collection within the veterinary public health domain, is the over-estimation of the effective population size when sampling animal populations. Whereas it is common practice to take into account random effects due to common environmental and/or management factors when performing statistical analyses, common genetic background is virtually never accounted for. Samples taken from livestock herds may be family-based samples, or random samples from genetically isolated populations, where due to, limited population size, relatives are likely to be present by chance (Aulchenko, 2011). Genetic relationships between pair of individuals change the genotypic distribution of a population. Such distribution or population structure consists of several genetic structures on the distribution of genotypes in a population that need to be considered. Inbreeding (the preferential breeding between relatives), which is created by geographical reasons or husbandry practices increase the frequency of homozygous. Thus, if the genetic structure of population is not taken into account, the standard-statistical association tests tend to be inflated (Aulchenko, 2011), but also, the evaluation of random effects may hide relationships with population genetics models that might have not been thoroughly established (i.e. heritability estimation) by the statistics measured in analyses (Olivier and Vekemans, 1999).

Modelling systems may provide insights into how ecological and evolutionary processes lead up to emergence of diseases, influence the spread of pathogens, and how evolutive forces are likely to be affected by changes in specific features of the risk environment. Mathematical models help to understand the biology of the host and pathogen (Medley and Anderson, 1992). The objectives of mathematical and statistical models are on the one hand prediction, on the other inference (understanding), or a combination of both (Keeling and Rohani, 2008; James et al., 2014). They thus help to predict, understand and describe the impact of a specific biological phenomenon. There is a growing body of modelling efforts, dealing with pathogen emergence, pathogen evolution, and more general transmission aspects, such as (e.g.) the dependence of pathogens on contact networks

and routes of transmission (Medley and Anderson, 1992; Ngonghala et al., 2014; Read and Keeling, 2003; Keeling and Rohani, 2008). Dynamic models (by means of differential or difference equations) have proved useful tools to understand epidemiological or genetic processes in populations, and in this way, have been used when developing decision tools to understand and control epidemics, and to study evolutive forces in game and livestock populations, allowing the development of strategies to be followed in control campaigns (Keeling and Rohani, 2008). Strictly statistical models are concerned with finding models and patterns from the available data. Statistical analyses result in predictive and classification models that can find patterns, associations, clusters and subgroups (Lavrac et al., 2007).

Therefore, given the above mentioned elements, related to animal and public health, that risk managers are confronted with in the agricultural and veterinary sectors, there is a necessity to find strategies, indicate possible measures and analyse data and risk factors (co-variables) that permit the development of mathematical and statistical models in order to understand the relationships between variables and the effective risk. There is also a need to understand the dynamical processes affecting the susceptibility of livestock in terms of host-pathogen interaction, and in terms of the innate response of the host. Those topics will give to decision makers better tools to improve internal breeding practices and the possibility to improve the public and animal health.

1.2 Livestock Neglected Zoonoses

Infectious diseases in livestock are a major threat to global animal health and welfare and their effective control is crucial for agronomic health, and for alleviating rural poverty in developing countries (Tomley and Shirley, 2009). Livestock diseases have devastating outcomes on animal health and impact on national and international trade. Among a vast list of diseases that animals can contract, zoonoses are the ones that affect also to humans in general, and livestock keepers in particular. These diseases affect human health and wellbeing directly because of the disease, and indirectly through impacts on livelihoods and food security as a result of livestock production losses (Halliday et al., 2015). Because of the huge differences in their kind of production systems and the inputs between low-intensity subsistence livestock farming and the highly-organised intensive livestock industry, the zoonoses may be classified in two groups: one group embraces emerging and re-emerging zoonoses and the other group embraces the endemic neglected zoonoses (Tomley and Shirley, 2009). As mentioned by Maudlin et al. (2009), emerging and re-emerging zoonoses like Bovine Spongiform Encephalopathy, Avian Influenza A H5N1, Severe Acute Respiratory Syndrome CoronaVirus (SARS-CoV), Nipah Virus, and Ebola, attract international attention. In contrast, endemic neglected-zoonoses have reduced importance in the eyes of administrators and funding agencies (Maudlin et al., 2009; Halliday

et al., 2015; Tomley and Shirley, 2009). Additionally, those diseases tend to be located in marginalised communities where animals are often kept under scavenging conditions with little attention to disease control, housing or feed supplementation, and are associated with people living in close proximity to livestock sources (ICONZ, 2015). The latter are mainly found throughout the developing world where the conditions for their maintenance and spread exist. Neglected zoonoses, such as anthrax, rabies, brucellosis, bovine TB, zoonotic trypanosomiasis, echinococcosis, cysticercosis and leishmaniasis are major causes of ill-health in the poorest communities in developing countries in Africa, Latin America and Asia (ICONZ, 2015). Additionally, those diseases have been mentioned as the “Major and Neglected Diseases in Developing Countries” according to the European Parliament (Maudlin et al., 2009). In Ecuador, for instance, the main zoonoses that can be attributed to the presence of unhealthy livestock, and as of the public health concern, are brucellosis and taeniasis/cysticercosis. Thus, the battle against brucellosis and cysticercosis represents a serious challenge for livestock keepers as well as for the veterinary and public health sectors and an increasing awareness about of their causes and how they may be prevented –often with simple technologies– can reduce their incidence in many endemic zones (Maudlin et al., 2009).

1.2.1 Brucellosis

Brucellosis is a bacterial disease caused by members of the genus *Brucella* affecting mainly mammals (Spickler, 2009). Although brucellosis is essentially a disease of wildlife and domesticated livestock, humans are also accidentally a host, turning the disease in a zoonosis. At present several *Brucella* species, more or less host-specific, are responsible for the disease in animals, but cross-species infections also occur (Bricker et al., 2003; Atluri et al., 2011; FAO/WHO/OIE, 2006). *Brucella abortus* is normally associated with cattle, *B. melitensis* with sheep and goats and *B. suis* with pigs; *B. ovis* causes infection specifically in sheep, *B. canis* in dogs; *B. neotomae* is found in desert wood rats, *B. microti* in common voles (*Microtus arvalis*), *B. pinnipedialis* in seals, *B. ceti* in dolphins, porpoises and whales; the non-human host of *B. inopinata* is unknown (Atluri et al., 2011); recently, a new species (*B. papionis*) was discovered in baboons (Whatmore et al., 2014). *Brucella* strains isolated from marine mammals have been joined in the species *B. maris* (Cloeckert et al., 2001). In humans, the most important causative bacteria of brucellosis in decreasing order are: *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis* (Lucero et al., 2008).

In animals, the disease affects especially sexually mature mammals, where the infection tends to be localised in reproductive tissues and typically produces placentitis (followed by abortion) in pregnant females and epididymitis and orchitis in males (FAO/WHO/OIE, 2006; Spickler, 2009). Prematurely born, weak calves are also observed (FAO/WHO/OIE (2006)). In some parts of Africa, hygromas and abscesses are the major clinical signs

in nomadic or semi-nomadic cattle herds infected with *B. abortus* biovar 3. In horses, local abscess formation in bursæ may be the only clinical sign and infection, although this species is often asymptomatic (FAO/WHO/OIE, 2006). In cattle, *B. abortus* causes abortions, stillbirths and weak calves; abortions usually occur during the second half of gestation (England et al., 2004; Yamamoto et al., 2008). The placenta may be retained and lactation may be decreased. After the first abortion, subsequent pregnancies are generally normal, and the infected cows are often asymptomatic (Ragan, 2002). In bulls epididymitis, seminal vesiculitis, orchitis and testicular abscesses have been seen (Spickler, 2009). In sheep and goats infected with *B. melitensis* abortion occurs only once, and acute orchitis and epididymitis occur in males. At the end of the disease, it may result in infertility of animals. *B. ovis* affects sheep but not goats and also causes epididymitis, orchitis and impaired fertility in rams where testes may atrophy; few abortions have been registered. In pigs, the most common symptoms are abortion, which can occur at any time during gestation, and weak or stillborn piglets; additionally, swollen joints and tendon sheaths, accompanied by lameness and incoordination, can occur in both sexes (Spickler, 2009; FAO/WHO/OIE, 2006).

In humans, brucellosis is associated with substantial residual disability and has a wide spectrum of clinical manifestation; however depending on the disease stage, fever is the most common feature (Franco et al., 2007; Pappas et al., 2006). In the acute phase, there is acute febrile illness with nonspecific flu-like signs such as undulant fever, headache, malaise, back pain, myalgia and generalised aches. Profuse sweating occurs at night time. Likewise, osteo-articular manifestations such as sacroiliitis, spondylitis, peripheral arthritis and osteomyelitis and chronic fatigue have been observed during the chronic phase of the disease (Franco et al., 2007). In men, additionally genito-urinary complications such as orchi-epididymitis (Ron-Román et al., 2012), glomerulo-nephritis, and renal abscesses have been reported (Franco et al., 2007). Asymptomatic infections are common in humans (Celebi et al., 2007). When a patient is ill from brucellosis, this has a strong impact on the household economy in terms of out-of-pocket contributions to health costs and changes in income (Dean et al., 2012).

Brucellosis has multiple economic implications across agriculture and public health spheres (Zinsstag et al., 2005; Dean et al., 2012). Costs incurred because of brucellosis in humans are related to the treatment, diagnosis, disability time due to loss of work or time devoted to agricultural tasks for farmers. Economic impacts of brucellosis vary depending on the main livestock species, management systems and on the capacity of the country's veterinary and medical systems (McDermott et al., 2013). Higher productivity losses are associated with higher prevalence. For instance, in low-income countries, brucellosis is endemic and neglected, with large disease and livelihood burdens in animals and people. Seropositive animals have higher rates of abortion, stillbirth, infertility and calf mortality, as well as reduced growth and longer calving intervals, and although cows give birth,

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infected cows produce $\pm 10\%$ below their potential (McDermott et al., 2013).

Transmission of brucellosis to humans occurs through the consumption of infected, unpasteurised animal milk products, through direct contact with infected animal parts (such as the placenta causing infection through ruptures of skin and mucous membranes), through the inhalation of infected aerosolised particles and through the accidental injection of live brucellosis vaccines (Pappas et al., 2005; Spickler, 2009). Brucellosis is an occupational disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals and personnel in microbiologic laboratories. Cattle and other Bovidae, *Brucella* is usually transmitted from animal to animal by contact following an abortion (FAO/WHO/OIE, 2006). The mode of transmission is by contact with infected tissues, blood, urine, aborted tissues and foetuses (Ragan, 2002; Gonzalez-Guzman and Naulin, 1994). Additionally, vertical transmission is an important route of transmission for calves and heifers. These heifers do not present seropositivity until they have reached sexual maturity and may abort suddenly (a syndrome known as “Heifer syndrome”) (Dobson and Meagher, 1996; Cheville et al., 1998; Yamamoto et al., 2008; England et al., 2004). Other potential routes of transmission, with little research-based evidence so far, are the possibility of environmental and wildlife reservoirs and sexual contact both in cattle (Cheville et al., 1998; Uhring et al., 2013; Ainseba et al., 2010; Amin et al., 2001) and in man (Ron-Román et al., 2012). Figure 1.1 shows the potential zoonotic routes in brucellosis transmission within animals and for humans.

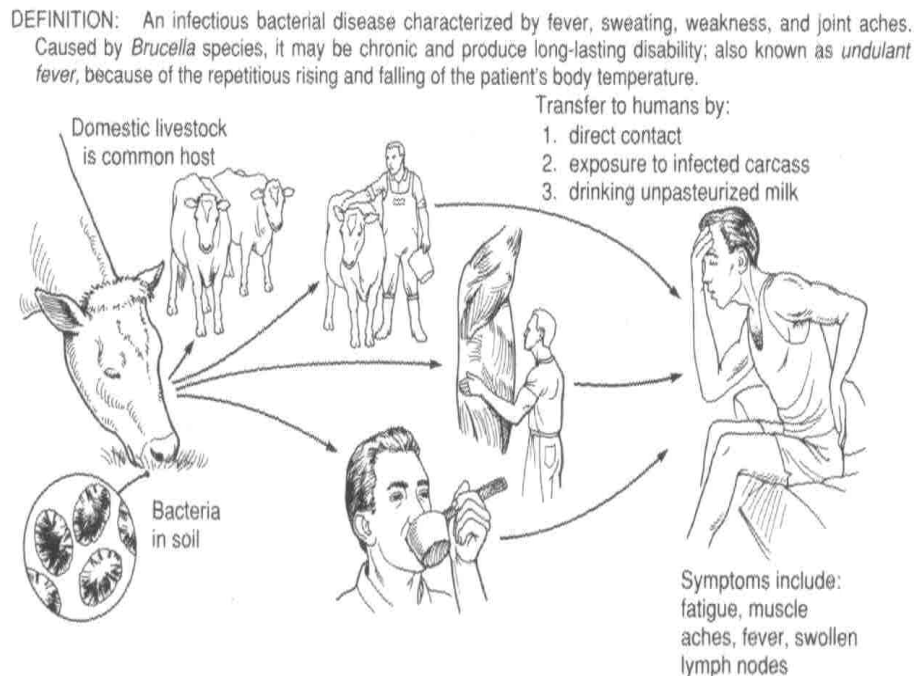


Figure 1.1: Zoonotic routes in brucellosis transmission.
<http://m2002.tripod.com.brucellosis.jpg>

Brucellosis is distributed worldwide, although a lower disease incidence is seen in devel-

oped countries when compared to low- and middle-income countries (Pappas et al., 2006; Spickler, 2009; McDermott et al., 2013). Clinical disease is common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin and the Caribbean (Pappas et al., 2006). *Brucella* species vary in their geographic distribution. For example, *B. abortus* is found worldwide in cattle raising regions, although in the majority of developed countries the disease have been eradicated (Ragan, 2002; Pappas et al., 2006; England et al., 2004). In Mediterranean countries places where sheep and goats are raised *B. melitensis* is the most frequent cause of brucellosis (FAO/WHO/OIE, 2006; Lucero et al., 2008; Pappas et al., 2006). *B. ovis* probably occurs in most sheep-rearing regions of the world and it has been reported from Australia, New Zealand, North and South America, South Africa and many countries in Europe (FAO/WHO/OIE, 2006; Spickler, 2009). In the past, *B. suis* was found worldwide in pig-rearing regions, at present the bacterium continues to occur in domestic herds in some countries of South and Central America and Asia (Spickler, 2009).

Nationwide eradication programs for *B. abortus*, *B. melitensis* and *B. suis* include quarantining infected herds, vaccination, test-and-slaughter and/or depopulation techniques, cleaning and disinfection of infected farms and various forms of surveillance and tracebacks (England et al., 2004; Gonzalez-Guzman and Naulin, 1994; Ragan, 2002; Saegerman et al., 2010; Spickler, 2009; Yamamoto et al., 2008; Zinsstag et al., 2005). Brucellosis may be avoided by employing good sanitation and management practices. The use of approved vaccines (i.e. RB51 and S19 for *B. abortus* and Rev 1 for *B. melitensis*) can increase protection of herds (FAO/WHO/OIE, 2006). Vaccination is an extremely important and effective way to reduce the prevalence in long-term programs (Gonzalez-Guzman and Naulin, 1994). However, vaccination has the disadvantage that its use may confuse diagnosis by serological tests and also because it can cause abortions in vaccinated animals (i.e. Strain 19) (FAO/WHO/OIE, 2006; Ragan, 2002). For eradication purposes, intensive national surveillance and the removal of seropositive animals is required (England et al., 2004; Yamamoto et al., 2008). Surveillance include frequently serological testing, bulk-milk testing, abortion notifications in herds, livestock markets, slaughterhouses, but a system of tracing back and identification is required (Ragan, 2002; England et al., 2004). Disease control and eradication are either probably not feasible or probably not economically justifiable in countries with inadequate veterinary resources, the inability to control livestock movement, widespread brucellosis in feral animals or wildlife and farmers who are not strongly committed to public disease control efforts (McDermott et al., 2013). Additionally, herd additions must come from brucellosis-free areas or accredited herds, as well as certified semen sources; animals from other sources should be isolated and tested before adding them to the herd (McDermott et al., 2013; Ragan, 2002).

1.2.2 *Taenia solium* taeniasis/cysticercosis

Taeniasis and cysticercosis refer to food-borne zoonotic infections with adult and larval stages of tapeworms (*Taenia solium* and *Taenia saginata*) (Murrell, 2005b). In particular, cysticercosis is a tissue infection with the larval stages of *T. solium* or *T. saginata* (called cyst or metacestode) that occurs most commonly in pigs and cattle (Murrell, 2005b). The infection occurs when these animals eat tapeworm eggs. Tapeworm eggs are produced by the adult stages of *T. solium* or *T. saginata* usually released in the environment in human faeces of human tapeworm carriers (Pawlowski et al., 2005). Thus, cysticercosis is, in fact, a faecal-borne infection that is produced by human tapeworm carriers. On the other hand, taeniasis is a food-borne zoonotic parasitic disease where the adult stage (after being a larva or metacestode) develops in the intestine of a human host. Humans acquire the adult stage through eating improperly cooked infected meat (Murrell, 2005b; Flisser et al., 2005; Pawlowski et al., 2005).

Human cysticercosis is also a faecal-borne infection caused by ingestion of *T. solium* eggs dispersed by a human *T. solium* tapeworm carrier, where humans harbour the cyst stage in their tissues, causing cysticercosis (Pawlowski et al., 2005). Neurocysticercosis (NCC) occurs when recently hatched larvae of this parasite migrate to the brain to develop a cyst. This parasitosis has been reported as the most frequent helminthic infection of the central nervous system in humans (Ndimubanzi et al., 2010; Hotez et al., 2008). Figure 1.2 presents the total cycle of life of *T. solium* and the diseases caused by this parasite.

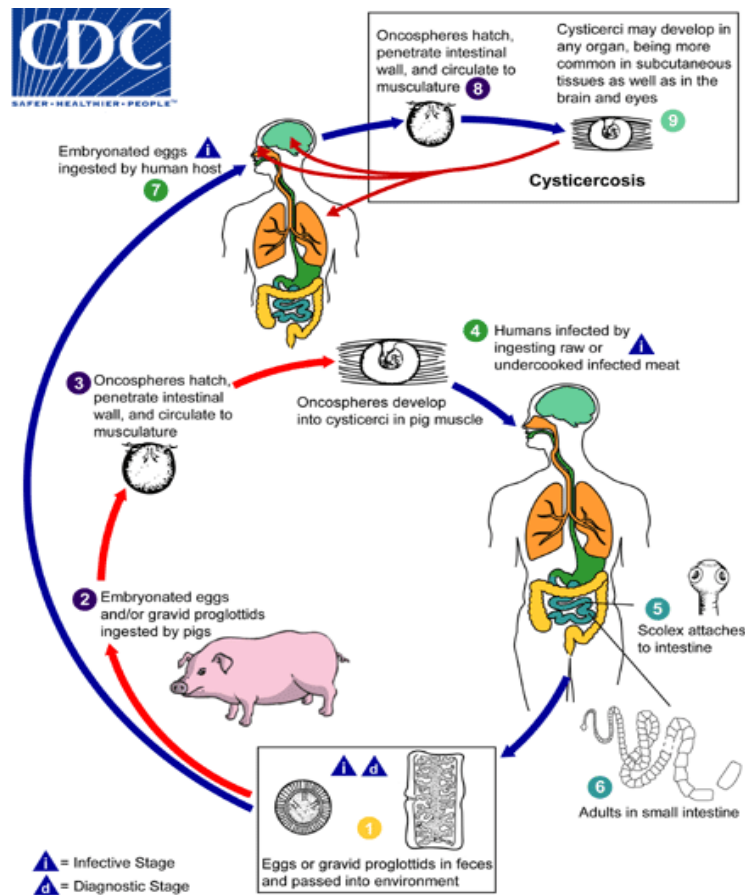


Figure 1.2: Taeniasis/cysticercosis cycle.

<http://www.cdc.gov/parasites/cysticercosis/biology.html>

In humans, the pathology caused by adult intestinal *Taenia* spp. is generally mild and infection is often unnoticed, except when some proglottids (gravid segments of tapeworm) are discharged. In the case of the infection by *T. solium*-cysticerci, any organ and tissue can harbour it, but when the infection is localized in the central nervous system serious pathologies start especially NCC. This disease is one of the main causes of epileptic seizures in many less developed countries (García et al., 2003; Del Brutto et al., 2005). In some cases, it has been observed that some symptoms are non-specific, but in other cases the disease is asymptomatic, which mean that only one part of the disease effects is seen through the NCC cases or even worst through epileptic cases (Nash et al., 2004; García et al., 2003; Pawlowski et al., 2005).

Taenia solium is transmitted mainly in rural areas where pigs have access to untreated human sewage or faeces and infected pork is widely available (García et al., 2003). The tapeworm that causes cysticercosis is found most often in rural zones where with poor hygiene, poor pig management and lack or absence of meat inspection and control (Eddi et al., 2003). But the disease is not only a serious problem in rural communities but also in urban areas where many infected pigs are transported and consumed. For animal cysticercosis, tapeworm carriers in rural areas (pig small-holders in particular) help to

keep the *T. solium* life cycle perpetual, but those living in urban areas with little access to pigs, play a considerable role as a source of human neurocysticercosis (Pawlowski et al., 2005).

Human cysticercosis is a disease associated with poverty where people eat pork and traditional pig husbandry is practiced. It is endemic and an old problem in Latin America where national programs against *T. solium* cysticercosis rarely exist. Likewise, due to the increasing pig production industry and with similar tendency in smallholder pig farms, *T. solium* NCC has become in a serious emerging public health issue in Eastern and Southern Africa and many countries in East Asia. In developed countries, the majority of *T. solium* taeniosis/NCC cases are attributed to immigration and travel (Murrell, 2005a).

The principal economical losses in porcine cysticercosis are due to pigs confiscated in abattoirs when veterinarian inspection exist. Small-scale pork producers usually avoid veterinary inspection and minimise commercial losses associated with infected carcass condemnation in official abattoirs by selling their pigs in clandestine or local markets. For instance, in developing countries nearly to 40% of pork is provided by those meat markets in fairs (Pawlowski et al., 2005; Flisser et al., 2005). Smallholders in poor areas usually do not invest in maintenance of pigs and the pigs are waste-fed or are left to roam freely (Murrell, 2005b). The global economic costs imposed by *T. solium* are difficult to evaluate, but the majority of NCC cases occur in individuals of productive age who are frequently unable to work because of the symptoms. Disability rates due to NCC and epilepsy are considerable but difficult to quantify (Pawlowski et al., 2005). Additionally to the disability costs, economic costs involved in diagnosis (neuro-imaging), medical treatment and loss of working days are of economic relevance (Pal et al., 2000).

Prevention measures like the development of improved sanitation and hygiene practices have a great impact in developing countries (García et al., 2003; Kyvsgaard and Murrell, 2005). Additionally, to avoid the contact between pigs and humans faeces through the adoption of safe animal husbandry practices and the installation of adequate pens for pigs are necessary (piglets must be preventing from escaping). Meat inspection avoids the increase of tapeworm carriers and development of safe slaughtering facilities is necessary in the communities themselves. Chemotherapy or pre-slaughter drug treatment of pigs has been proposed as an effective strategy to interrupt the transmission cycle, thereby reducing the tapeworm infection in humans, although the period prior to slaughter and consumption should be respected to avoid drug residues (Gonzalez et al., 2001; Pawlowski et al., 2005). Another preventive measure is the screening of farmers and farm workers for taeniasis treatment although this measure should be focused towards tapeworm carriers rather than carried out in the general population (Kyvsgaard and Murrell, 2005; Pawlowski et al., 2005). However, the availability of copro-antigen tests and specialised laboratory facilities in field research limit their application (Pawlowski et al., 2005; Dorny et al., 2005). Finally, increasing the role of health education can play a critical role in

prevention and control of cysticercosis (Cao et al., 1997; Pawlowski et al., 2005; Murrell, 2005b). Thus if farmers, rural communities and population in general learn about the consequences of insanitary rearing of pigs, the methods to recognise infected pork and in general the modes of transmission of the disease would make a great effort in prevention of future cases (Pawlowski et al., 2005).

For control and eradication purposes, all the measures mentioned in the prevention of the disease have to be applied, but the detection of tapeworm carriers is a crucial point since the removal of adult tapeworms breaks the parasite's life cycle (Pawlowski et al., 2005). Three of the most important conclusions found in epidemiological studies about *T. solium*/cysticercosis are: (i) the cases are clustered in endemic foci, so that for planning control measures research to identify those clusters is needed; (ii) in endemic areas late onsets of epilepsy is a strong predictor of neuro-cysticercosis and (iii) the detection of tapeworm carriers is very effective in reducing incidence rates. Unfortunately, vaccination as a control measure is not yet available for pigs (García et al., 2003; Pawlowski et al., 2005)

1.3 Effective Population Size and its influence on sample size

A fundamental fact in a closed population (i.e. without immigration), where the presence of only a small number of individuals is maintained over several generations, is that the finite sampling of gametes leads to the depletion of genetic variation (Lande and Barrowelough, 1987). In effect, the phenomenon that is happening is that the number of alleles out of the original population ($2N$) diminishes and after a certain period only one of these original gene copies is preserved, so that the population is fixed for that one allele (Felsenstein, 2015). This phenomenon, known as *random genetic drift*, tends to diminish genetic variation. Figure 1.3, shows a simulation with the effects of genetic drift on a population of 40 individuals when the initial gene frequency was 0.5. As it can be seen, during a period of 80 generations some populations fixed or lost one of the alleles. It is less obvious that the lost alleles are replaced with other alleles which come from other individuals in the population; therefore the replacement of alleles is made with alleles identical by descent (*IBD*), and thus the individuals in each future generation become relatives and consanguineous (inbreeding), so that genetic drift is synonymous with increasing levels of inbreeding.

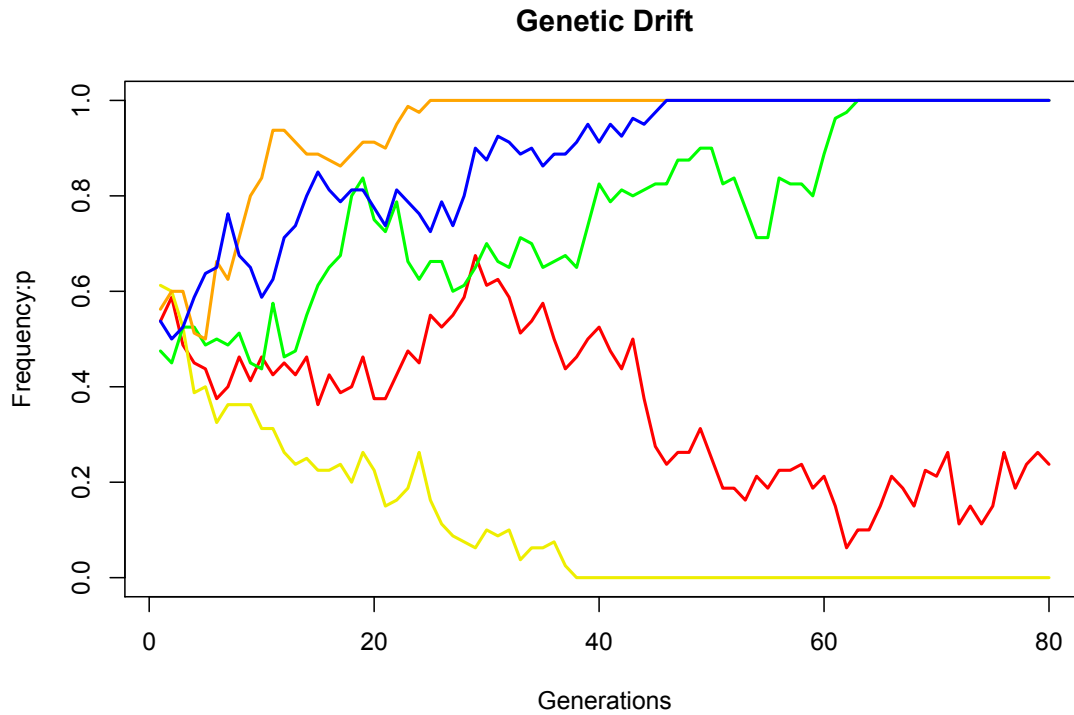


Figure 1.3: Effects of Genetic Drift on a population of 40 individuals and a initial allele frequency of 0.5

In a population, offspring results of the random union of gametes generated in a previous generation, so that the probability that those gametes are *IBD* (inbreeding) is given in the next equation (with f_t = homogeneity in generation t ; $2N$ = size original population):

$$f_t = \frac{1}{2N} + f_{t-1} \left(1 - \frac{1}{2N}\right)$$

The solution of this equation is:

$$f_t = 1 - \left(1 - \frac{1}{2N}\right)^t$$

The probability of not to be *IBD* is $h_t = 1 - f_t$, therefore in each generation the heterozygosity (h_t), falls in a fraction equivalent to $(1 - \frac{1}{2N})$; and the new inbreeding introduced each generation is: $\Delta f = \frac{1}{2N}$. The above model is also known as the *Wright-Fisher Model*. It was developed for an ideal population (i.e. a population with discrete generations where selfing is allowed and the individuals are not differentiated in sexes). In real populations there is a way to find equivalent size to an ideal population that would have the same level of inbreeding as the one we observe in the real population; this measure is know as (inbreeding) Effective Population Size (N_e) (Felsenstein, 2015).

1.3. EFFECTIVE POPULATION SIZE AND ITS INFLUENCE ON SAMPLE SIZE

Thus, the correct way to describe a population size in genetic terms is not the same as the census of animals. The effective breeding number is a measure directly related to the average inbreeding value, and it is an important population descriptor that enables managers to predict if possible problems may occur as the result of inbreeding depression or loss of genetic variance (Tave, 1999). The maintenance of genetic variation in livestock populations is a function that dependen directly on N_e (Brotherstone and Goddard, 2005). When carrying out genetic improvement within a breed livestock owners desire to maximize the intensity of selection by using as few animals as possible as parents of the next generation and therefore N_e tends to be low. This is the case of some cattle breeds where closed herdbooks are used in breeding programs. For instance, in Holstein and Jersey breeds in USA an N_e of 50 has been estimated, which creates rates of inbreeding of 0.2% per year (Brotherstone and Goddard, 2005).

Estimation of effective population size (N_e) when pedigree records are available, can be accomplished by calculating the change in inbreeding (Δf), i.e. by solving the expression $\Delta f = \frac{1}{2N_e}$ (Wright, 1931). When pedigree information is highly incomplete, using the data at hand and making certain assumptions, several formulas are available to estimate N_e (a review of this topic was made by Caballero (1994)). For example, when distinct-parent number is divided by the number of females (N_f) and males (N_m) an estimation of N_e is:

$$N_e \approx \frac{4N_f N_m}{N_f + N_m} + \frac{1}{2}$$

When the population size varies across generations (t), N_e is the harmonic mean of the populations sizes (N_i) (Felsenstein, 2015):

$$N_e = \frac{t}{\sum_{i=0}^{t-1} \frac{1}{N_i}}$$

According to the variation in offspring numbers or gametes surviving, N_e is estimated by:

$$N_e = \frac{4N - 2}{2 + V_n}$$

where V_n is the variance in the number of effective gametes (n_i) generated by the individuals.

Hill (1979) found an expression for the estimation of N_e when populations have overlapping generations and different variances in family sizes of males and females mating in the population:

$$\frac{1}{N_e} = \frac{1}{16ML} \left[2 + V_{mm} + 2 \left(\frac{M}{F} \right) C_{mm,mf} + \left(\frac{M}{F} \right)^2 V_{mf} \right] + \frac{1}{16FL} \left[2 + V_{ff} + 2 \left(\frac{F}{M} \right) C_{fm,fff} + \left(\frac{F}{M} \right)^2 V_{fm} \right]$$

1.3. EFFECTIVE POPULATION SIZE AND ITS INFLUENCE ON SAMPLE SIZE

where M and F are the number of sires and dams in a population, L is the generation interval, and V and C are dispersion parameters of family size (Variances and covariances of male progeny among sires and dams, i.e. V_{mm} variance of male progeny among sires).

Alongside this parameter, inbreeding and kinship describe the genetic structure of the population in terms of phenotypical similarity and resemblance (Felsenstein, 2015). A common mistake consists of overestimating the effective population size when sampling animal populations by considering them as independent samples. Thus, whereas it is common practice to take into account random effects due to common environmental and/or management factors when performing statistical analyses, the genetic background is virtually never accounted for. Samples taken or measured in livestock herds usually are family-based samples, where relatives are very likely to be present and therefore, due to the phenotypical resemblance, similar responses are expected (Lynch and Walsh, 1998). Therefore this resemblance must be taken into account in terms of the genetic structure of populations analysed. This can be the case when an hypothesis about a specific parameter has been formulated.

Testing a null hypotheses about the mean of the population $H_0 : \mu = c$ in contrast to an alternative one-side hypotheses $H_a : \mu > c$ is based on the sample size. The power of a statistical test for a δ difference under normality and sampling independence assumptions for the mean of a population is given for the next expression:

$$Power = 1 - \beta = 1 - P(z < z_{(\beta)})$$

where:

$$z_{(\beta)} = z_{(1-\alpha)} + \frac{\delta\sqrt{n}}{\sigma}$$

and $z_{(1-\alpha)}$, is the cutoff where significance (α) of the test was fixed.

However, for instance, when a population under study is based on half sibs or on cousins (see pedigree structure in Figure 1.4), the statistical power due to genetic resemblance in the characteristic, for a trait with a phenotypical standard deviation of 5 units and a heritability of 0.2 in the trait, are given in Table 1.1a, Table 1.1b, and Table 1.1c:

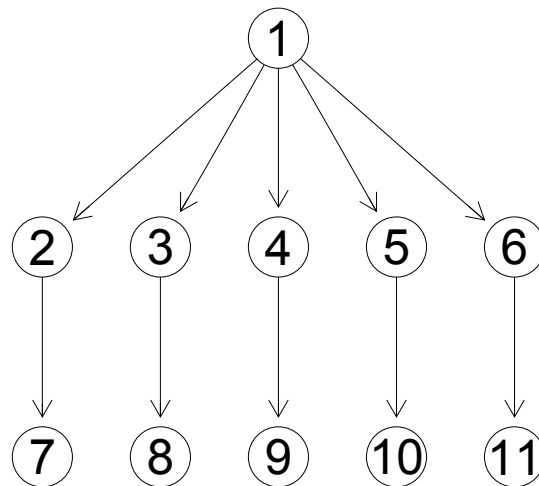


Figure 1.4: Population of cousins with only one common ancestor

The variance estimator of the mean for dependent samples is $Var(\hat{x})$ is estimated as $(\mathbf{X}'\Sigma^{-1}\mathbf{X})^{-1}$, where \mathbf{X} matrix represents the design matrix (in this example a vector of 1's of size n , and n is the sample size), and Σ matrix represents the covariance matrix among the individuals. In this case $\Sigma = \sigma_A^2 \mathbf{A} + \sigma_e^2 \mathbf{I}_n$ where σ_A^2 is additive genetic variance (due to additive effects in the phenotype), and σ_e^2 is the environmental variance due to environmental effects. \mathbf{A} is the matrix of additive relationships among the individuals (1 for non inbred individuals in the diagonal, or $\frac{1}{4}$ for half sibs, and $\frac{1}{16}$ for cousins out the diagonal of the matrix), and \mathbf{I}_n is the Identity matrix of size $n \times n$.

Table 1.1: Statistical Power

| (a) Independent samples | | | | (b) Halfsibs family | | | | (c) Cousins family | | | |
|-------------------------|--------------|--------------|--------------|---------------------|--------------|--------------|--------------|--------------------|--------------|--------------|--------------|
| n | $\delta = 1$ | $\delta = 2$ | $\delta = 5$ | n | $\delta = 1$ | $\delta = 2$ | $\delta = 5$ | n | $\delta = 1$ | $\delta = 2$ | $\delta = 5$ |
| 10 | 0.156 | 0.352 | 0.935 | 10 | 0.130 | 0.272 | 0.828 | 10 | 0.148 | 0.327 | 0.912 |
| 50 | 0.408 | 0.882 | 1.000 | 50 | 0.187 | 0.448 | 0.983 | 50 | 0.300 | 0.719 | 0.999 |
| 100 | 0.639 | 0.991 | 1.000 | 100 | 0.204 | 0.50 | 0.992 | 100 | 0.380 | 0.848 | 0.999 |
| 200 | 0.882 | 0.999 | 1.000 | 200 | 0.214 | 0.524 | 0.995 | 200 | 0.448 | 0.917 | 1.000 |
| 500 | 0.997 | 1.000 | 1.000 | 500 | 0.221 | 0.544 | 0.996 | 500 | 0.507 | 0.954 | 1.000 |
| 1000 | 0.999 | 1.000 | 1.000 | 1000 | 0.224 | 0.550 | 0.997 | 1000 | 0.531 | 0.964 | 1.000 |

1.4 Methods

1.4.1 Analysis of Point Patterns

The interest in analysing spatial point patterns is in whether observed events exhibit any systematic pattern or events are distributed at random way in a specific area. Usually, the

spatial point analysis tries to determine whether sampled points form a spatial aggregation (clusters) or if they have a regular or uniform distribution (Bailey and Gatrell, 1995). In this way, the spatial interaction may be used as an indicator of the interaction between individuals and the environment (Pfeiffer, 2004).

The spatial structure of a phenomenon usually is the result of stochastic punctual process. The point pattern formed with samples taken in field may give information about the initial process and it can be inferred. Actually, the point pattern analysis is founded in the comparison between a theoretical random distribution (usually an homogeneous Poisson process) and the observed distribution. Thus, under the assumptions of isotropy and stationarity, the intensity rate of the process ($\lambda(\mathbf{s})$) can be tested in subregions and the results will give an indication of how the intensity of the process is changing over the total region R . A problem with sub-dividing the counts is that although they may give a global idea of sub-regions with high or low intensity they throw away much of the spatial detail in the observed pattern. A way around this problem is through the use of counts per unit area in a 'moving window', where a suitable 'window' size is moved continuously over the region and the intensity is estimated from the event count per unit area within the 'window' centre on that point. To reduce the complexity in the form and the directions, circular zones are preferred and therefore within a distance h the expected number of events would be $\lambda\pi h^2$. Scanning statistics is commonly used to detect clusters in a point process (Kulldorff, 1997). An important characteristic of a spatial scan test is that it can detect the location of the clusters and make inferences about the hypothesis. For this case, under the null hypothesis, the probability p or the rate of cases inside of a circular area is the same as the probability outside of this circle q , ($H_0 : p = q$).

SaTScan (www.satscan.org) analyses spatial, temporal and space-time data using the spatial, temporal or space-time scan statistics. It was designed to perform geographical surveillance of diseases, to detect spatial or space-time disease clusters and to see if they are statistically significant. Applications include the evaluation of the statistical significance of disease cluster alarms and performing prospective real-time or time-periodic disease surveillance for the early detection of disease outbreaks (Kulldorff, 2015). SaTScan can be used for discrete as well as continuous scan statistics. For discrete scan statistics the geographical locations where data are observed are non-random and fixed by the user. These locations may be the actual locations of the observations, such as houses, schools or ant nests, or it could be a central location representing a larger area, such as the geographical or population weighted centroids of postal areas, counties or provinces. For discrete scan statistics, SaTScan uses a discrete Poisson-based model, where the number of events in a geographical location is Poisson-distributed, according to a known underlying population at risk. Data may be either aggregated at the census tract, zipcode, county or other geographical level or there may be unique coordinates for each observation. SaTScan adjusts for the underlying spatial inhomogeneity of a background population (Kulldorff,

2015).

1.4.2 Regression Models for Count Data

Since data in epidemiology include the collection of nonnegative integer values, several random discrete models may be applied to study the different phenomena observed. The Poisson distribution is often used to model count data (Dobson, 2002; Zeileis et al., 2008). This model is used for counts of events that occur randomly over time or space at a given rate λ (Agresti, 1990). Thus, if a variable y follows a Poisson distribution with parameter λ , it has the following distribution function:

$$f_P(k|\lambda) = Pr(y = k) = \frac{\lambda^k e^{-\lambda}}{k!} \quad \lambda > 0, k = 0, 1, 2, 3, \dots$$

Although Poisson distribution is commonly used for modelling count variables, it is very restrictive because, the mean and variance are the same (λ). However, in the practice, it often happens that variance exceeds the mean, a phenomenon known as *overdispersion*. A commonly used model for such count data is Negative Binomial (NB) model (Tang et al., 2012). This distribution is a binomial experiment describing the number of k failures needed to achieve r successes, where each trial is independent and there is a p probability of success. The following expression describes the distribution:

$$f_{NB}(y = k|p, r) = \frac{\Gamma(r + k)}{k! \Gamma(r)} p^r (1 - p)^k$$

Where $\Gamma(\cdot)$ is a Gamma function. The NB distribution is also often specified by a set of equivalent parameters, $\alpha = \frac{1}{r}$ and $\mu = \frac{1-p}{p}r$, so that the distribution can be written as follows:

$$f_{NB}(y|\mu, \alpha) = \frac{\Gamma(y + \frac{1}{\alpha})}{y! \Gamma(\frac{1}{\alpha})} \left(\frac{1}{1 + \alpha\mu} \right)^{\frac{1}{\alpha}} \left(\frac{\alpha\mu}{1 + \alpha\mu} \right)^y, \alpha > 0, y = 0, 1, 2, 3, \dots$$

Under this parametrisation, the mean and variance of y are: $E(y|\mu, \alpha) = \mu$ and $Var(y|\mu, \alpha) = \mu(1 + \alpha\mu)$. Therefore, the NB model adds a quadratic term to the variance of the Poisson model to account for overdispersion, including that the parameter α has to be bigger than zero (Tang et al., 2012).

The negative binomial distribution can also be viewed as a Poisson distribution where the Poisson parameter (λ) is itself a random variable, distributed according to a Gamma distribution ($\Lambda \sim \Gamma(\alpha, \beta)$); in this way, a negative binomial distribution is known as Poisson-Gamma mixture:

$$\begin{aligned}
P(Y = k|\Lambda = \lambda)g(\lambda) &= \frac{e^{-\lambda}\lambda^k}{k!} \frac{\alpha^\beta}{\Gamma(\beta)} \lambda^{\beta-1} e^{-\alpha\lambda}; \\
P(y = k) &= \int_0^\infty P(y = k|\Lambda = \lambda)g(\lambda)d\lambda \\
&= \int_0^\infty \frac{e^{-\lambda} \lambda^k}{k!} \frac{\alpha^\beta}{\Gamma(\beta)} \lambda^{\beta-1} e^{-\alpha\lambda} d\lambda \\
&= \int_0^\infty \frac{\alpha^\beta}{k!\Gamma(\beta)} \lambda^{k+\beta-1} e^{-(\alpha+1)\lambda} d\lambda \\
&= \frac{\alpha^\beta}{k! \Gamma(\beta)} \frac{\Gamma(k + \beta)}{(\alpha + 1)^{k+\beta}} \int_0^\infty \frac{(\alpha + 1)^{k+\beta}}{\Gamma(k + \beta)} \lambda^{k+\beta-1} e^{-(\alpha+1)\lambda} d\lambda \\
&= \frac{\alpha^\beta}{k!\Gamma(\beta)} \frac{\Gamma(k + \beta)}{(\alpha + 1)^{k+\beta}} \\
&= \frac{\Gamma(k + \beta)}{\Gamma(k + 1)\Gamma(\beta)} \left(\frac{\alpha}{\alpha + 1}\right)^\beta \left(\frac{1}{\alpha + 1}\right)^k \\
&= \binom{k + \beta - 1}{k} \left(\frac{\alpha}{\alpha + 1}\right)^\beta \left(\frac{1}{\alpha + 1}\right)^k; \quad k = 0, 1, 2, \dots
\end{aligned}$$

Zero-inflated models are another model class capable of dealing with excess zero counts (Dobson, 2002; Zeileis et al., 2008). These models have two-component mixture models combining a point mass at zero with a count distribution such as Poisson or negative binomial. The probability of observing a zero count is inflated with probability π to zero inflation part (structural zeros). The parameter π can be model using the covariates for a logistic regression. For instance, a Poisson model with zero inflation probability distribution is given by:

$$\begin{cases} P(Y = 0) & \text{with probability } \pi + (1 - \pi) e^{-\lambda} \\ P(Y = k) & \text{with probability } (1 - \pi) \frac{e^{-\lambda}\lambda^k}{k!}; \quad k = 1, 2, \dots \end{cases}$$

Function `glm()` implemented in **stats** package with the specification that the variance increases in quadratic way (`glm.family = quasi(variance="mu^2", link="log")`) and function `glm.nb()` of **MASS** package (Venables and Ripley, 2002), both implemented in R Core Team (2015), were used to analyse data in Poisson and Negative Binomial models. `bic.glm()` function implemented in **BMA** package (Raftery et al., 1997) was used to average among the 2^p possible models when p potential covariates are included risk analysis, and function `stepAIC()` of **MASS** package was used to choose models by AIC (Akaike Information Criterion) in a stepwise algorithm. To fit ZIP and ZINB models the functions `zeroinfl(formula ~ ., data = dt, dist = "negbin")` were applied within **pscl** package (Jackman, 2008). Algorithms were also ran using `zip` and `zinb` commands

in STATA v.12 software (StataCorp LP, College Station, Texas).

1.4.3 Tree-Based Models

Tree-based methods involve the stratification or segmentation of the predictors or covariates into a number of simple regions (subsets) which explain observations where they belong (James et al., 2014). In this way, a tree is built by the following process: first a single variable is found which best splits the data into groups. Later on, subgroups are generated recursively until no improvement can be made (Therneau and Atkinson, 1997). Using a more formal language, it is necessary to divide the predictor space, i.e. the set of possible values for X_1, X_2, \dots, X_p predictors, into J distinct and non-overlapping regions R_1, R_2, \dots, R_J ; for every observation that falls into the region R_j the prediction is simply the mean of the response values in R_j region. The goal of the regions is to minimise in general the Residual Sum of Squares (RSS) given by (y = observed values; \hat{y} = predicted values):

$$\sum_{j=1}^J \sum_{i \in R_j} (y_i - \hat{y}_{R_j})^2$$

Unfortunately, it is computationally infeasible to consider every possible partition in the feature space (dependent variable); for this reason, an approach is to make a recursive binary splitting. At the beginning, at the top of the tree an algorithm chooses among the predictors, one of them X_j and a cut-point s such that splitting the predictor space into the regions $\{X|X_j < s\}$ and $\{X|X_j > s\}$ leads to the greatest possible reduction in RSS. The first predictor and its cut-point are chosen such that the resulting partition has the lowest RSS. Afterwards, the process is repeated looking for the best predictors and their best cut-points in order to split the data further so as to minimise the RSS within each of the resulting regions. In this way, a decision tree is built; it can be displayed graphically and its results are easy to interpret (James, 1977). Under the R environment R Core Team (2015), the function `rpart()` of the **rpart** package, specifying `method = "poisson"` for count variables was used (Therneau and Atkinson, 1997).

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Chapter 2

OBJECTIVES

2.1 General Objective

The general objective of this thesis is to apply statistical and mathematical tools to support decision making in public and animal health sectors in the context of neglected zoonotic diseases (Brucellosis and Cysticercosis) and lack of knowledge of genetic diversity management that can be present in extensive and small livestock holders systems in developing countries such as Ecuador.

2.2 Specific objectives

- To determine the spatio-temporal distribution of incident human brucellosis cases in Ecuador at municipality level based on reported cases of human brucellosis between 1996 and 2008, and to assess the effects of important risk factors on the space distribution of the disease around the country.
- To determine spatio-temporal distribution in Ecuadorian municipalities with high incidence of hospitalized Neurocysticercosis (NCC) and epilepsy human cases between 1996 and 2008 and to assess the effect of several socio-economic and landscape variables in the macro-epidemiology of NCC and epilepsy at municipality level.
- To develop a mathematical model and a computational user-friendly interface for *Brucella abortus*-brucellosis epidemiological dynamics in dairy cattle herds that cover the majority of insights in the disease transmission and where the model outputs can be used for decision makers without strong mathematical background to facilitate the comprehension in epidemiological and economical terms.

- To provide understandable recommendations about how to avoid the accumulation of inbreeding and genetic drift in livestock populations for supporting decision making for with a few background in genetics but the need of applying genetics and breeding principles to broodstock management.

Experimental section

Chapter 3

APPLICATION OF SCANNING TOOLS AND REGRESSION MODELS IN SURVEILLANCE OF BRUCELLOSIS-HUMAN CASES

Modified from "Spatio-temporal clusters of incident human brucellosis cases in Ecuador". (2013). Lenin Ron-Garrido, Washington Benitez, Niko Speybroeck, Jorge Ron, Claude Saegerman, Dirk Berkvens, Emmanuel Abatih. *Spatial and Spatio-temporal Epidemiology*, 5: 1-10. Reference citation: Ron-Garrido et al. (2013)

3.1 Abstract

This study aimed to determine whether variations in the incidence of reported cases of human brucellosis in Ecuador were clustered in space and time. In addition, the effects of cattle and small ruminant population density and other socio-economic factors on the incidence were investigated. Significant space-time clusters were found in the northern and southern highlands and parts of Ecuadorian Amazonia. Customs of people, cattle, goat, and sheep population density appeared to influence the incidence of brucellosis. In this study, the incidence of reported cases of human brucellosis was found to be higher in highlands (sierra) and in municipalities near to Perú and Colombia. The results of this study highlight the need for prevention and control measures aimed at abating the incidence of brucellosis among livestock and humans.

3.2 Introduction

Brucellosis is one of the world's major zoonosis (Pappas et al., 2006). Four *Brucella* species are mainly responsible for the disease: *B. abortus* typically found in cattle; *B. melitensis* in goats and sheep; *B. suis* in swine; and *B. canis* in raised dogs (Fosgate et al., 2002; Sauret and Vallisova, 2002). Even though these four species of *Brucella* can infect humans; *B. melitensis* has been mentioned as the most pathogenic and frequent in humans (Lucero et al., 2008). Humans contract the disease through consumption of infected and unpasteurized milk and milk products, through direct contact with infected tissues such as placenta and through inhalation of infected aerosolized particles (Pappas et al., 2005). Human brucellosis is associated with chronic debilitating infections and is often characterized by fever of unknown origin, a less specific symptom (Sauret and Vallisova, 2002; Almuneef et al., 2004; Martins et al., 2009).

Brucellosis in cattle and in small ruminants remains a significant animal health problem in many countries (Sauret and Vallisova, 2002; OIE, 2005; D'Orazi et al., 2007; Martins et al., 2009). The disease mainly affects reproduction and fertility in females, reduces survival of newborns, and reduces milk yield (Sewell and Brocklesby, 1990; Zinsstag et al., 2005). In developed countries with prevalence of this disease, strong control measures are implemented for eradication, including intensive national surveillance systems in animals (serologic test) and removal of infected livestock (Yamamoto et al., 2008; Lithg-Pereira et al., 2004; Martins et al., 2009; Lee et al., 2009). However, developing countries rarely have national programs to prevent, control, monitor, and eradicate brucellosis in animal populations.

Ecuador, with nearly 5 million cattle, 1.2 million of sheep, 1.7 million of pigs, and 0.15 million of goats has no structured system for livestock disease management. Slaughterhouses and National Veterinary Service reports frequently include foot and mouth disease cases, distomatosis, metritis, and mastitis in cattle, goats, sheep and pigs that are slaughtered (Anonymous, 2008c). The seroprevalence of bovine brucellosis was officially estimated to range from 1.92% to 10.62% among the provinces in the highlands (Sierra) and from 4.12% to 10.62% among provinces in the Coast (Torres, 2008). The seroprevalence of bovine brucellosis was estimated to be 2.17% and 9.42% using the Rose Bengal Test (RBT) and indirect enzyme-linked immunosorbent assay (iELISA), respectively in Santo Domingo (Pichincha) and 1.08% and 9.73% respectively in El Carmen (Manabí) (Angulo and Tufiño, 2005). Furthermore, in Ecuador, an average of 12 hospitalized human brucellosis cases are reported through the National Office of Statistics (INEC) every year (INEC, 2008).

A Study carried out among farmers in Peru (bordering country of Ecuador) have estimated a brucellosis seroprevalence to be between 1.5% and 4.5% in humans (Mendoza-Nunez et al., 2008), and a pilot study conducted in the northern part of Ecuador estimated

a true prevalence between 24% and 48% in cattle (Ron-Roman, 2003). In both studies, the consumption of unpasteurized milk and milk products, permanent contact with animals, and the occupation of the people were the main factors found to be associated with brucellosis seropositivity among humans. The identification of the municipality and time period with an elevated risk of the infection may contribute to our understanding of the underlying risk factors for the disease. For example, the results of comparing and contrasting clusters with information on the population density of livestock or on the ethnic groups in the population may be used to explain observed clusters (Fosgate et al., 2002; DeChello and Sheehan, 2007).

The aim of this study was to determine the spatio-temporal distribution of incident human brucellosis cases in the continental Ecuadorian territory using municipality level data on reported cases of human brucellosis between 1996 and 2008. This will enable the identification of areas with a high incidence of the disease and also to assess the effects of important risk factors such as ethnicity and cattle, sheep and goat population densities on the space-time distribution of the disease.

3.3 Materials and Methods

3.3.1 Study region and data

The study unit was the municipality. Information on the number of incident human cases of brucellosis and total human population between 1996 and 2008 for each municipality was provided by the National Office of Statistics (INEC, 2008). It was assumed that all cases originated at patients municipality of residence. Brucellosis cases were identified based on presumptive clinical diagnosis. The map showing the political division of the country at municipality level was provided by the agricultural office of geographical information systems (Anonymous, 2008a). Since the data were aggregated at the municipality level, each record was designed to contain the total number of reported human cases, the population, the year and the coordinates of the centroid of each municipality. In addition, the number of cattle, goats, sheep, and swine population by municipality were collected for each year. Information was also available for some potential municipality level risk factors for the presence of human brucellosis: climatic zone (tropical or highlands), percentage of farms with technical assistance (fraction of the number of farms which were visited by veterinarians or agronomist), number of people in the municipality, percentage of indigenous people in each municipality, percentage of grazing land in the municipality, proportion of farms with artificial irrigation in the municipality, percentage of households with tubing water, percentage of people living in extreme poverty and literacy level in the municipality (Anonymous, 2008b). We decided to include the percentage of indigenous people in the municipalities because the majority of indigenous people still live in rural

areas in Ecuador, and because the majority of them are smallholders of land and livestock, so that traditional systems of husbandry and consumption habits of animal products are maintained in those populations.

The study was reviewed and approved by the ethical committee of the Biomedical Center of the Central University of Ecuador (COBI/CBM/UCE).

3.3.2 Zero-inflated Poisson regression model

The data used for this study are reported cases of incident human brucellosis based on hospital records of clinically diagnosed patients and risk factors at municipality level were obtained from national databases. For most of the municipalities, no human brucellosis cases were reported. Therefore, use of the Poisson and negative binomial regression models on this type of data may lead to biased estimations as they do not take account of the overabundance of zeros. To overcome these drawbacks, zero-inflated Poisson (ZIP) models first proposed by Lambert (1992) can be used. The ZIP model accounts for excess zeros by distinguishing between two types of zeros namely structural and random. For the data at hand, structural (true) zeros arose in municipalities where individuals were not predisposed to brucellosis for example not consuming unpasteurized milk products or not involved in occupations that increase their risk of acquiring brucellosis. Random (false) zeros on the other hand are believed to have arisen from confusing brucellosis symptoms with those of other health problems, not seeking treatment due to lack of hospitals or because individuals though subject to activities that expose them to the infection are not infected. To determine which of the ZIP and zero-inflated negative binomial models (ZINB) models best fits the observed data, both models were fit to the data with no covariates and the expected counts were obtained. The model with the fitted counts closest to the observed counts was selected as the most appropriate model to start with (Zaninotto and Falaschetti, 2011). To assess the influence of the selected risk factors (section 3.3.1) on the incidence of reported cases of incident human brucellosis, the ZIP (or ZINB) model models the non-zero counts and those that can be expected under a Poisson model using a Poisson distribution (or Negative Binomial distribution) and the zero counts using a logistic regression model to model the probability of a municipality being in the structural zero group (Long and Freese, 2001). A manual forward stepwise model building approach was employed with the Akaike's Information Criterion (AIC) as the calibrating parameter to select the count part of the final model and Vuong's to determine whether the ZIP (ZINB) model performed better than a standard Poisson regression model (Negative binomial model), again using a manual forward stepwise selection procedure. A significant p-value of this test will indicate that the ZIP (or ZINB) model provides a better fit (Long and Freese, 2001). The models were built using the zip command in STATA, version 12, software (SataCorp LP, College station, Texas).

3.3.3 Regression tree analysis

In addition to the zero-inflated Poisson model, Poisson regression tree analysis (Rosikova et al., 2011) was used to explore the effects of potential socio-economic variables and their interactions on reported brucellosis cases within municipalities. The response variable of interest in this case was the number of brucellosis cases combined with the total population (2 columns matrix) and the explanatory variables used are listed in section 3.3.1. The process of tree building involves determining, for each node, which of the many possible splits best explains the variability in brucellosis incidence risk, and then deciding whether a node should be terminal or should be further split into sub nodes (Breiman et al., 1984). Pruning was applied to obtain a simpler tree in which the splits significantly reduced the variability within subgroups. The R software (R version 2.10.1) package `textitpart` was used with Poisson method as splitting criterion and for generating the trees (Therneau and Atkinson, 1997).

3.3.4 Space-time analysis

The space-time scan software (Kulldorff, 1997) was used to search, test for significance and identify approximate locations of areas with an increased risk for the occurrence of human brucellosis cases. Poisson distributed case incidence with population size as exposure variable was assumed. The search for significant space-time clusters was performed using cylindrical moving windows of variable sizes that moved in space and in time across the study region. The circular base of the cylinder represented the spatial dimension and varied from 0 up to a specified maximum value which allowed for the inclusion of as much as 50%, 25% and 10% of the total number of centroids in the study region respectively. The height of the cylinder represented the temporal dimension with a maximum of up to 50% of the study period with a time precision of 1 year. Assuming for each cylinder that cases were Poisson-distributed (with population size as the exposure factor), space-time clustering was assessed by comparing the incidence risk of brucellosis within the cylinder with the risk expected if brucellosis cases were randomly distributed in space and time. The likelihood ratio for each cluster was calculated based on the number of cases inside and outside the cylinder. The cylinder with the maximum likelihood ratio was selected as the most likely cluster. The significance of identified space-time clusters was tested using the likelihood ratio test statistic and p-values of the test were obtained through Monte Carlo simulations. In this study, 999 simulations were used and significance was arbitrated at the 5% level. Only secondary clusters that do not overlap with the most likely cluster were requested from the SaTScan Software. It is worth noting that, it is possible to detect smaller sized clusters when the maximum scanning window is set at 50% if they have a greater likelihood. The smaller sized windows were therefore applied to explore more localized clusters by eliminating larger clusters which could have a higher

likelihood ratio. The resulting space-time clusters were contrasted with raster geographic data of livestock density in municipalities found in Geonetwotk generated by the FAO-AGA project FAO/AGRA (2009). All significant clusters were visualized using Manifold System (version 8) (CDA International Ltd.).

3.4 Results

A total of 163 reported cases of human brucellosis between 1996 and 2008 in the 217 municipalities of Ecuador were analyzed. One case reported in Galapagos Islands in 2002 was not included in the analysis since the island is detached from the national territory and because with one case alone, a separate analysis for the island could not be done. Figure 3.1 shows the reported incidence risk of human brucellosis between 1996 and 2008 for Ecuador. Overall, the reported incidence risk of brucellosis was observed to vary across the years with peaks in 1996, 2000 and 2007. In addition, a steeply increasing trend was observed from 2002 to 2007.

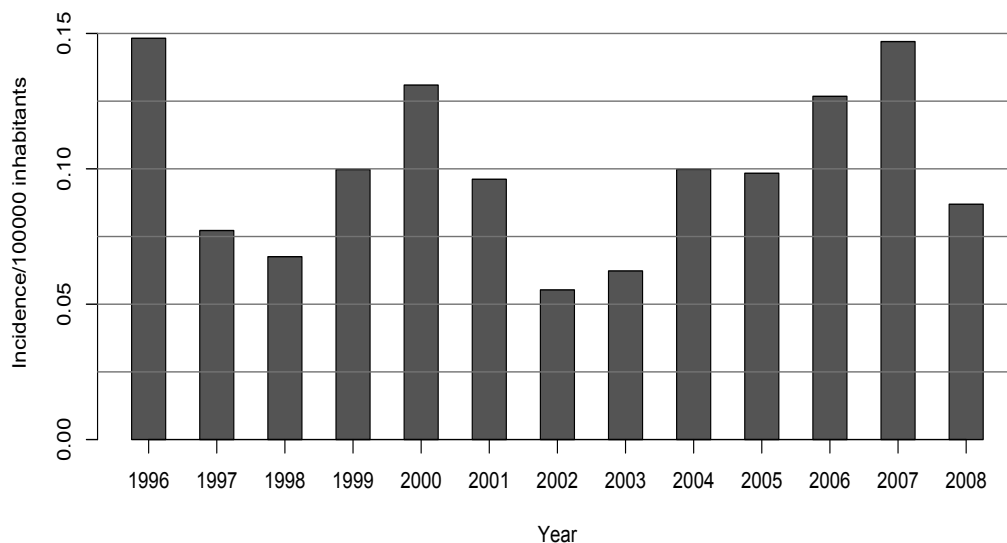


Figure 3.1: Incidence of reported cases of human brucellosis per 100.000 inhabitants in Ecuador between 1996 and 2008.

The plot of the distribution of the observed and expected counts revealed that the ZINB was underestimating the number of excess zeros and over estimating the number of 1's whereas the ZIP model provided expected counts closer to the observed number of reported human brucellosis cases (Figure 3.2). The ZIP model was therefore considered during the model building process. Vuong's test suggested that the ZIP model performed better than the standard Poisson regression model ($Z = 3.59, P - value < 0.001$). Based

on the final model, the number of cattle, the number of sheep, the number of goats and the climatic zone (tropical or highlands) had significant relationships with the incidence of reported cases of human brucellosis and no significant relationships with the probability of not having human brucellosis Table 3.1.

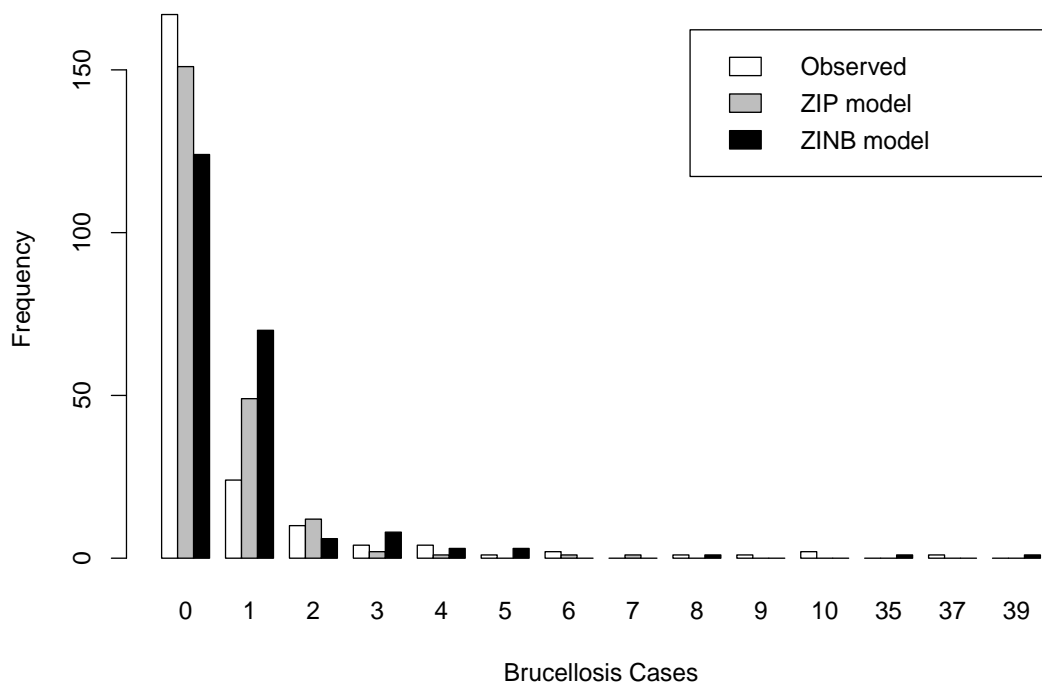


Figure 3.2: Distribution of observed cases by municipality, expected cases based on a zero-inflated poisson (ZIP) model and those based on zero-inflated negative binomial (ZINB) model of human brucellosis in Ecuador between 1996 and 2008. There were 167 municipalities based on observed data (about 77%), 151 based on the ZIP model and 124 based on the ZINB model with no reported cases of human brucellosis and the remaining municipalities had between 1 and 39 cases.

It can be seen from the results that whereas the number of sheep; number of goats and the climatic zone or origin of the cases had significant effects on the expected incidence of reported human brucellosis cases, they had no influence on the probability of the absence of human brucellosis. On the other hand, the number of cattle seemed to influence both the presence of human brucellosis and the probability of its absence. However, the coefficient is positive for the count model part and negatives for the logistic regression model part. This is an indication that an increase in the number of cattle is associated with a corresponding increase in the expected incidence of human brucellosis whereas an increase in the number of cattle leads to a decrease in the odds of a municipality not having human brucellosis.

Among the municipalities that were exposed to brucellosis, the expected incidence of reported cases of human brucellosis was 1.64 times higher for those municipalities in the

highlands as compared to those in the tropics keeping all other factors constant. On the other hand, the incidence of reported cases of human brucellosis appeared to increase by a factor of 1.13 with an increase in the bovine population whereas the incidence decreased by a factor of 0.77 for each unit increase in the number of sheep.

Table 3.1: Parameter estimates, their 95% confidence intervals and expected incidence of a zero-inflated Poisson model for the number of human brucellosis hospitalized cases in Ecuador between 1996-2008.

| | Estimate | 95% C.I. | p-Value | $e^{b \cdot se^*}$ |
|--|----------|-----------------------|---------|--------------------|
| <i>Count part: Poisson model</i> | | | | |
| Intercept | -4.65 | (-5.04,-4.27) | < 0.001 | |
| Climatic zone (Highlands vs. tropical) | 1.50 | (1.45,1.55) | < 0.001 | 1.64 |
| Number of cattle | 0.000005 | (0.000001, 0.000008) | 0.007 | 1.13 |
| Number of sheep | -0.00002 | (-0.00003, -0.00001) | 0.001 | 0.77 |
| Number of goats | 0.00013 | (0.00003, 0.00024) | 0.013 | 1.96 |
| <i>Inflated part: Logistic model</i> | | | | |
| Intercept | 1.00 | (0.25,1.76) | 0.009 | |
| Climatic zone (Highlands vs. tropical) | 0.58 | (-0.35, 1.51) | 0.219 | 1.33 |
| Number of cattle | -0.00003 | (-0.00005, -0.000003) | 0.023 | 0.53 |
| Number of sheep | -0.00002 | (-0.00006, 0.00002) | 0.333 | 0.75 |
| Number of goats | 0.0001 | (-0.00014, 0.00033) | 0.416 | 1.64 |

* $e^{b \cdot se}$: factor change in expected count for a one standard deviation increase in the value of interest for those municipalities having human brucellosis (Poisson model part) or for those not having the disease (Inflated part).

According to the results obtained from the SaTScan software, there were significant circular space-time clusters with a maximum of 50% and 25% of the total centroids included in the scanning window respectively, a maximum of up to 50% of the study period included and a time precision of 1 year. The results obtained using a maximum of 10% of the data in the scanning window were exactly the same as those obtained with 25% of the centroids included in the scanning window. Only the results with 25% of the centroids included in the scanning window were discussed. The most likely space-time cluster (p -value < 0.001) based on the circular window with 50% of the centroids in the scanning window spanned the time frame between 2004 to 2008 with 12 human brucellosis cases where only 1 case was expected (Table 3.2). The relative risk of this cluster was 10.4 indicating that humans in this region are 10 times more likely to get infected with brucellosis as compared to the rest of the study region and time period not included in the cluster. Two significant secondary clusters were also reported with one of the secondary clusters covering about 50% of the entire study region. This cluster is located in the southern part of the country, near the boundary with Perú. More localized clusters were obtained when only up to a maximum of 25% of the centroids were included in the circular scanning window. The large cluster previously obtained with the window size of 50% was observed to dissociate into 4 smaller clusters with a window size of 25% (Figure 3.3). The

primary cluster however remained unchanged (Table 3.2). Likewise, Figure 3.3 shows the brucellosis status of cattle farms carried out in 14 provinces based on 2000 farms and 20000 animals.

Table 3.2: Characteristics of significant space-time clusters of human brucellosis in Ecuador between 1996-2008 with up to a maximum of 50% and 25% of the centroids included in the circular scanning window respectively.

| Window size (%) | Type | Municipalities | Period | O(E) | RR | p-Value |
|-----------------|-----------|---|-----------|-----------|------|---------|
| 50 | Primary | Nangaritza, Palanda, Zamora, Loja, Yantzaza, Centinela del Cóndor, Quilanga | 2004-2008 | 12 (1.2) | 10.4 | 0.001 |
| | Secondary | Aguarico, Cuyabeno, Shushufindi, Joya de los Sachas, Orellana, Arajuno, Lagoagrio, Pastaza, Loreto, Cascales, El Chaco, Quijos, Gonzalo Pizarro, Santa, Clara, Archidona, Carlos Julio Arrocemena, Tena, Sucumbios, Tulcán, San Pedro de Huaca, Bolívar, Mira, Montúfar, Espejo, Pimampiro, Ibarra, Antonio Ante, Otavalo, Urcuquí, Cotacachi, Cayambe, Quito, Pedro Moncayo, Rumiñahui, Mejía, San Miguel de los Bancos, Mera, Baños, Patate, Ambato, Santiago de Pillaro, Mocha, Cevallos, Quero, Tisaleo, San Pedro de Pelileo, Salcedo, Latacunga, Pujilí, Sigchos, Saquisilí, Colta, Guano, Riobamba, Penipe, Guamote, Chambo, Palora, Taisha, Humaboya, Pablo VI, Morona, Sucúa, Logroño, Tiwinza, Santiago | 2003-2008 | 57 (27.9) | 2.6 | 0.002 |
| | Secondary | Milagro, Yahuachi | 2001 | 5 (0.2) | 27.0 | 0.025 |
| 25 | Primary | Nangaritza, Palanda, Zamora, Centinela del Cóndor, Loja, Quilanga, Yantzaza | 2004-2008 | 12 (1.2) | 10.4 | 0.001 |
| | Secondary | Archidona, Quijos, Tena, Carlos Julio Arrosemena, El Chaco | 2007-2008 | 6 (0.2) | 32.2 | 0.002 |
| | Secondary | Tulcán | 2003-2008 | 7(0.5) | 14.8 | 0.017 |
| | Secondary | Milagro, Yahuachi | 2001 | 5 (0.2) | 27.0 | 0.025 |
| | Secondary | Cevallos, Quero, San Pedro de Pelileo, Patate, Ambato, Baños, Mocha, Tisaleo, Colta, Chambo, Guano, Alausí, Riobamba, Penipe, Guamote, Pallatanga, Guaranda, Chimbo, San Miguel, Mera, Palora, Pablo VI | 2003-2006 | 16 (4.0) | 4.4 | 0.048 |

O stands for observed; E for expected and RR for relative risk.

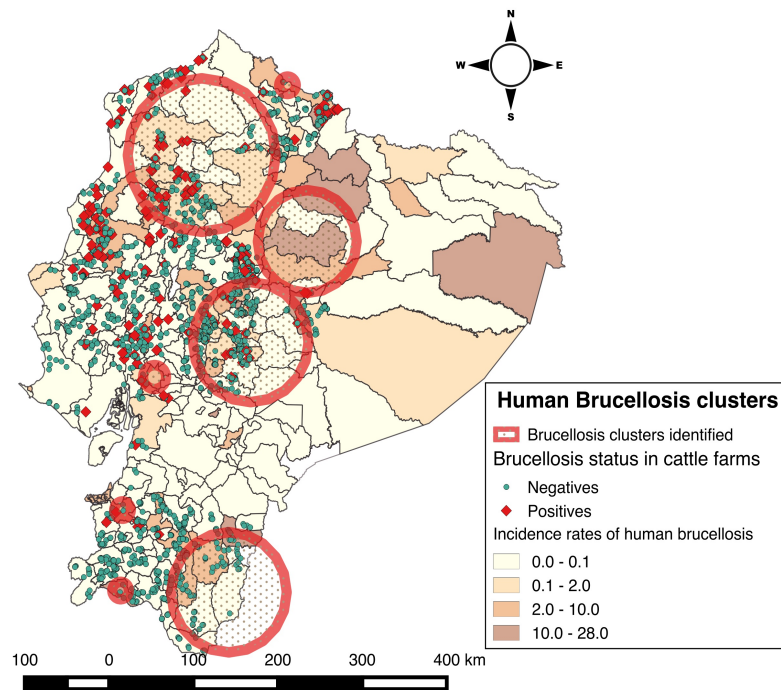


Figure 3.3: Human Brucellosis clusters identified and Positive Brucellosis herds. Space clusters of human brucellosis cases with a maximum of 25% of the total centroids used in the circular scanning window between 1996 and 2008.

Figure 3.4 shows the map of the cattle density and the significant space-time clusters. It can be seen from the map that cattle were mostly found on the western part of the country. The location of the significant cluster in the northern part of the country was found to coincide with areas of high cattle population density. The significant clusters in the southern and central parts of the country only partially featured in areas with high cattle population density. The low cattle population densities in the eastern part of the country reflect the limited cattle breeding activities in the Amazonia. Just as for the case with cattle, small ruminant density seems to be highest in the western part of the country (Figure 3.5). However, the significant clusters in the northern, central and southern parts of the country did not coincide with areas of high small ruminant population density. This is an indication that the observed high incidence of human brucellosis maybe due in part to high cattle population density. Later on, the decision tree model evaluated this result.

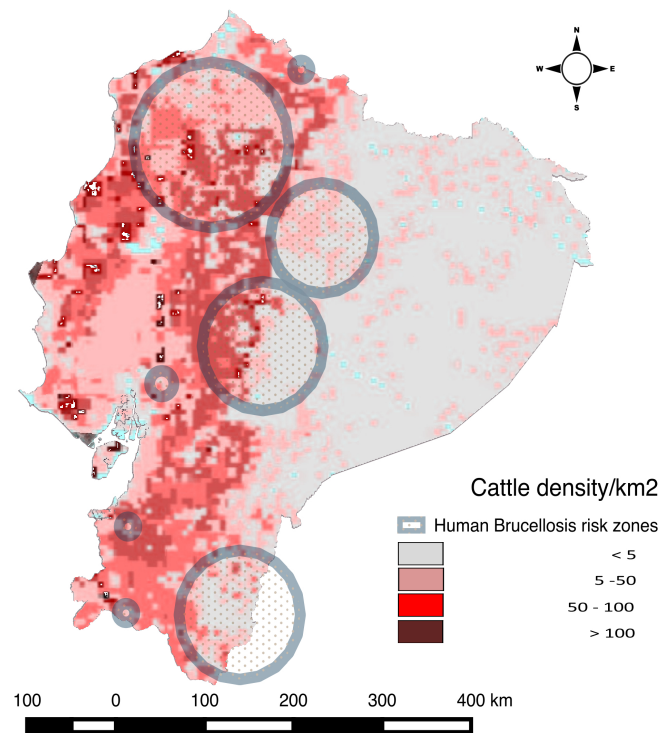


Figure 3.4: Possible effects of cattle density on the space-time distribution of human brucellosis cases based on circular clusters.

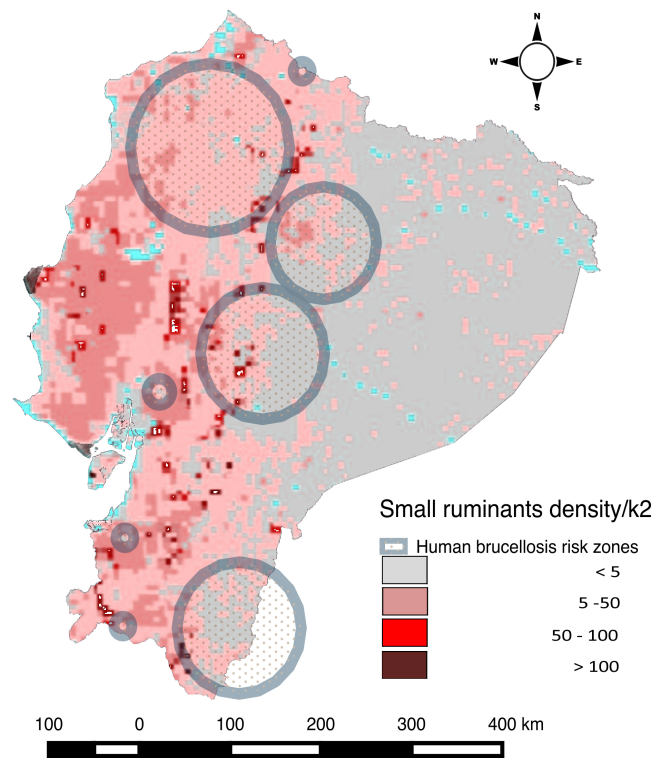


Figure 3.5: Possible effects of small ruminants density on the space-time distribution of human brucellosis cases based on circular clusters.

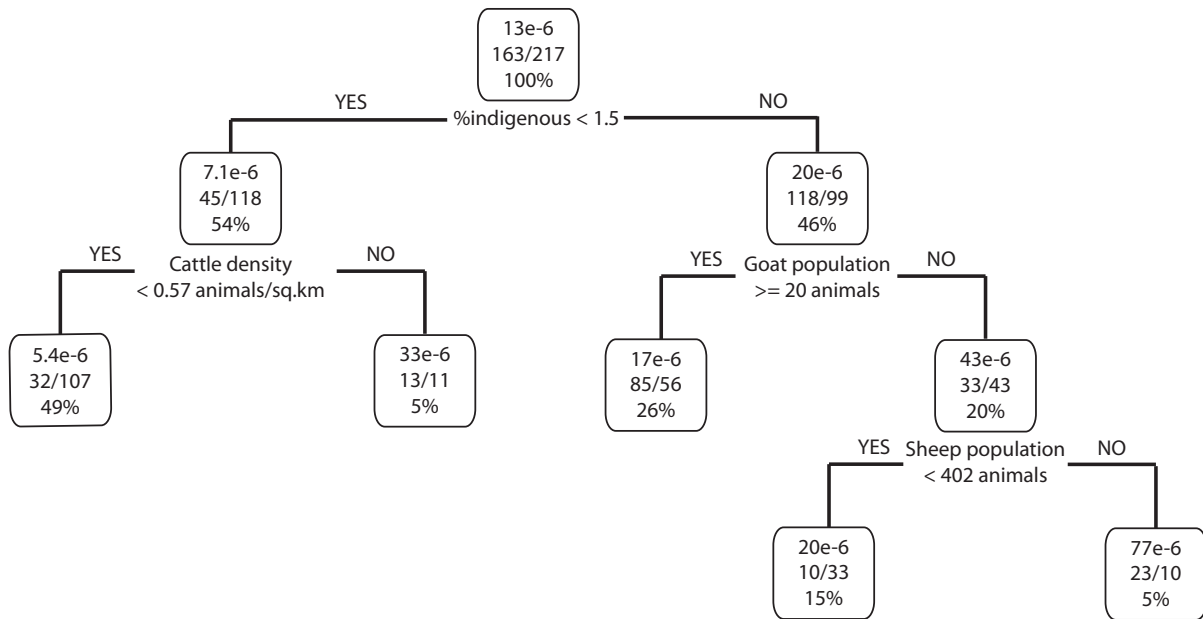


Figure 3.6: Regression tree analysis for human brucellosis incidence risk in Ecuador using several socio-economic and demographic variables in 217 municipalities. At each node, the first line represents the incidence and the second line represents on the lefthand side the number of human cases and on the righthand side the number of municipalities from the parent node for which the splitting criterion is true. Each node also has the percentage of municipalities having the given criteria.

The application of the regression tree methodology to study the relationship between the incidence risk of brucellosis and several socio-economic and demographic features of the municipalities revealed that only 6 out of 42 variables play an important role in brucellosis dynamics (Figure 3.6). Of these, the percentage of indigenous population appeared to be the most important splitting variable. For municipalities where the percentage of indigenous population was less than 1.5, the incidence risk depended on the density of cattle in the population. When the cattle population density was greater than 0.57, the incidence was 3.3 whereas it was 0.54 when the population density was lower than 0.57. On the other hand, for municipalities where the % of the indigenous people was greater than 1.5, the incidence depended on the number of goats in the municipality. When the number of goats was less than 21, the incidence was 1.7 whereas when the number of goats in the municipality was greater than 21, the incidence was determined by the number of sheep in the municipality. For municipalities where the number of sheep was greater than 402, the incidence was 7.7. With less than 402 sheep, the incidence was further determined by the proportion of farms with artificial irrigation in the municipality. Municipalities with more than 5% of the farms with artificial irrigation presented a higher incidence (4.1) as compared to municipalities where less than 5% of the farms had experienced artificial irrigation (0.3).

3.5 Discussion

In this paper, an analysis of the space-time distribution of human brucellosis cases was performed to identify areas with high risks of the disease. We also investigated the effects of cattle and small ruminant densities on the space-time distribution of human brucellosis cases. In addition, the effects of socio-economic variables and their interactions at the municipality level on the expected incidence of reported cases of human brucellosis were investigated using the zero inflated Poisson regression and regression tree analyses. The presence of brucellosis among humans may be an expression of a more widespread problem among livestock (Omer et al., 2002; Sauret and Vallisova, 2002; Avdikou et al., 2005; John et al., 2010).

The most significant cluster was observed in the northern part of the Ecuador. It is located in the highlands and at the beginning of the Amazonia region where dairy cattle and sheep are raised. The cluster existed between 2003 and 2008, a time period which coincided with the period of steep increase in the incidence of brucellosis. Parts of this cluster lie in the boundary between Ecuador and Colombia. This cluster should therefore be reevaluated in order to confirm the species most frequently found in these areas. This is so because *B. suis* is endemic in Colombia (Arambulo, 1998; Lucero et al., 2008). One of the secondary clusters was located in the southern part of the country bordering Peru. This cluster existed between 2004 and 2008 which also ties with the period of steep increase in the incidence of brucellosis. Given that *B. melitensis* is endemic in Peru (Mendoza-Nunez et al., 2008), the closeness of this cluster to the boundary begs for an investigation of the presence of *B. melitensis* in order to determine whether the increased incidence might have been due to transborder infections. Spread of brucellosis across countries has been well documented in places where illicit movements of flocks and of animal products are permitted (Avdikou et al., 2005).

In the Mejía municipality in the South of Pichincha province, the disease in cattle has been considered as endemic for *B. abortus*, in spite of the continuous vaccination (Gonzalez, 2008). In this municipality there is at least 1 hospitalized brucellosis case every year. The presence of brucellosis should be confirmed in places where the disease is no longer reported, such as El Carmen in Manabí province, Macará in Loja and Milagro in Guayas Province. Many studies have reported that surveillance in places where the disease has apparently disappeared can indicate sporadic re-emergence (Pappas et al., 2006; Zinsstag et al., 2005; Abernethy et al., 2006). It is important to point out that the rates reported here are based only on hospital cases. As it is known, human brucellosis has a wide spectrum of clinical manifestations, and asymptomatic cases are very common in the acute form of the disease (Sauret and Vallisova, 2002; Gonzalez-Guzman and Naulin, 1994; Almuneef et al., 2004; Hasanjani et al., 2004; Cutler et al., 2005). Therefore only a small percentage of the patients go to the hospitals.

There was evidence of a correlation between the significant space-time clusters of human brucellosis cases and the distribution of cattle and small ruminant density. The results from the ZIP model and regression tree analysis further confirmed that cattle and small ruminant density were important factors for the infection. De Massis et al. (2005) demonstrated that in Italy, human brucellosis was more widespread in areas where the prevalence of infection in sheep and goats was highest. Furthermore, using regression analysis they showed that the relationship between human and animal infections between 1997 and 2002 was statistically significant (De Massis et al., 2005). This is therefore an indication that, abating the prevalence in animals will reduce the risk of human infection. The Poisson regression tree analysis also indicated that the percentage of the indigenous population in the municipality was the most important risk factor for human brucellosis in Ecuador. The high influence of this factor is manifested in the dietary habits of the population such as consumption of unpasteurized milk or cheese, and also the type of herd management practices. Other studies have identified the composition of the population as an important risk factor for human brucellosis (Fosgate et al., 2002).

In conclusion, the variations in the incidence rates of brucellosis were clustered in space and time with significant clusters in the northern and southern highlands and parts of the Ecuadorian Amazonia. Customs of people, cattle density, and goat and sheep populations were found to be the main factors influencing the dynamics of brucellosis within municipalities. Since a reduction of the disease in livestock will reduce the incidence in humans (Saegerman et al., 2010), specific programs for prevention, control and eradication such as vaccination should be implemented by national authorities in the livestock production industry in Ecuador. In addition, public sensitization campaigns on the epidemiology of brucellosis should be launched in order to better educate the indigenous population on the epidemiology of the disease and on the risks of consuming unpasteurized milk products. Finally, it is recommended to investigate more suspected brucellosis human cases, especially with blood cultures and subsequent biotyping of *Brucella* strains to obtain more detailed epidemiological information concerning the circulating *Brucella* strains among humans in relation with available data among animals.

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Chapter 4

APPLICATION OF SCANNING TOOLS AND REGRESSION MODELS IN SURVEILLANCE OF HUMAN CYSTICERCOSIS CASES

Modified from “Distribution and Potential Indicators of Hospitalized cases of Neurocysticercosis and Epilepsy in Ecuador from 1996 to 2008”. (2015). Lenin Ron-Garrido, Marco Coral-Almeida, Sarah Gabriel, Washington Benitez O., Claude Saegerman, Pierre Dorny, Dirk Berkvens, Emmanuel Abatih.

Reference citation: Ron-Garrido et al. (2015)

4.1 Abstract

4.1.1 Background

Epilepsy is one of the most common signs of Neurocysticercosis (NCC). In this study, spatial and temporal variations in the incidence of hospitalized cases (IHC) of epilepsy and NCC in Ecuadorian municipalities were analyzed. Additionally, potential socio-economic and landscape indicators were evaluated in order to understand in part the macro-epidemiology of the *Taenia solium* taeniasis/cysticercosis complex.

4.1.2 Methodology

Data on the number of hospitalized epilepsy and NCC cases by municipality of residence were obtained from morbidity-hospital systems in Ecuador. SatScan software was used

to determine whether variations in the IHC of epilepsy and NCC in space and time. In addition, several socio-economic and landscape variables at municipality level were used to study factors intervening in the macro-epidemiology of these diseases. Negative Binomial regression models through stepwise selection and Bayesian Model Averaging (BMA) were used to explain the variations in the IHC of epilepsy and NCC.

4.1.3 Principal findings

Different clusters were identified through space and time. Traditional endemic zones for NCC and epilepsy, recognized in other studies were confirmed in our study. However, for both disorders more recent clusters were identified. Among municipalities, an increasing tendency for IHC of epilepsy, and a decreasing tendency for the IHC of NCC were observed over time. In contrast, within municipalities a positive linear relationship between both disorders was found. An increase in the implementation of systems for eliminating excrements would help to reduce the IHC of epilepsy by 1.00% ($CI_{95\%}$; 0.2% - 1.8%) and by 5.12% ($CI_{95\%}$; 3.63%-6.59%) for the IHC of NCC. The presence of pig production was related to IHC of NCC.

4.1.4 Conclusion/Significance

Both disorders were related to the lack of an efficient system for eliminating excrements. Given the appearance of recent epilepsy clusters, these locations should be studied in depth to discriminate epilepsies due to NCC from epilepsies due to other causes. Field studies are needed to evaluate the true prevalence of cysticercosis in humans and pigs in different zones of the country in order to better implement and manage prevention and/or control campaigns.

4.2 Introduction

Humans are the definitive hosts of *Taenia solium* harboring the intestinal adult tapeworm, which causes taeniasis (Lescano et al., 2009; Flisser et al., 2005). Humans acquire the tapeworm through consumption of improperly cooked infected pork. The intermediate pig host gets infected by ingestion of parasite eggs, passed in the stool of a tapeworm carrier. The metacestode larval stage establishes in the pig's muscles, brain and other tissues (cysticercosis) (Flisser et al., 2005). Unfortunately, humans can also serve as dead-end intermediate hosts by accidentally ingesting parasite eggs and developing the metacestode larval stage (Flisser et al., 2005). In humans, the parasite tends to locate in the central nervous system (neurocysticercosis (NCC)) causing a variety of neurological symptoms, such as seizures, headache and in many cases epilepsy (Mafojane et al., 2003; Pawlowski et al., 2005; Pondja et al., 2010).

In developing countries, NCC is often an underrecognized and neglected disease (Foyaca-Sibat et al., 2009; Praet et al., 2009). The large variety of clinical signs and symptoms, and the inaccessibility of highly sensitive tests, like Computerized Tomography (CT) or Magnetic Resonance Imaging (MRI) (due to high costs involved and the unavailability of neuroimaging facilities), have contributed to the underreporting of NCC.

Different measures such as education, improvement of household sanitation, changes in meat inspection practices, identification and treatment of tapeworm carriers, mass drug administration and modifications in pig-rearing methods have proven, at least at short term, to be effective in lowering levels of transmission of NCC (Keilbach et al., 1989; Sarti et al., 2000; Morales et al., 2008). However, practices like free roaming pig management, clandestine marketing of living pigs and pork, open defecation, and the use of residual waters in irrigation (Murrell, 2005; Morales et al., 2008), make the disease still prevalent in many regions.

In developing countries, *T. solium* NCC has been found to be the leading cause of acquired epilepsy. In fact, NCC was found to be responsible for at least half or a third of acquired epilepsy cases (Cruz et al., 1999; Ndimubanzi et al., 2010). In Ecuador, an average of 480 and 1670 patients are hospitalized each year because of NCC and epilepsy, respectively INEC (2008). Notification of hospitalized cases for both disorders is mandatory in Ecuador. Tools for diagnosing causes of acquired epilepsy, including parasitic and infectious diseases are available in the country; however, these diagnostic tools are not regularly used because of their high costs, thus, as in other NCC endemic countries, in Ecuador more than 82% of epilepsies do not have definitive diagnosis and the cause remains unknown (Mwape et al., 2015; INEC, 2008; Preux and Druet-Cabanac, 2005; WHO, 2015).

Symptomatic and asymptomatic cases of NCC have been studied in urban (Kelvin et al., 2012; Goodman et al., 1999; Alarcón and Del Brutto, 2012) and rural (Cruz et al., 1989; Coral-Almeida et al., 2014; Rodríguez-Hidalgo et al., 2003, 2006; Praet et al., 2009; Cruz et al., 1999) areas of Ecuador in different epidemiological studies. Endemic areas were found mostly in the highlands, where the population has a high exposure to the parasite as measured by specific antibody detection. Up to one third of the population is seropositive in some of these areas Coral-Almeida et al. (2014). In some endemic communities in these highland areas, human cysticercosis active infections, as measured by antigen detection are present in up to five percent of the population (Rodríguez-Hidalgo et al., 2003). However, in the past three decades, a decreasing trend in the incidence of hospitalized cases (IHC) of NCC has been observed in the country, apparently due to an improvement in sanitary conditions and the use of better diagnostic tools (Alarcón and Del Brutto, 2012).

Epidemiological surveillance data in the Ecuadorian health status reports only include ambulatory cases from the public health care system. The public registers from the Min-

istry of Public Health are the only official health reports available. The consultations at any level attended in the public health system have been estimated to be around 30% of all the medical consultations in the country (Flores and Castillo, 2012). However, for the ambulatory cases the reporting is not always done properly, mainly due to the lack of synchronization among the different types of health care services in Ecuador (MSP, 2015). Figure 4.1 explains the reporting system for NCC and epilepsy cases in Ecuador. Additionally, and in a parallel way, the National office of Statistics INEC (2008)(INEC) collects all information on morbidity registered in hospitals and clinical centres (from public, private or social security systems) where patients are attended at the secondary health care level and also in case of emergencies irrespective of their underlying sources. In these registers, both NCC and epilepsy are frequent causes of hospitalization. Thus, epidemiological data generated by the Ecuadorian office of statistics offers the possibility of studying important variables related to the macro-epidemiology of NCC and epilepsy in Ecuador. Here, we use macro-epidemiology in terms of the determinants of disease, including economic, social, and climatological factors into national patterns in risk assessment (Hueston and Walker, 1993).

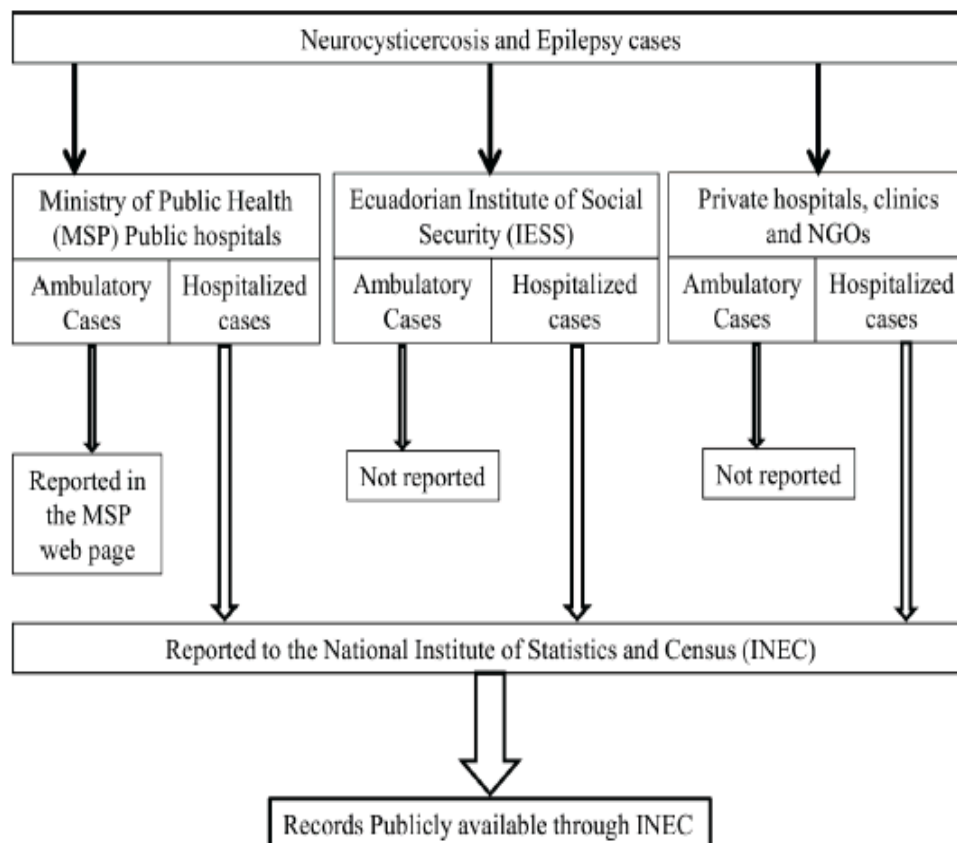


Figure 4.1: Neurocysticercosis and epilepsy reporting flow chart in the Ecuadorian health system.

NGO: Non-governmental organization

In this study we aimed at identifying areas (municipalities) with a high IHC of NCC and epilepsy between 1996 and 2008. In addition, given the fact that the IHC of NCC and epilepsy have been related to several socio-economic, and landscape variables, we evaluated the macro-epidemiology of epilepsy and NCC in Ecuador at the municipality level. Finally, spatio-temporal analysis was implemented in order to investigate the distribution of the IHC of epilepsy and NCC in Ecuador.

4.3 Materials and Methods

4.3.1 Ethics statement

Ethical approval was not required for this study. All information used in this study came from public sources freely available on the referenced websites. Reports of human cases belonged to the public health surveillance system, the anonymity of clinical histories is guaranteed by legal mandate.

4.3.2 Study region and data

The study unit was the municipality. The number of patients hospitalized in different institutions with diagnosis of epilepsy and NCC, from 1996 to 2008 was obtained from hospital morbidity and mortality databases managed by the National Office of Statistics (INEC, 2008). This time period was chosen because of the availability of digitized morbidity data (www.inec.gob.ec), and because data on agricultural and life conditions of the population were available for that time period (INEC, 2002, 2010). Additionally, we did not consider the data of the period after 2008 because biases in the number of cases were expected given the fact that public health systems increased their coverage and became more accessible and free of charge, including the distribution of parasitic drugs (Yáñez, 2013). The cases were identified through the ICD-10 codes for disease classification (WHO, 2010). In Ecuador, the protocol to declare a patient with NCC is defined by the neurology department of hospitals. Briefly, the diagnosis of NCC follows the directions of the Del Brutto et al. (2001) criteria. The diagnosis is based on patient' clinical symptoms and signs (seizures, headache, dementia, hydrocephalous, among other neurological disorders), serology (detection of antibodies directed to *T. solium* metacestodes and/or circulating antigens of *T. solium* metacestodes in serum or cerebrospinal fluid) and imaging (CT, MRI) (Del Brutto et al., 2001). In the public sector there exist 39 neuroimaging facilities (CT-Scans) the majority of them are in the provincial capital cities (24 provinces). The private sector also has neuroimaging capacity but the number of scanners is unknown. The database has information about each hospitalized patient with cause-specific morbidity, hospital of attendance, and the place (parish or municipality)

where the patient lives (access to official databases are included in the Appendix to the paper).

All municipalities in the continental part of the country were included in this study (217 municipalities). The registers of the Galapagos Islands were excluded because they might distort the spatial analysis, although both disorders have also been reported there. Each record was designed to contain the total number of NCC and epilepsy cases, the total population, the year and the geographical coordinates of the centroid of each municipality. Time trends of hospitalized reported cases for both disorders were tested using Negative Binomial regression models. Furthermore, the relationship between the number of hospitalized cases of epilepsy and NCC was evaluated using correlation analysis on *Log* transformed data.

Additionally, data on several explanatory variables were gathered using governmental databases about several socio-economic indices, information from the agricultural census conducted in 2000, and climatological information from the National Hydrometeorological Service (INAMHI (2015)). The information on explanatory variables possibly associated with NCC and epilepsy at municipality level were grouped into different classes. Climatic variables: (tropical or highlands) (ZONE), rainfall (RAIN) and number of days without precipitations (DRYD). For each municipality, values of RAIN and DRYD were inferred on the basis of 205 weather stations, and ordinary Kriging was used to interpolate values. The similarity with rainfall maps published by the National Hydrometeorological Service (INAMHI) let us choose the proper model (INAMHI, 2015). Population variables: population number (POPULATION), percentage of indigenous population (%INDG), and percentage of rural population (%RURAL); Educational level: mean number of years of schooling (SCHOOL), mean number of years of schooling for farmers in the municipality (FSCHOOL), and percentage of farms receiving technical assistance (TECHASIS); Sanitary conditions: % of families with piped water (TUBWAT), % of dwellings with systems for eliminating excrements (EXCR), and physicians per 10,000 inhabitants (PHYS); Poverty indices such as: percentage of families with unsatisfied basic necessities (UBN) (Number of people or families that live under poverty conditions with respect to the population in a specific year, referring to the lack of dwelling, health, education and employment (2015)), and percentage of people under extreme poverty (EXTPOOV); Livestock: percentage of agriculture land dedicated to pastures (%GLS) and pig population (PIG) (2008). In Ecuador, the majority (58.8%) of the pig population is raised under the traditional husbandry system if we consider smallholder producers in Ecuador as those farmers owning ≤ 10 pigs (INEC, 2002). The agricultural office of geographical information systems provided the map showing the political division of the country at municipality level. A description of all the variables considered for this study is presented in Table 4.1 (access to official databases are included in the Appendix to the paper).

Table 4.1: Socio-economic and demographic factors used to model the incidence risk of hospitalized human cases of epilepsy and neurocysticercosis in Ecuador from 1996 to 2008.

| Variable | Description |
|------------|---|
| POPULATION | Population |
| EPI | Number of epilepsy cases (1996-2008) |
| CYS | Number of NCC cases (1998-2008) |
| Zone | Tropical (1) or temperate zone (0) |
| RAIN | Rainfall during the year |
| DRYD | Number of days during a year with precipitations $\leq 1mm$ |
| %GLS | Percentage of agriculture land dedicate to pastures |
| PIG | Number of pigs (Criollo bred) |
| SCHOOL | Average number of years on formal education in people ≥ 24 years old |
| PHYS | Number of physicians per 10.000 inhabitants |
| TUBWAT | Percentage of dwellings with piped water |
| EXCR | Percentage of dwellings with some kind of system for eliminating excrements |
| UBN | Percentage of families with unsatisfied basic necessities |
| EXTPOOV | Percentage of families living in extreme poverty |
| %INDG | Percentage of indigenous population |
| %RURAL | Percentage of rural population |
| TECHASSIS | Percentage of land with technical assistance |
| FSCHOOL | Years of education of farmers ≥ 24 years |
| <i>N</i> | 217 municipalities |

4.3.3 Space-time analysis

Space-time analysis was used to determine whether municipalities with high incidence of hospitalized cases (IHC) of epilepsy and NCC are clustered in space and in time (Kulldorff, 1997; Patil and Taillie, 2003). The space-time scan software (Kulldorff, 2015) was used to search, and test for significance and identify approximate locations of areas with an increased risk for the occurrence of NCC and epilepsy cases. The analysis was run in SatScan Software v9.4, with case file as the number of hospitalized reported cases, population file as the estimated total number of individuals in each municipality per year and as the coordinate file, the latitudes and longitudes of the centroids of each municipality. The spatial dimension varied from 0 up to 25% of the total number of centroids in the study region. The temporal dimension was established with a maximum of up to 50% of the study period with a time precision of 1 year. Poisson distributed case incidence with population size as the exposure variable was assumed. Space-time clustering was assessed by comparing the *iRR* (incidence rate ratio) of epilepsy and NCC IHC within a specific area and time in contrast to an expected *iRR* of hospitalized NCC and epilepsy cases if their incidences were randomly distributed. The cylinder with the maximum likelihood ratio was selected as the most likely cluster (Primary cluster), and others no overlapping significant clusters were also selected. The significance of identified space-time clusters was tested using the likelihood ratio test statistic and *p-values* of the test were obtained using 999 Monte Carlo simulations. The significance was arbitrated at the 5% level. All

significant clusters were visualized using **QGIS** software (version 2.8).

4.3.4 Regression analysis

Multivariable-count regression models were used to assess the relative contribution of different socio-economic and demographic variables to the IHC of epilepsy and NCC from 1996 to 2008 across the country. For the case of hospitalized NCC cases, an excess of zero cases were observed. For this reason, Zero inflated negative binomial models (ZINB) were used to explain the over-abundance of zero cases (Hu et al., 2011).

A manual forward stepwise procedure was implemented to select the set of independent variables that describe the number of NCC hospitalized cases or the probability of not observing any hospitalized case of NCC using the `zinb` command in STATA, version 12 software (StataCorp LP, College Station, Texas). The procedure started with the null model (with no covariates), and subsequently, covariates were added and evaluated for their importance. The Akaike Information Criteria (*AIC*) was used as the calibrating parameter and models with lower *AIC* values and few parameters were preferred. Two models were considered to be significantly different whenever the difference in *AIC* was greater than 3 (Raftery, 1995). In the forward selection procedure, each covariate was added in either the linear predictor for the count part or in the *logit* function for the absence of the disease, and the covariate with the best explanation preserved for the second round. If the addition of a covariate improved the model explanation through the reduction in *AIC*, the variable was captured and the process was repeated until the *AIC* value could not be reduced further (Zuur et al., 2012). The significance level for the covariates in the model was set at 0.05. For the case of NCC Vuong's test was applied in order to evaluate if zero-inflation was more appropriate as compared to the standard negative binomial model. Coefficient interpretations in the text are given in terms of *iRR* (incidence rate ratios) and *OR* (odds ratios). *iRR* and *OR* were obtained by exponentiating the regression coefficient, and represent the increase/decrease of the risk rate or risk chance when there is a unit of increment in the co-variable, the other co-variables remaining fixed.

To assess the influence of the selected indicators on the IHC of epilepsy, a Poisson regression model was used, and due to the presence of over-dispersion, Negative Binomial models were also evaluated through a likelihood ratio test for over-dispersion using the function `odTest` (in `pscl` package, in the R software) to test the null hypothesis that the restriction implicit in the Poisson model is true (Jackman, 2008). The approach of bidirectional elimination of variables was applied for variable selection, which also used the *AIC* as calibrating parameter. The functions `glm.nb` and `stepAIC`, in the **MASS** package under the R environment were used (Ripley et al., 2015).

In addition, for the study of epilepsy, Bayesian model averaging (BMA) was used in

order to deal with the uncertainty about the “correct” model (Hoeting et al., 1999). BMA chooses the better model according to the best posterior probabilities among the models using the Occam’s window principle (Raftery, 1995). The inference about the explanatory variables in the best model is expressed as posterior effect probabilities, which indicate evidence of the importance of the effects of each variable in the model. The function `bic.glm()` in the **BMA** package of the R software was used (Raftery et al., 2010) with the specification that counts follow a quasi-Poisson distribution, and that variance increases with the square of the mean: an equivalent version of NB regression (Ver Hoef and Boveng, 2007). No explicit prior distributions for models and model parameters were assumed implying that all models were equally likely.

4.4 Results

4.4.1 Space-time analysis

Figure 4.2 and Figure 4.3 display the histogram of the cumulative incidence of hospitalized epilepsy (Figure 4.2) and NCC (Figure 4.3) cases, during the study period (between 1996 and 2008) over all the municipalities. For epilepsy, the distribution is more uniform compared to that of NCC; however, for NCC there are high proportions of zero cases throughout the municipalities. Figure 4.4 presents the Incidence of hospitalized cases (IHC) with both health problems over time in Ecuador. In the case of NCC, from 1996 to 2008, there was an overall decreasing trend with around 5 cases per 100,000 inhabitants in 1996 to around 3 cases per 100,000 inhabitants in 2008. The decreasing trend was statistically significant ($p < 0.001$); thus, annually a reduction of 5.68% ($CI_{95\%}$: 4.5%-6.4%) in hospital cases is expected. In contrast, for the case of epilepsy, the incidence appeared to be slowly and steadily increasing from 1996 to 2008. The increasing trend was statistically significant ($p < 0.001$). Annually an increase of 4.7% ($CI_{95\%}$: 3.7%–5.7%) in reported cases is expected. It was also observed that as long as the incidence of epilepsy increased through time, the annual IHC of NCC appeared to decrease slowly; and the IHC for epilepsy was always higher than that of NCC.

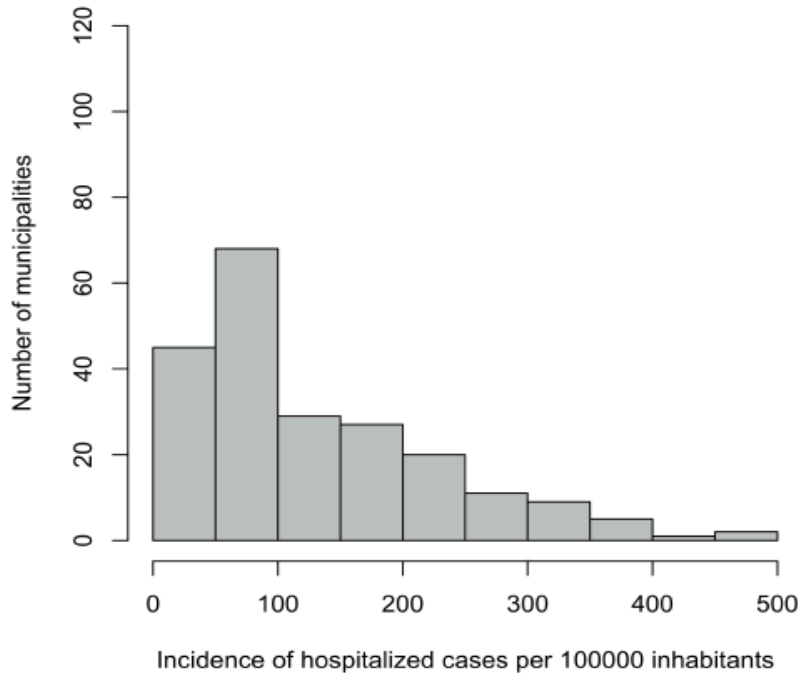


Figure 4.2: Histogram of the incidence of hospitalized epilepsy cases per 100.000 inhabitants between 1996 and 2008 in Ecuadorian municipalities.

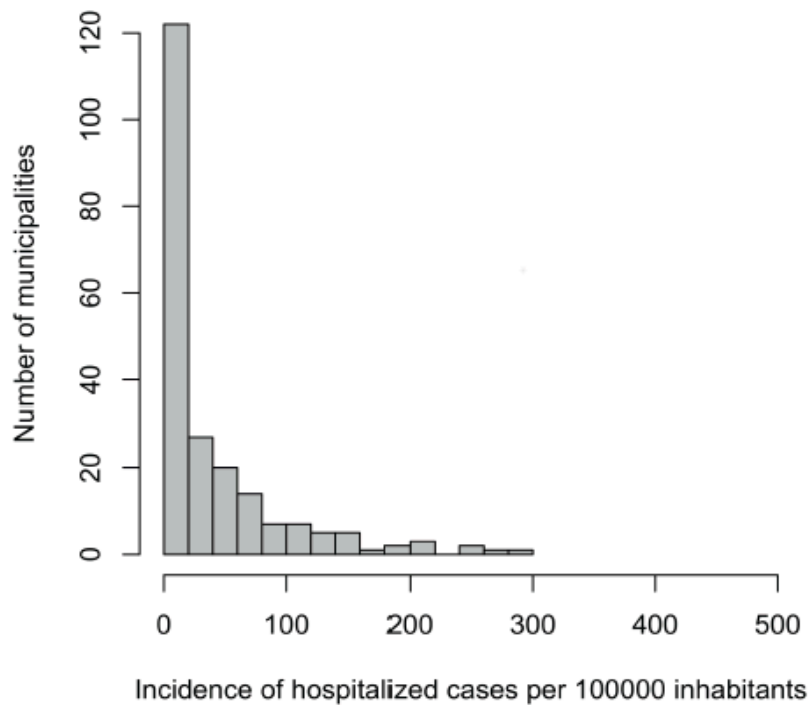


Figure 4.3: Histogram of the incidence of hospitalized neurocysticercosis cases per 100.000 inhabitants between 1996 and 2008 in Ecuadorian municipalities.

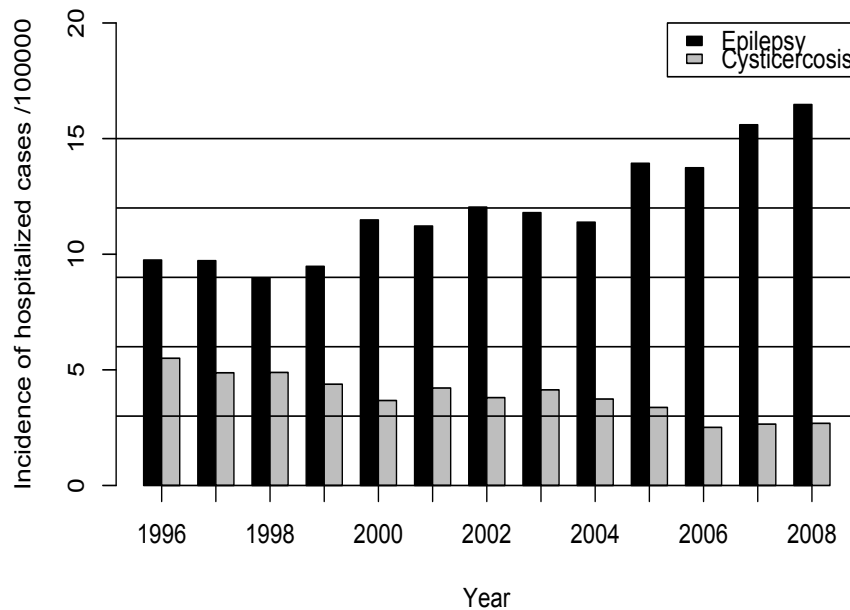


Figure 4.4: Incidence of hospitalized neurocysticercosis and epilepsy cases per 100.000 inhabitants in Ecuador between 1996 and 2008.

Table 4.2 presents a list of the top 15 municipalities with the highest IHC of epilepsy and NCC. It can be observed that municipalities with a high IHC of epilepsy did not necessarily have a high IHC of NCC. However, an overall (all years combined for each municipality), highly significant positive linear correlation ($r = 0.78$, $CI_{95\%} : 0.72 - 1.00$, $p < 0.0001$) was found on $\log(x+1)$ transformed number of hospitalized cases of epilepsy and NCC reported in each municipality. Similar significant positive linear trends within municipalities were observed for each year evaluated separately. The IHC of epilepsy varied from 0 to 505 cases per 100,000 with an average number of 127.1 cases. On the other hand, for NCC, the IHC varied from 0 to 282.54, and the average number of cases was 35.1. Out of the top 15 municipalities with high IHC for epilepsy, six also featured amongst the top 15 for municipalities with high IHC for NCC. It should be highlighted that Quito (capital city) presented the highest number of patients (3123 and 1829) in hospitals for epilepsy and NCC during the study period, but the rates were diluted because of the city's large population size.

Table 4.2: Municipalities having the highest incidence of hospitalized cases (/100.000 in habitants) of epilepsy and of neurocysticercosis from 1996 to 2008.

| Municipality | Highest IHC for epilepsy | | Municipality | Highest IHC for NCC | |
|--------------|--------------------------|---------|--------------|---------------------|---------|
| | IHC Epilepsy | IHC NCC | | IHC Epilepsy | IHC NCC |
| Paulo VI | 505.0 | 0.0 | Chinchiipe | * | * |
| Chinchiipe | 494.4 | 282.5 | Loja | * | * |
| Zamora | 472.7 | 183.6 | Quilanga | 43.6 | 218.2 |
| Sta. Clara | 396.2 | 0.0 | Espíndola | 273.0 | 215.8 |
| El Chaco | 391.3 | 130.4 | El Tambo | 218.2 | 206.0 |
| Sucúa | 381.6 | 13.8 | Azogues | * | * |
| Santiago | 375.9 | 0.0 | Zamora | * | * |
| Baños | 367.1 | 0.0 | Cañar | 268.1 | 171.7 |
| Azogues | 359.0 | 204.9 | Paltas | 206.4 | 165.9 |
| Sta. Rosa | 341.1 | 28.1 | Calvas | 282.6 | 159.4 |
| Biblián | 328.1 | 144.7 | Biblián | * | * |
| Loja | 327.4 | 241.6 | Gonzanamá | 126.8 | 140.1 |
| Morona | 321.8 | 51.0 | Cuenca | 288.8 | 138.8 |
| Riobamba | 320.7 | 135.5 | Riobamba | * | * |
| Penipe | 308.0 | 61.0 | Ibarra | 134.4 | 204.2 |

Legend: IHC: Incidence of hospitalized cases; NCC: Neurocysticercosis. * Data reported in previous columns.

4.4.2 Significant space-time clusters

Four clusters were detected for the iRR of epilepsy when up to a maximum of 25% of centroids were included in the scanning window Figure 4.5. The most likely cluster extended from the center to the northern part of the country, involving municipalities from Tungurahua, Cotopaxi, Napo and Pichincha provinces. This cluster lasted between 2003 and 2008 with an iRR of 1.57 in contrast to centroids outside the cluster ($p = 0.001$). A secondary cluster was located in the Guayaquil municipality in Guayas province in the Southern coast of the country with an iRR of 1.69 ($p = 0.001$). This cluster existed between 2005 and 2008 with 1745 hospitalized cases when only 1070 cases were expected. A third secondary cluster was located in the southern part of the country in municipalities in the provinces of El Oro, Loja Zamora, Caar, Azuay, Morona Santiago, and Chimborazo. This cluster was the biggest cluster found, and the iRR was 1.60 ($p = 0.001$) compared to the municipalities outside the cluster. This cluster existed from 2002 to 2007. Finally, in the western coast of the country, a secondary cluster covering 11 municipalities of Manabí province was detected in 2008, which had an iRR of 1.62 ($p = 0.001$).

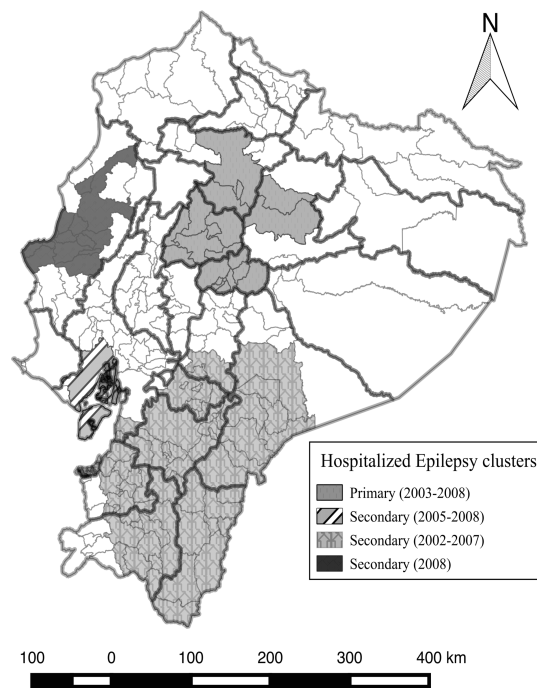


Figure 4.5: Significant space-time clusters of hospitalized epilepsy cases with up to a maximum 25% of the total centroids included in the scanning window between 1996 and 2008.

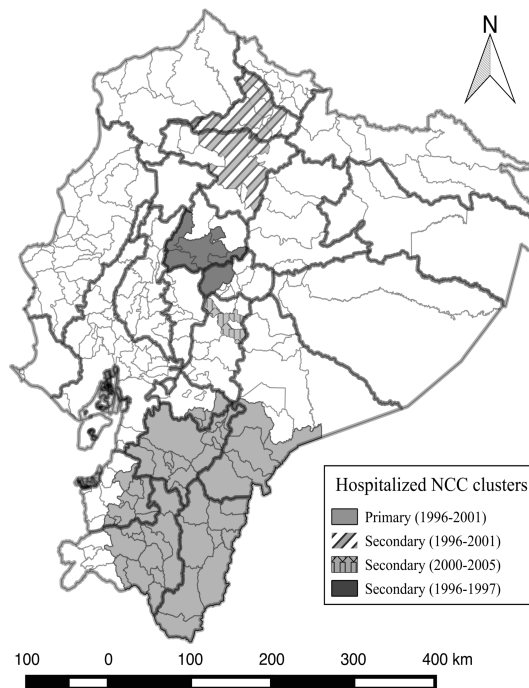


Figure 4.6: Significant space-time clusters of hospitalized NCC cases with up to a maximum 25% of the total centroids included in the scanning window between 1996 and 2008.

Like for epilepsy, four significant clusters were identified for NCC. The most likely cluster (primary) was found in the southern part of the country, which existed from 1996 to 2001 with an iRR of 3.78 ($p = 0.001$) (Figure 4.6). Municipalities of El Oro, Loja, Zamora, Azuay and Morona Santiago provinces were part of this cluster within the given time frame. Likewise, in the central-northern part of the country, some municipalities from Imbabura, and Pichincha provinces were part of an important secondary cluster that lasted from 1996 to 2001. The iRR for the municipalities within this cluster was 2.75 ($p = 0.001$). Additionally, there was a secondary cluster in the municipality of Riobamba in Chimborazo Province in the middle of the country that lasted between 2000 and 2005. The iRR for this cluster was 3.15 ($p = 0.001$). Finally, besides the last zone, some municipalities from Cotopaxi and Tungurahua provinces were part of an additional cluster that had an iRR of 2.09 ($p = 0.001$) and had a relatively high incidence starting in 1996 until 1997. Figure 4.7 makes a comparison between the municipality clusters for Epilepsy and NCC when the scanning window sizes were set at 25% and 10% of the total centroids included in the country, and the base map was the pig population density

around the country.

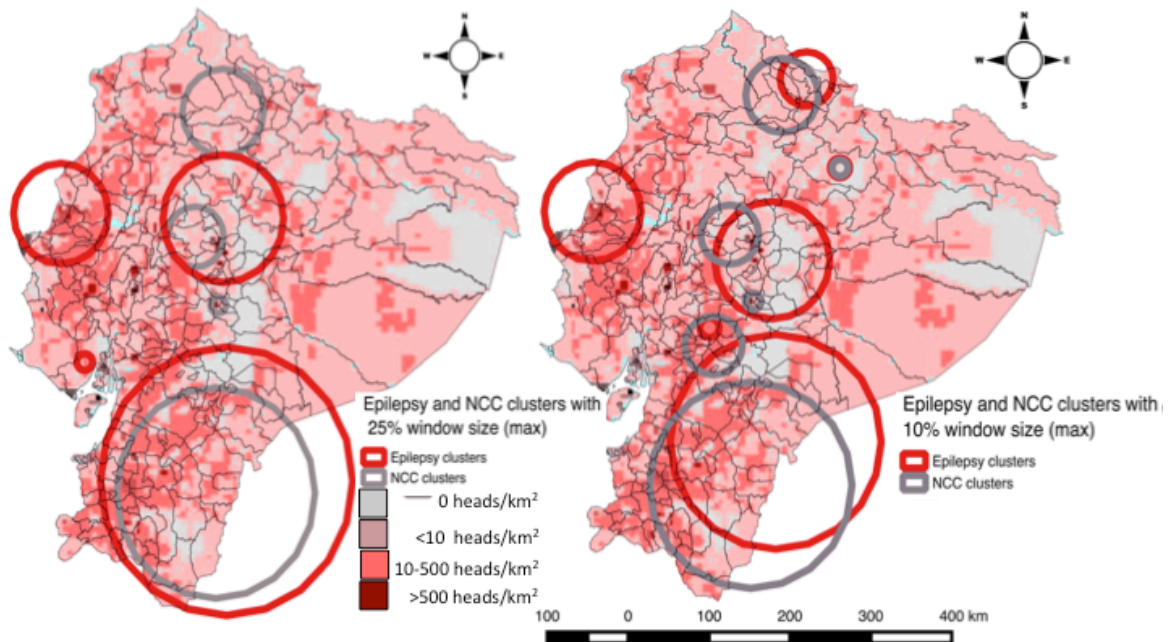


Figure 4.7: Significant space-time clusters of hospitalized Epilepsy and NCC cases with up to a maximum 25% and 10% of the total centroids included in the scanning window between 1996 and 2008. The base map is the pig population density.

4.4.3 Potential indicators associated with the incidence of hospitalized epilepsy and neurocysticercosis cases

The analysis of several socio-economic and demographic variables affecting the IHC of epilepsy between 1996 and 2008 is presented in Table 4.3 and Table 4.4. Table 4.3 presents the set of covariates selected by stepwise procedure and Table 4.4 presents the total set of variables included in this set of potential indicators and their partial contribution according to the BMA methodology. Posterior effect probabilities $P(\beta \neq 0|D)$ were included to show the evidence of an effect for each covariate when model uncertainty was incorporated. According to the results, the variables that significantly increased the IHC of epilepsy in municipalities were: the number of physicians per 10,000 inhabitants [PHYS] $iRR = 1.045$ ($CI_{95\%}: 1.031-1.059$), and the percentage of families having tubing water (TUBWAT, not necessarily drinking water) $iRR=1.022$ ($CI_{95\%}: 1.014-1.029$). On

the other hand, the only variable that apparently led to a significant reduction in the IHC of epilepsy in municipalities was the percentage of houses having any kind of system to eliminate excrements [EXCR] $iRR=0.986$ ($CI_{95\%}: 0.979-0.993$). The climatic variable (Zone) showed that municipalities located in the highlands presented an increased risk of epilepsy in contrast to municipalities located in tropical zones ($iRR = 1.02$ ($CI_{95\%} : 0.855 - 1.207$)) even though it was not statistically significant at a 5% level. For the BMA variable selection, out of 15 variables evaluated, 3 had substantial evidence ($P(\beta \neq 0|D) > 90\%$) of being different from zero Table 4.4; [PHYS], [TUBWAT], and [EXCR]. Overall, the BMA and stepwise selection methods yielded similar results.

Table 4.3: Potential indicators associated with the incidence of hospitalized epilepsy cases in Ecuador from 1996 to 2008 (negative binomial chosen by *AIC* criterion).

| Coefficients: | Estimate | Std. Error | z value | p-value |
|--|----------|------------|---------|---------|
| (Intercept) | 6.77 | 0.199 | -33.95 | < 0.001 |
| Zone (Tropical) | -0.16 | 0.088 | -1.82 | 0.069 |
| PHYS | 0.04 | 0.007 | 6.37 | < 0.001 |
| TUBWAT | 0.02 | 0.004 | 5.53 | < 0.001 |
| EXCR | -0.01 | 0.004 | -3.85 | < 0.001 |
| Dispersion parameter for Negative Binomial (3.5) | | | | |
| <i>AIC</i> : 1769.8 | | | | |

Legend: PHYS: Number of physicians per 10.000 inhabitants; TUBWAT: Percentage of dwellings with piped water; EXCR: Percentage of dwellings with some kind of system for eliminating excrements; *AIC*: Akaike Information Criterion; Std. Error: Standard Error.

Table 4.4: Potential indicators associated with the incidence of hospitalized cases in Ecuador from 1996 to 2008 based on Bayesian model averaging (BMA).

| Coefficients: | $P(\beta \neq 0 D)$ | Mean | SD |
|---------------|---------------------|-------|-------|
| (Intercept) | 100 | -6.84 | 0.699 |
| PHYS | 100 | 0.05 | 0.008 |
| TUBWAT | 96.7 | 0.02 | 0.006 |
| EXCR | 94.0 | -0.01 | 0.005 |

Legend: TUBWAT: Percentage of dwellings with piped water; PHYS: Number of physicians per 10.000 inhabitants; EXCR: Percentage of dwellings with some kind of system for eliminating excrements; SD: Standard deviation; $P(\beta \neq 0|D)$: Posterior effects probability (%).

Table 4.5 presents the results of the potential indicator factors associated with NCC in hospitals. In the binary part, three covariates were chosen [EXCR, PIG, %RURAL]. According to this selection procedure, the covariates that positively influenced the odds of hospitalized NCC cases in the communities were the implementation of systems for eliminating excrements [EXCR] ($OR = 0.94$; $CI_{95\%}: 0.89 - 1.0$), and pig population [PIG] ($OR = 0.999$; $CI_{95\%}: 0.999-1.0$). In contrast, the higher the proportion of rural population

in a community the lower the odds ratio of reporting NCC hospitalized cases ($OR = 1.073(CI_{95\%}: 1.01 - 1.14)$).

Table 4.5: Potential indicators associated with the incidence of hospitalized neurocysticercosis cases in Ecuador from 1996 to 2008 based on a zero-inflated negative binomial regression model.

| Count model | Coef. | Std. Err. | Z | $P > z $ | [95% Conf. Interval] | |
|-----------------------|--------|-----------|-------|-----------|----------------------|---------|
| (Intercept) | -4.32 | 1.290 | -3.35 | 0.001 | -6.8430 | -1.7900 |
| Zone (Tropical) | -1.72 | 0.170 | -9.69 | < 0.001 | -2.0700 | -1.3700 |
| SCHOOL | 0.19 | 0.092 | 2.09 | 0.037 | 0.0120 | 0.3720 |
| EXCR | -0.05 | 0.008 | -6.62 | < 0.001 | -0.0680 | -0.0370 |
| TUBWAT | 0.02 | 0.008 | 1.98 | 0.047 | 0.0002 | 0.0300 |
| TECHASSIS | 0.05 | 0.015 | 3.46 | 0.001 | 0.0222 | 0.0800 |
| %GLS | 0.01 | 0.004 | 2.52 | 0.012 | 0.0024 | 0.0190 |
| EXTPOOV | -0.02 | 0.008 | -2.33 | 0.020 | -0.0362 | 0.0030 |
| %RURAL | -0.01 | 0.005 | -2.03 | 0.042 | -0.0192 | -0.0001 |
| Zero inflation | | | | | | |
| Intercept | -1.97 | 3.099 | -0.64 | 0.525 | -8.0451 | 4.1020 |
| EXCR | -0.06 | 0.028 | -2.09 | 0.036 | -0.1134 | -0.0030 |
| PIG | -0.001 | 0.0003 | -2.46 | 0.014 | -0.0011 | -0.0001 |
| %RURAL | 0.070 | 0.032 | 2.13 | 0.033 | 0.0056 | 0.1310 |
| $\log(\alpha)$ | -0.070 | 0.1453 | -4.74 | < 0.001 | -0.9734 | -0.4030 |
| α | 0.50 | 0.0730 | | | 0.3777 | 0.6670 |

Legend: SCHOOL: Average number of years on formal education in people ≥ 24 years old; EXCR: Percentage of dwellings with some kind of system for eliminating excrements; TUBWAT: Percentage of dwellings with piped water; TECHASSIS: Percentage of farms with technical assistance; %GLS: Percentage of agriculture land dedicated to pastures; EXTPOOV: Percentage of extreme poverty; %RURAL: Percentage of rural population; PIG: Number of pigs (Criollo breed); Coef: coefficient; Std.Error: Standard Error.

On the other hand, for the count model, the variables that positively influenced the number of hospitalized cases were [SCHOOL], [TUBWAT], [TECHASSIS], and %GLS, and the variables that were associated with a decrease in the number of hospitalized cases were Zone (Tropical), [EXCR], [EXTPOOV], and [%RURAL]. The temperate zone (highlands) had by far higher NCC cases compared to the tropical zones, so that the possibility of having a NCC diagnosis in Andean zone hospitals is higher ($iRR=5.6$ times ($CI_{95\%}: 3.95-7.92$)). Variables such as, the schooling in municipalities [SCHOOL] ($iRR = 1.212$; $CI_{95\%}: 1.012-1.451$), the percentage of dwellings having tubing water [TUBWAT], ($iRR = 1.020$; $CI_{95\%}: 1.001 - 1.031$), the proportion of farms with technical assistance [TECHASSIST] ($iRR = 1.051$; $CI_{95\%}: 1.022 - 1.084$), and the percentage of land dedicated to pastures [%GLS] ($iRR = 1.010$; $CI_{95\%}: 1.002 - 1.019$) were positively associated with an increase in the IHC of NCC. In contrast, covariates that negatively affected the number of NCC hospitalized cases in hospitals were: the percentage of dwellings having any system for eliminating excrements [EXCR] ($iRR = 0.951$; $CI_{95\%}: 0.934 - 0.964$), extreme

poverty [EXTPOOV] ($iRR = 0.980$; $CI_{95\%}: 0.965 - 0.997$); and [%RURAL] population ($iRR = 0.990$; $CI_{95\%}: 0.9812 - 0.999$). The implementation of systems for eliminating excrements [EXCR] reduced the IHC of NCC in average by 4.87% ($CI_{95\%}: 3.6\% - 6.6\%$).

The model chosen for the iRR of epilepsy fitted better than the model without over-dispersion. The likelihood-ratio test for the over-dispersion parameter was significantly different from zero ($p < 0.001$), meaning that Negative binomial regression was preferred over Poisson regression. For the case of NCC, when the zero-inflated negative binomial model was contrasted against the zero-inflated Poisson model, the likelihood-ratio test was significant in favour of taking into account the overdispersion (different from zero) ($p < 0.0001$). In the same way, Vuong's test confirmed that the assumption of zero inflation in the model for NCC was preferred over a model without this assumption ($p = 0.02$).

4.5 Discussion

During the study period, NCC still had an impact on the general health status of the population in Ecuador. Our findings indicate that 6294 cases of NCC and 19821 cases of epilepsy were hospitalized between 1996 and 2008. Additionally, there was a significant increasing time-trend for IHC of epilepsy, but a decreasing time-trend for IHC of NCC overall. In contrast, within municipalities a positive linear relationship between both disorders was found. Also, the number of hospitalized cases (both for epilepsy and NCC) was related to some potential indicators evaluated. A general reduction in the IHC of both conditions was observed with an increasing percentage of systems to eliminate excrements. Moreover, the presence of pig production was related to the IHC of NCC.

4.5.1 Epilepsy

In the case of epilepsy, according to the spatio-temporal analysis, all significant clusters of municipalities with high incidence of epilepsy existed between 2002 and 2008. The incidence risk ratio (iRR) of the epilepsy clusters were not so high (they ranged from 1.57 to 1.69), meaning that epilepsy can be almost classified as an endemic disorder in Ecuador. A possible explanation for the epilepsy clusters since 2002 is that during these years, health facilities could have improved in municipalities leading to a better coverage of hospitalization.

Clustered epilepsy municipalities were located in the Sierra region, mainly in the southern and some in the central-northern part of the country. Some of those municipalities were known as endemic for epilepsy and as epilepsy-NCC related zones, with some new clusters in the Sierra region that have not been pointed out before as endemic areas in previous studies (Cruz et al., 1999, 1989; Rodríguez-Hidalgo et al., 2003, 2006; Del Brutto

et al., 2005). The appearance of this new epilepsy cluster could also be related to the presence of other infectious and non-infectious diseases present in coastal tropical zones (Idro et al., 2010; Sander, 2004), but the evaluation of the newer clusters become urgent as this could be a strong predictor for NCC (Pawlowski et al., 2005).

Based on two selection methods, there was a positive association between number of physicians and number of hospitalized cases of epilepsy. This effect occurs mainly in provincial capital cities where more health services exist and consequently specialized physicians are available to people. The implementation of systems for eliminating excrements, such as latrines, septic tanks, or sewage systems, seemed to have an impact on the occurrence of epilepsy, which suggests that a part of hospitalized epilepsy cases might be due to NCC or other fecal-related causes of epilepsy (Sander, 2004). In this study period, the majority of the epilepsy cases were classified as non-specific cases (ICD codes: G408 and G409), and they were between 81% and 92% every year. As in other places, the majority of epilepsy cases apparently do not have a recognized etiological origin well identified (Preux and Druet-Cabanac, 2005; WHO, 2015). However, not all types of acquired epilepsy can be attributed to NCC (Sander, 2004; Preux and Druet-Cabanac, 2005; Pawlowski et al., 2005). Other causes related to poverty, such as poor nutrition, neurological sequels due to infectious agents, and head traumas related to occupational accidents and violence can also contribute to the number of hospitalized epileptic cases (Sander, 2004; Yemadje et al., 2011; Vaid et al., 2012; Raghava et al., 2010). The other variable was the percentage of dwellings with piped water. Having piped water not necessarily refers to water that is drinkable. According to estimations, in Ecuador, 30% of the piped water in urban zones is not potable water (OAS, 2010).

4.5.2 NCC

In Ecuador, the protocol to declare a patient with NCC depends on the diagnostic capacity of the neurology department, which in many cases, only exists in the main cities (Del Brutto et al., 2001). Based on national hospital data, 35.5% of the NCC cases were reported by the public sector, 39.4% were reported by the private sector and 23% by the systems of social security insurance. Furthermore, the appearance of clinical signs of NCC in patients might occur several months or even years after infection, so that some etiological factors could not have been measured correctly at the beginning of this study. Likewise, it is worth to mention that only a part of the cases of human cysticercosis are symptomatic, and therefore the statistical relationships found in this study are valid only for the hospitalized cases reported. A wider spectrum might be found with the addition of asymptomatic cases and people suffering from chronic headaches who do not often consult physicians, which only can be found in field studies (Nash et al., 2004; Pawlowski et al., 2005; Bhattarai et al., 2012; Carabin and Traoré, 2014; Cruz et al., 1995).

NCC clusters in Ecuador apparently appeared earlier compared to epilepsy clusters and the majority of them existed between 1996 and 2001. Only one of them was identified from 2000 to 2005 in Chimborazo province and in some surrounding municipalities in Bolívar Province. This area presents ideal conditions for the *T. solium* life cycle, as it is located in the highlands with a high percentage of rural population and lack of basic services. In this cluster, more than 90% of the pig producers are smallholders INEC (2002). According to the last Agricultural National Census carried out in 2000, 80.5% of the smallholders (≤ 10 pigs) are located in the Sierra region and 18.6% in the Coastal region. Porcine Cysticercosis has not been reported in slaughterhouses by the National Veterinary Services Anonymous (2008) since 2001 (OIE, 2005). However, only 30% of slaughtered pigs in these facilities is provided by smallholder (AGROCALIDAD, 2010).

Based on a zero-inflated negative binomial model, the percentage of rural population in municipalities was associated with a reduction in the IHC of NCC; so that urban zones increased their incidence in contrast with previous studies published (Placencia et al., 1992). Nowadays, the rural population in Ecuador accounts for less than 38% of the total population, which is a significant reduction compared to previous decades when the rural population was over 50%. Translocated rural communities tend to settle in slum zones of big cities (INEC, 2010). However, rural and sometimes poor communities might have not been well represented in the data, as they may refrain from hospitalization due to the high costs of diagnosis and treatments (Pal et al., 2000). This concern is a limitation of hospital-based registers (Raghava et al., 2010). In our study only 7% of patients appeared to belong to rural communities, although, if we consider the patients not living in the provincial capital cities this percentage increases to 26.7%. The structure of the data makes it difficult to differentiate people from peri-urban zones or slums, or semi-rural towns and communities from urbanized areas. On the other hand, housemaids and food vendors coming from endemic rural zones have a higher chance to be tapeworm carriers and can be at the origin of spreading *T. solium* infection among the urban population (García et al., 1998; Kelvin et al., 2012; Huisa et al., 2005). Another possible explanation is that traditional livestock systems are still preserved on a small scale in urban slums, although this presumption has not been quantified.

The presence of pigs was the most important positively associated with the appearance of hospitalized-symptomatic NCC cases. Industrialization of pig production, in many cases is not responsible for the increase in NCC cases. On the other hand, the presence of free roaming pigs has been associated with an increased risk for the occurrence of cysticercosis (Assana et al., 2010; Rodríguez-Hidalgo et al., 2006). In Ecuador, despite 58.8% of pigs are raised in traditional production systems it has been estimated, that it only represents nearly 30% (50% in year 2000) of pork available in markets (AGROCALIDAD, 2010).

In the negative binomial count model, eight variables were associated with the IHC of

NCC. As in the case of epilepsy, the implementation of systems for eliminating excrements was involved in a reduction in the IHC of NCC. More in depth studies are needed to evaluate the real scale of those variables in the macro epidemiology of NCC, although some of them express the lack of quality in offering services.

4.5.3 Epilepsy & NCC

It has been mentioned that the difficulties of identifying the etiology of epilepsy could play an important role in the sub-notification of NCC (Cruz et al., 1999; Ngoungou and Preux, 2008; Medina et al., 1990). The condition most commonly associated with NCC is epilepsy, but many cases of NCC are asymptomatic or manifest chronic headaches or other neurological disorders (Pawlowski et al., 2005; Cruz et al., 1995; Bhattarai et al., 2012; Carabin and Traoré, 2014). In *T. solium* endemic communities in Ecuador an important proportion of acquired epilepsy cases were due to NCC (Del Brutto et al., 2005; Cruz et al., 1989). Although extrapolating this quantity to the current reality in the country may be biased; zones with an apparent increase of epilepsy cases may elucidate the origin of new suspected NCC cases (Pawlowski et al., 2005; Flisser et al., 2005). Additionally, epilepsy and NCC in developing countries have been reported to be clustered (Lescano et al., 2009; Raghava et al., 2010; Sarti-Gutierrez et al., 1988), but the presence of imported cases has also been mentioned as an important factor in urban zones.

NCC underreporting might be due to a misdiagnosis in the epilepsy etiology. However, in our case both disorders were linearly related. This positive relationship is an indicator of an apparent constant relationship between epilepsy and NCC. This relationship has to be further studied and the meaning of this pattern has to be elucidated.

Additionally, given that the lack of sewage systems was demonstrated to be associated with an increase in the incidence of both conditions. Increasing the sewage systems could be used as an important control tool to reduce the incidence of the hospitalized cases.. The installation of these systems, at municipal level varied from 20.6% to 96.3% of coverage with a median value of 68.5%, so there are still many municipalities that lack basic services.

The zone where the municipality was located was one of the principal indicators affecting the IHC of NCC and epilepsy. Municipalities located in temperate zones (highlands) had a significantly higher number of hospitalized NCC cases. From the BMA procedure, in the case of epilepsy, the posterior effect had a small probability (11.8%), so we argue that in tropical zones lack of appropriate diagnostic tools and specialized knowledge of health staff might make it difficult to properly identify NCC cases (Yemadje et al., 2011). Likewise, the levels of coverage of basic services is lower in tropical zones of Ecuador (INEC, 2010). Thus, the presence of the life cycle of *T. solium* in these zones traditionally considered to be NCC free cannot be ruled out.

A limitation of this study was that peri-urban zones where poverty belts of cities are frequently located could not be analyzed separately given that records do not use this residence category for patients. The conditions in peripheral zones can differ from city to city, thus the assumption that the origin of the hospitalized cases in big cities come from peripheral zones should not be extrapolated in all cases. As in other studies based on hospital data, rural communities might not be appropriately represented in the sample. This could be a major limitation of our study, and also because of the asymptomatic human cysticercosis cases, migraine-type and chronic headaches (Pawlowski et al., 2005; Cruz et al., 1995; Bhattarai et al., 2012). However, due to the fact that ambulatory cases do not offer reliable data, hospital data is a better attempt to represent the situation of the disease in a municipality. Another limitation in this study might be the presence of duplicated cases in the data base. These cases might be due to the fact that a patient was hospitalized more than once, or because some NCC cases were diagnosed as epilepsy before. But given the mentioned limitations, the present results are still reliable due to their apparent representation of the municipalities in Ecuador, and because they are based on the appropriate statistical tools. So given the data constraints, the methods used to identify risk indicators and/or areas based on available data presents valuable results for veterinary and public health sectors at no cost. There is a need to re-evaluate the current situation for both disorders throughout the country as life conditions have been changing over time (MSP, 2012; Flores and Castillo, 2012; Yáñez, 2013). Given the recent changes in the organization of the public health sector, new trends need enough data collection-time to be evaluated again.

In conclusion, NCC might still have a relevant presence in Ecuador and might play an important role as a cause of acquired epilepsy in Ecuador (Del Brutto et al., 2005). Although the real burden of NCC is still unknown, we found that the hospitalization rate of patients with epilepsy has been increasing in recent years (Figure 4.4, Figure 4.7). Traditional NCC and epileptic endemic zones were recognized as high risk zones even though more recent clusters of both diseases seem to have appeared. Although the lack quality of basic services was related to the IHC in both disorders, one important finding of this study was that the implementation of systems for eliminating excrements helped to reduce the incidence of hospitalized cases of both epilepsy and NCC, which could be used as an indicator strategy for planning control programs. More specific studies linking human NCC with epilepsy and their respective factors in field conditions are needed to evaluate the prevalence of the disease in humans throughout the country and generate data that could be used for estimation of the burden disease.

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Chapter 5

APPLICATION OF DECISION TOOLS USING DYNAMIC MODELS FOR CONTROLLING *BRUCELLA* *ABORTUS*-BRUCELLOSIS IN DAIRY CATTLE

Modified from “Modeling the transmission dynamics of bovine Brucellosis in dairy cattle herds”. (2015) Lenin Ron-Garrido, Norberto Bartoloni, Famke Jansen, Washington Benitez , Claude Saegerman, Emmanuel Abatih, Dirk Berkvens.

5.1 Abstract

Abortion is the most significant symptom caused by *Brucella abortus* in cattle. Losses due to abortions and the consequent lactation delays make this disease a serious problem for dairy farmers and, because of a considerable reduction in animal replacements, for cattle production in general. This paper presents a mathematical model for the transmission dynamics of bovine brucellosis which takes into account disease stages of infectious cattle, making a distinction between abortive and non-abortive compartments, as well as the incorporation of vertical, sexual (and Artificial Insemination), direct and indirect routes of transmission. The aim of this study is to describe the dynamics of brucellosis in a cattle

herd and analyse the behaviour of the system under different scenarios. The effects of control programs such as vaccination and culling were investigated. A tool for managers and decision makers was developed, so that the consequences of control measures and changes in model parameters may be evaluated directly through a **shiny** application using the **R** environment and **RStudio** as platform. We argue that in healthy herds without reproduction problems, a proportion near to $\frac{2}{3}$ female calves have to be preserved as future replacements, which means that annually $\frac{1}{3}$ of female calves are to be sold in order to maintain the population near the carrying capacity of the system. If a brucellosis infection appears into the herd, a deficit of female calves becomes evident and milk production losses start to appear. The application helps to predict losses in terms of abortions and milk losses. The model predicts that in absence of control measures and depending on the initial infective disease stage, in a whole herd with a milk yield of 5000 kg per lactation per cow, 2.14 to 2.25 abortions per cow are expected and between 2.1 and 3.7 kg milk per cow per day are lost on average in a period of 10 years. According to parameters used in our model, and given the current available tools for control and eradication, brucellosis in cattle is an eradicable disease. However, a combination of measures are necessary to be implemented. For example, in endemic zones, strict vaccination of female calves and biannual serological testing of part (at least half) of the population with culling of seropositive animals may reduce the disease prevalence to a negligible proportion in less than 10 years with minimal economic losses, but continuous epidemiological surveillance must be implemented afterwards.

5.2 Introduction

Brucellosis is a complex of infectious diseases, caused by species of the genus *Brucella*, that mainly affect mammals, including humans. The disease is distributed worldwide, although developing countries of Africa, western Asia and Latin America have the highest incidence rates (Pappas et al., 2006; FAO/WHO/OIE, 2006). Abortion in domestic livestock is probably the most significant symptom and reason for economic losses. Brucellosis is also of special importance due to its zoonotic potential and the possibility of inter-species transmission (Godfroid, 2004; Xie and Horan, 2009).

Brucellosis is transmitted when susceptible animals come into direct contact with infective animals, especially with mucosae or infected fluids from placenta and/or foetus, and this usually by touching, licking or ingestion (Cheville et al., 1998; Xie and Horan, 2009; England et al., 2004). Additionally, young animals can contract the disease during birth or through ingestion of infected milk, the former being the main way of vertical transmission. It has been argued that this infected young stock tends to abort upon reaching sexual maturity, but they do not show serum reactions before developing reproductive tissue (the so-called heifer syndrome) (Cheville et al., 1998; England et al., 2004; Yamamoto

et al., 2008). On the other hand, brucellosis is considered a venereal disease that can be transmitted by infected males, although little evidence exists that natural mating is a frequent route for brucellosis transmission (Uhring et al., 2013). By contrast, when unsafe artificial insemination (A.I.) is used, infected semen placed directly in the uterus often leads to transmission (Cheville et al., 1998). Likewise, when infected females evict the placenta or foetus during abortion or parturition, vast quantities of bacteria are shed into the environment, making this route an indirect way of transmission due to the creation of environmental reservoirs (England et al., 2004; Ainseba et al., 2010; Xie and Horan, 2009; FAO/WHO/OIE, 2006). Environmental survival rates of *Brucella* species under different conditions have been reported to range from a few days to several years (FAO/WHO/OIE, 2006; Cheville et al., 1998). As a result, climatological conditions probably influence the transmission of the disease in the long term. Thus, transmission of brucellosis may occur either directly (i.e. horizontally, vertically or through sexual transmission) or indirectly when animals feed on contaminated feedstuffs (e.g. grass).

Methods for controlling the disease in livestock include good hygiene, biosecurity, vaccination and elimination of seropositive animals (Gonzalez-Guzman and Naulin, 1994; Olsen and Stoffregen, 2005; Saegerman et al., 2010). For eradication purposes, intensive national surveillance and removal of test-positive animals are required (England et al., 2004; Yamamoto et al., 2008). Surveillance strategies for this disease in cattle include serological testing, bulk-milk testing, as well as abortion notifications (England et al., 2004; Ragan, 2002). Vaccination is very effective when aiming to reduce the disease prevalence to low levels Gonzalez-Guzman and Naulin (1994). The final eradication stages should be based on an effective test-and-removal of seropositive individuals strategy (Olsen and Stoffregen, 2005; Olsen, 2013). Deciding when to change from vaccination to eradication is not always a simple matter. The inability of available serological tests to detect all infected animals (imperfect tests) requires specifically adapted programs, involving simultaneous use of different diagnostic tests. Furthermore, vaccines against *Brucella abortus* still do not offer a perfect protection, and sometimes they even induce abortion in pregnant animals. Finally, it must be pointed out that the use of antibiotics in animals is strictly forbidden, for fear of creating resistant strains, which are then impossible to control in humans (FAO/WHO/OIE, 2006).

Mathematical models help to understand the biology of the host and pathogen and consequent epidemiological patterns of disease (Medley and Anderson, 1992). In this way, several attempts to model the dynamics of brucellosis in livestock populations have been made (Carpenter et al., 1987; Gonzalez-Guzman and Naulin, 1994; Dobson and Meagher, 1996; England et al., 2004; Yamamoto et al., 2008; Zinsstag et al., 2005; Ainseba et al., 2010; Xie and Horan, 2009; Hou et al., 2013). The most important aspects treated by these models can be summarised in the following categories: control and eradication strategies, transmission routes, whether direct or indirect transmission or both; modelling the dy-

namics of infective cattle that abort and those that give full-term birth, modelling of vertical transmission, estimation of the transmission rate parameters, modelling the interspecies spread and evaluation of surveillance strategies. One of the earliest models was developed by Gonzalez-Guzman and Naulin (1994), who proposed modelling the disease assuming that transmission results from direct contact between susceptible and infective cattle and they differentiated infective cattle into abortive and non-abortive. This distinction is important in that the bacterial discharge is several logs higher after abortion than after full-term parturition (Williams et al., 1962). Furthermore, Gonzalez-Guzman and Naulin (1994) assumed that after the first abortion a non-abortive, carrier state ensued. In the same way, the model developed by Dobson and Meagher (1996) for the transmission dynamics of bovine brucellosis in bison assumed that disease transmission was via direct contact or through vertical transmission. Ainseba et al. (2010) proposed a model for ovine brucellosis, which incorporated direct (horizontal and vertical transmissions) and indirect or environmental transmission (see also Hou et al. (2013)), without specifically distinguishing abortive from non-abortive infected sheep. It is important to mention, that abortion is not always the first symptom, as the placenta does not always preferentially allow for *Brucella* localisation (Samartino and Enright, 1993). Thus, the spread of brucellosis is closely related to the incidence of abortions or parturitions, and therefore its transmission dynamics depends a lot on animal's reproductive cycle and husbandry conditions.

Traditionally models are tools for mathematically inclined people, performing sensitivity analyses to evaluate the importance and effects of parameter values and uncertainty and model stability and the like. However, currently there exist tools that allow the development and running of mathematical models in the background, exposing the end-user only to a user-friendly interface to select parameter values and immediately see the effect on output, in tabular and graphical format. More specifically the R environment (<http://www.r-project.org>), using RStudio (<https://www.rstudio.com>) and the package `shiny` allow decision makers to interact with mathematical models without necessarily having to understand the finer details of the underlying model.

Given the characteristics of the above mentioned models, there is a need for a mathematical model to unify the important features of bovine brucellosis dynamics described in relation to dairy cattle herds. This paper therefore proposes a model for the transmission dynamics of brucellosis which takes into account partitions of infectious cattle into abortive and non-abortive compartments, the incorporation of vertical, sexual (including artificial insemination), and direct and indirect sources of transmission such as a contaminated environment. The aim of this study was to describe the dynamics of brucellosis in a dairy cattle herd in an ordinary differential equations system and analyse the behaviour of the system in different scenarios. The stability of the system was evaluated numerically and estimates of losses due to the disease were presented. Finally, the effects of control

programs such as vaccination and culling were investigated. A tool for managers and decision makers was developed, so that the consequences of control measures and changes in models parameters may be evaluated directly through the `shiny` application. As a result, the model presents a valid tool for decision makers in order to study brucellosis dynamics in dairy cattle herds and thereby it proposes some strategies to control and eradicate the disease using current knowledge in the disease transmission and available technologies.

5.3 Model Formulation and Compartments

5.3.1 Compartments

The model depicts various traits of individuals within different disease stages. In cattle, population is divided according to the sex and to the disease stage. In females, susceptibility of individuals starts when they reach sexual maturity (S). After infection, infected primiparous cows (P_1) might abort or they might calve at full term, those compartments are represented by the stages I_b and I_f respectively. Following an abortion or a birth, infected cows get newly pregnant having again the possibility to either abort or to give birth. These pregnancy stages are named in the model as the infected multiparous cows P_n . By the males side, susceptible bulls (M) may contract the disease becoming in infective bulls (I_m). Additionally, infected females and males may come from vertical transmission route. Similarly, sexual transmission is represented by the presence of infected males (I_m) in the system, or by the presence of *Brucella*-infected semen straws (Δ). Furthermore, in the model the environmental pool of *Brucella* (B) is fed by infected stages especially when aborting or calving stages evict the bacteria on pastures and feedstuffs. Two additional compartments compose the system which include the presence of wildlife (w) and the presence of humans (h). In the case of the application of control strategies two additional compartments are needed in the model: R_f and R_m which represent bull and cow immunized by vaccine effects. A full description of each stage and model parameters is given in Table 5.1. Figure 5.1 represents the flow diagram of the disease and the full system. The following compartments conform the system:

- S : Susceptible cows
- M : Susceptible bull
- P_1 : Infected Primiparous cows
- I_f : Infected cows calving at full term
- I_b : Infected cows aborting
- P_n : Infected multiparous cows

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- I_m : Infected bulls
- B : Environmental pool of bacteria
- w : Wildlife population
- h : Human population
- Δ : Proportion of *Brucella*-infected semen straws
- R_f Cows immunized by the vaccine effect
- R_m Bulls immunized by the vaccine effect

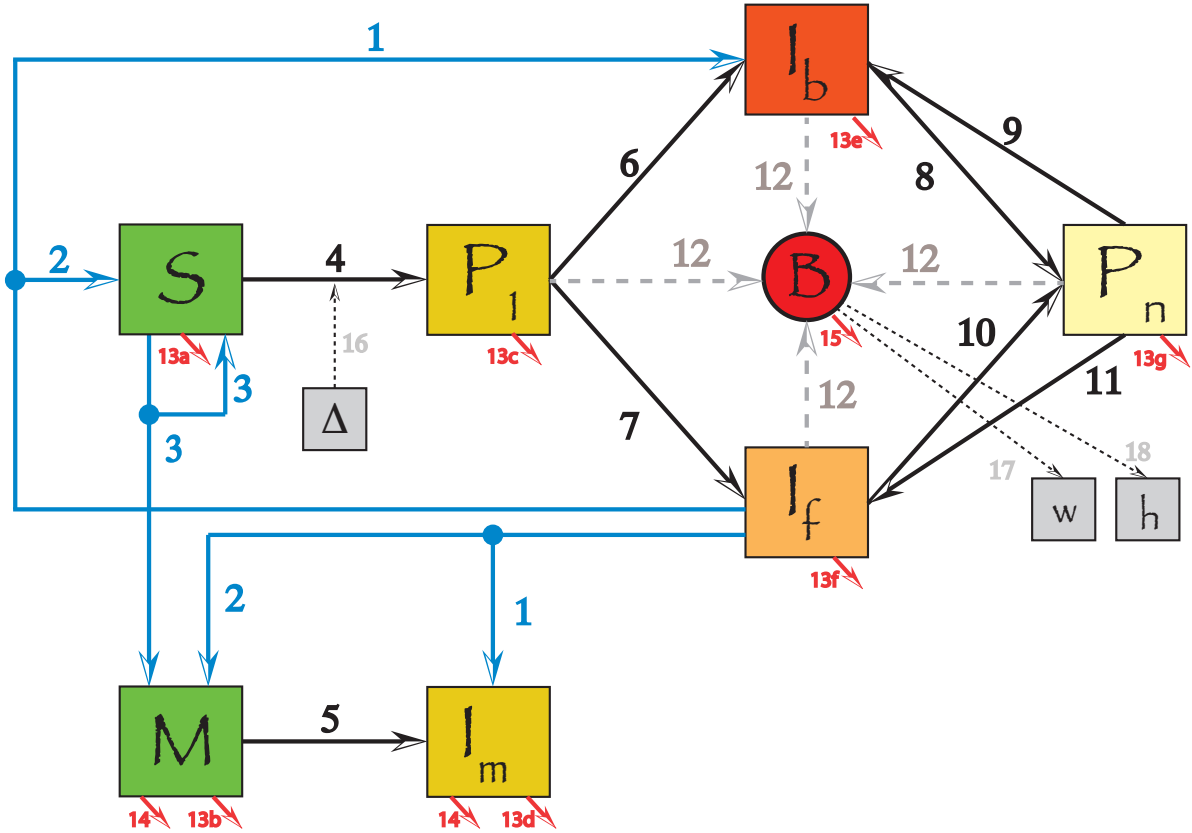


Figure 5.1: *Brucella abortus* transmission dynamics.

$$\begin{aligned}
 1 &= 0.5\gamma\delta(1 - \mu_c)^{18}; \quad 2 = 0.5\gamma(1 - \delta)(1 - \mu_c)^{18}; \quad 3 = 0.5\gamma(1 - \mu_c)^{18}; \quad 4 = \beta_1 + \beta_2 \\
 5 &= \beta_1 + \beta_3; \quad 6 = \lambda_1\xi_b; \quad 7 = (1 - \lambda_1)\xi_f; \quad 8 = \omega_b; \quad 9 = \lambda_n\xi_b; \quad 10 = \omega_f; \quad 11 = (1 - \lambda_n)\xi_f \\
 12 &= \tau; \quad 13a = \mu_S; \quad 13b = \mu_m; \quad 13c = \mu_{P_1}; \quad 13d = \mu_{I_m}; \quad 13e = \mu_{I_b}; \quad 13f = \mu_{I_f}; \quad 13g = \mu_{P_n}; \quad 14 = \sigma_s; \quad 15 = \\
 &\mu_b; \quad 16 = \eta\varepsilon; \quad 17 = \rho_w; \quad 18 = \rho_h
 \end{aligned}$$

Table 5.1: Model parameters values

| Symbol | Description | Value(s) | References |
|--------------------------------|---|------------------------|---|
| γ | Calving rate | $\frac{3}{40}$ | Yamamoto et al. (2008) |
| μ_c | Calf and heifer death and sales probability | $[0.0024 - 0.03775]^*$ | Svensson et al. (2006), Moran (2012) |
| μ_s | Mortality rate in susceptible females | 1/38 | England et al. (2004), Yamamoto et al. (2008) |
| μ_{P1} | Infected-primo-parous mortality rate | 1/38 | England et al. (2004), Yamamoto et al. (2008) |
| μ_{I1} | Infected full-term calving cows mortality rate | 1/38 | England et al. (2004), Yamamoto et al. (2008) |
| μ_{Ib} | Infected aborting cow mortality rate | 1/38 | England et al. (2004), Yamamoto et al. (2008) |
| μ_{Pn} | Infected-multiparous cow mortality rate | 1/32 | England et al. (2004), Yamamoto et al. (2008) |
| μ_{In} | Mortality rate of infected bulls | 1/32 | England et al. (2004), Yamamoto et al. (2008) |
| σ_m | Monthly sales rate of bulls | [1-4] | Assumption |
| η | Proportion of cows inseminated artificially (a.i.) | 0.1 | Scenario |
| κ_b | Carrying capacity of bovine population | 300 | Assumption |
| Transmission parameters | | | |
| φ_e | Effective infection rate of bacteria in environment | $3.13 \cdot 10^{-14}$ | Chevillat et al. (1998), Manthei and Carter (1950), McEwen et al. (1939), Kenyon et al. (1979)† |
| φ_c | Effective infection rate due to direct contact | 0.00025 | Dobson and Meagher (1996) |
| φ_{mf} | Effective sexual infection rate from ♂ to ♀ | 1e-8 | Uhring et al. (2013) |
| φ_{fm} | Effective sexual infection rate from ♀ to ♂ | 1e-7 | Assumption |
| φ_w | Effective infection rate due to direct contact with infected game | 0.00002 | England et al. (2004), Yamamoto et al. (2008) |
| ε | Effective infection rate due to artificial insemination | $4.4 \cdot 10^{-3}$ | Chevillat et al. (1998) |
| δ | Proportion of calves infected by vertical transmission | 0.1 | England et al. (2004), Yamamoto et al. (2008), Ainseba et al. (2010) |
| Inner parameters | | | |
| λ_1 | Proportion of primo-infected cows aborting | 0.6 | Dobson and Meagher (1996), Gonzalez-Guzman and Naulin (1994), Yamamoto et al. (2008) |
| λ_n | Proportion of abortions in subsequent gestations after infection | 0.1 | Gonzalez-Guzman and Naulin (1994), OIE (2005) |
| ω_f | Pre-conception rate after full-term pregnancy (days open) | $\frac{1}{3}$ | Moran (2012), Yamamoto et al. (2008) |
| ω_b | Pre-conception rate after an abortion (days open) | 1/3 | Moran (2012), Yamamoto et al. (2008) |
| ξ_b | Average time to abort | 6 (rate 1/6) | England et al. (2004), Yamamoto et al. (2008) |
| ξ_f | Average time for calving | 9 (rate 1/9) | England et al. (2004), Yamamoto et al. (2008) |
| α_f | Reduction in infectivity respect to abortive stage | 0.5 | Assumption |
| α_n | Reduction in infectivity respect to abortive stage | 0.05 | Derived + |
| Environmental Sources | | | |
| μ_b | Natural-environmental clearance rate of <i>Brucella sp.</i> | 1.0 | Aune et al. (2012) |
| τ_{Ib} | Bacterial discharge rate by cows in abortive stage | 10 ¹² | Williams et al. (1962) |
| τ_{If} | Bacterial discharge rate by cows in calving stage | 10 ⁹ | Ainseba et al. (2010) |
| τ_{P1} | Bacterial discharge rate by primo-infected cows | 10 ³ | Assumption |
| τ_{Pn} | Bacterial discharge rate by infected cows in subsequent pregnancies | 10 ³ | Derived + |
| τ_w | Bacterial discharge rate by infected game | 10 ³ | Assumption |
| ρ_b | Effective removal rate by cattle | 0.0025 | Assumption |
| ρ_w | Effective removal rate by wildlife | $1 \cdot 10^{-6}$ | Assumption |
| ρ_h | Effective removal rate by humans | $1 \cdot 10^{-8}$ | Assumption |
| Black boxes | | | |
| w | Game animal population around | 2 | in bovine units |
| t_w | Proportion of game animals that are infectious | 0.00001 | Assumption |
| Δ | Proportion of infected semen-straws | 10 ⁻³ | Assumption |
| h | Human population in the system (fixed) | Scenario | Scenario |
| Control measures | | | |
| ν | Vaccination proportion | [0-1] | Scenarios |
| X | Vaccine efficacy | 0.9 | Olsen and Stoffregen (2005) |
| S_p | Joint test Specificity | [0.5, 1.0] | |
| S_e | Joint test Sensitivity | [0.5, 1.0] | |
| ζ_f | Net culling rate for cows | | |
| ζ_m | Net culling rate in males | [1-5] | |

+ Rest period of pastures estimated to be 40 days (0.75 month⁻¹) times the inverse number of animals in the system (animal⁻¹) [100-1000 animals]
 † Probability of infection: $\pi = \frac{1}{1 + \exp(-0.566 - 4.179 \cdot 10^{-8} B)}$ via conjunctival route (Chevillat et al., 1998; Manthei and Carter, 1950). Thus, if the efficacy of the each bacterium via digestive route might be reduced 10⁶ times and the period of time to revisit the same place for each animal is 0.75month⁻¹ then, for time continuous model $\varphi_e = 3.13 \cdot 10^{-14}$

5.3.2 Model Equations

A set of ordinary differential equations (ODEs) that expresses the temporal changes of animal movements according to their disease-stage condition and the changes in the pool of environmental bacteria is given as follows:

$$\begin{aligned}\frac{dS}{dt} &= [0.5\gamma(1 - \mu_c)^{18}] \left[R_f + S + (1 - \delta)\frac{I_f}{\omega_f} \right] (1 - \nu\chi) - [\beta_1 + \beta_2 + \mu_s + (1 - Sp)\zeta_f] S \\ \frac{dM}{dt} &= [0.5\gamma(1 - \mu_c)^{18}] \left[R_f + S + (1 - \delta)\frac{I_f}{\omega_f} \right] (1 - \nu\chi) - [\beta_1 + \beta_3 + \mu_s + \sigma_m + (1 - Sp)\zeta_m] M \\ \frac{dP_1}{dt} &= (\beta_1 + \beta_2)S - [\lambda_1\xi_b + (1 - \lambda_1)\xi_f + \mu_{p_1} + Se\zeta_f] P_1 \\ \frac{dI_f}{dt} &= (1 - \lambda_1)\xi_f P_1 + (1 - \lambda_n)\xi_f P_n - (\omega_f + \mu_{I_f} + Se\zeta_f) I_f \\ \frac{dI_b}{dt} &= [0.5\gamma(1 - \mu_c)^{18}] \delta \frac{I_f}{\omega_f} + \lambda_1\xi_b P_1 + \lambda_n\xi_b P_n - (\omega_b + \mu_{I_b} + Se\zeta_f) I_b \\ \frac{dP_n}{dt} &= \omega_f I_f + \omega_b I_b - [(1 - \lambda_n)\xi_f + \lambda_n\xi_b + \mu_{p_n} + Se\zeta_f] P_n \\ \frac{dI_m}{dt} &= [0.5\gamma(1 - \mu_c)^{18}] \delta \frac{I_f}{\omega_f} + (\beta_1 + \beta_3)M - (\mu_{I_m} + \sigma_m + Se\zeta_m) I_m \\ \frac{dR_f}{dt} &= [0.5\gamma(1 - \mu_c)^{18}] \left[R_f + S + (1 - \delta)\frac{I_f}{\omega_f} \right] \nu\chi - [\mu_s + (1 - Sp)\zeta_f] R_f \\ \frac{dR_m}{dt} &= [0.5\gamma(1 - \mu_c)^{18}] \left[R_f + S + (1 - \delta)\frac{I_f}{\omega_f} \right] \nu\chi - [\mu_s + \sigma_m + (1 - Sp)\zeta_m] R_m\end{aligned}$$

$$\frac{dB}{dt} = \tau_{I_b} I_b + \tau_{I_f} I_f + \tau_{p_1} P_1 + \tau_{p_n} P_n + \tau_w l_w w - \rho B$$

$$N = S + M + I_f + I_b + P_1 + P_n + I_m + R_f + R_m$$

$$\beta_1 = \varphi_e B + \varphi_c [\alpha_f I_f + I_b + \alpha_n (P_1 + P_n)] + \varphi_w l_w w$$

$$\beta_2 = (1 - \eta)\varphi_{mf} I_m + \eta\varepsilon\Delta$$

$$\beta_3 = (1 - \eta)\varphi_{fm} (I_b + I_f)$$

$$\rho = \mu_b + \rho_b N + \rho_w w + \rho_h h$$

$$\mu_c = 0.00241 + \frac{(0.03775 - 0.00241)}{1 + 0.7 * \exp(-10 * (N/\kappa_b) - 1)^{25000}}$$

5.3.3 Model Description and Parameter estimation

The first equation in the model represents the dynamics of the susceptible cows in the population (S). New female calves, that will contribute to the susceptible population, come from three sources of adults cows: the susceptible adult cows (S), brucellosis-immunised cows (R_f) and infected cows calving at full term (I_f) but giving uninfected calves. This

last source is affected by the term $1 - \delta$ that represents the proportion of calves non-infected by vertical transmission and those contributions were divided by ω_f to compensate for the time they spend in stage I_f . Thus, the term $[0.5\gamma(1 - \mu_c)^{18}]$ represents the net birth rate of the adult female susceptible population after having survived the immature period (18 months). The term $1 - \nu\chi$ represents the proportion of individuals non-vaccinated or not effectively vaccinated that remain in the susceptible population. The susceptible population of cows are diminished because either they get the disease (interaction: $[\beta_1 + \beta_2]S$) or they are eliminated from the herd because of the mortality, sales or culling (interaction: $[\mu_s + (1 - Sp)\zeta_f]S$). The term $1 - Sp$ represents the lack of specificity (presence of false positives) in diagnostic tests.

The dynamics of susceptible males or bulls (M) is represented in the second equation. This compartment has almost the same terms as susceptible females with the exception that extra sales are allowed (σ_m), especially in dairy cattle where few males are maintained in the system.

From the third to the sixth equation the dynamics of infected females is described as in the compartment definition list for P_1 , I_b , I_f , and P_n . The effect of vertical transmission is seen in the fifth equation where new infected calves (δ proportion of infected newborns), coming from infected cows calving, reach sexual maturity. Losses in those compartments are expected due to mortality, sales or culling, but in this case the culling rate ζ_f is affected by the sensitivity of the diagnostic test (recognition of true positives).

The last differential equation describes the dynamics of the bacteria in the environment. As mentioned, this compartment is fed by infected stages, when they spread the bacteria into the environment, but also the contribution of external sources is allowed (term: $\varphi_w \iota_w w$), represented by the possible presence of infected wildlife. Likewise, the bacteria can diminish their population due to natural clearance factors or due to the feed consumption on pastures by the cattle population.

The estimation of specific reproduction, mortality, and transmission rates, and the losses expected due to the disease were obtained as follows:

- **Reproduction and mortality rates**

The demography of cattle population is an important determinant in brucellosis transmission because the majority of infectivity events are regulated by the animals' reproductive cycles. Therefore, defining the demographic parameters in terms of cattle reproductive and mortality rates correctly is important for the model. During the five-year lifespan of a cow, three calves are expected: γ represents the net calving rate in cows and a monthly value of 0.075 has been estimated (Scharrer et al., 2014) in Swiss cattle. This value is divided by 0.5 due to female and male births. Calf and heifer's mortality (μ_c) was estimated from field data collected and reported by Svensson et al. (2006) who estimated a natural mortality for heifers between 0 to 18 months of 4.34% meaning an instantaneous monthly rate of 0.0024 due

to natural causes. Usually in grass-based systems populations can increase until they have reached the carrying capacity (κ_b) of the system. An estimation of κ_b can be obtained from the yield of the farm pastures and from the farm size. In dairy cattle farms, to maintain stable the population size (N) a quantity near to $\frac{2}{3}$ of the new females must be preserved as replacements. This value is obtained by solving the first equation at stability in a disease free system, and by replacing the estimated values for γ and μ_s . In this way, when the population size is over carrying capacity, the excess of female calves has to be sold. Similarly, when population is on the system carrying capacity $\frac{1}{3}$ of the female calves must be sold; meanwhile, when abortions or reproductive problems produce a shortage of replacements, all the new female calves and heifers must be preserved. Thus, a logistic function for the mortality and sales of calves needs to be fitted according to either the excess or the lack of replacements. The last equation in the model and Figure 5.2 display the sales-mortality-logistic curve that describes the dynamics of μ_c as function of the $\frac{N}{\kappa_b}$ fraction. According to this function, when the cattle population in the farm is near to the carrying capacity, that is when $\frac{N}{\kappa_b} \approx 1$, the calf-survival function $(1 - \mu_c)^{18}$ returns a value close to $\frac{2}{3}$ (vertical and horizontal lines in the figure); otherwise, as in the case of brucellosis infection, the new borns tend to be preserved, and just natural mortality for young calves applies for μ_c when it takes a value of 0.0024, which means that more that 95% of the female calves will arrive to the sexual maturity.

Mortality in adults was estimated on the basis that a cow's adult life spans 38 months, so that a rate of 1/38 is used for the net mortality rate; given the fact that cows within the infected multiparous stage P_n have already passed by the previous infected stages, their μ_{P_n} was set at 1/32 to reflect this. A similar value was set for infected males μ_{I_m} because of their earlier replacement.

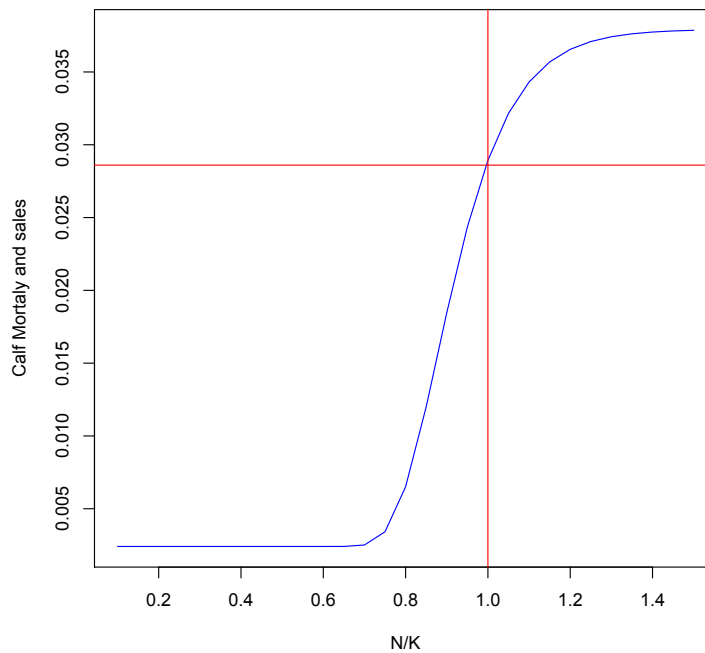


Figure 5.2: Logistic function for sales and mortality in calves and heifers as response to the carrying capacity of the system.

- **Transmission rates**

The epidemiology and transmission of bovine brucellosis are closely related to the incidence of abortions and parturitions in a herd. Thus, the contact and transmission rates will strongly depend on the reproduction rates in dairy cattle. For the model, touching, licking or ingestion of birthing or aborted materials are part of the direct contact transmission as soon as they occur. Similarly, all contacts at the oestrus period, when females exhibit sexual receptive behaviour towards other females and males (without coitus), are also part of the direct contact. In the model, direct transmission is represented by the parameter φ_c and the frequency of contacts are assumed to increase as population size increases, so the function $\varphi_c S[\sum(I_i + P_j)]$ represents the direct contacts between susceptible cows and infective stages (i.e. P_1, I_b, I_f, P_n). An instantaneous rate of 0.00025 indicates that an infective individual infects a number of individuals, given by a Poisson distribution with rate 2 during its lifespan, given a large enough population. We assume that after infection, only a proportion α of the newly infected individuals become infectious. Likewise, once a susceptible cow or heifer becomes infected, they enter a latent period which can last for 6 or 9 months. This period depends on the probability that a cow becomes abortive (λ_i) or gives a newborn at full term. In brucellosis, abortions occur with a high probability between between the fifth and eight month of pregnancy (Yamamoto et al., 2008; England et al., 2004; Samartino and Enright, 1993), yield-

ing a mean rate of $\frac{1}{6}$ per month for this event. Similarly, once a cow aborts, the waiting time for a new conception (ω_b) will be the same as for cattle calving (ω_f), and this parameter took a value of 3 months.

In the model there exist the possibility of modelling vertical transmission from infected cows giving birth at full term (I_f) to new infected heifers and young infected bulls. Infected heifers will become part of the abortive stage after their first pregnancy. This parameter has been represented by δ , and a proportion of 0.1 has been estimated in other studies (England et al., 2004; Yamamoto et al., 2008).

The possibility of environmental transmission occurs when animals come back to the same grazing zone in pasture-based production systems. Because the majority of dairy cattle are raised under pasture-based systems, the ability of *B. abortus* to survive in managed environments is an important factor determining the risk for brucellosis transmission because cattle return to the same places after a pasture growing period (Aune et al., 2012). It was assumed that on average 45 days (0.75 month^{-1}) are required to return to the same grazing place (pasture rest period) (Schlegel et al., 2000). Values for the net-environmental transmission rate (φ_e) were estimated from previous experiments carried out by Cheville et al. (1998) and Manthei and Carter (1950), where different bacterial doses were given to cows through the conjunctival route and we assume that a higher dose is necessary to produce infection through digestive route. The estimation of this quantity appears in the bottom of Table 5.1. Once *B. abortus* is picked up from environment, the bacterium can instantaneously infect susceptible animals and the infection capacity will depend on the ingested dose through the digestive route, which, as explained, requires higher doses than the conventional dose used for conjunctival vaccination (i.e. 10^{10}) (Hou et al., 2013; Olsen and Stoffregen, 2005). The quantity of bacteria shed during an abortion (net rate τ_{I_b}) is assumed to be larger than that shed by cows calving down at full-term (τ_{I_f}). Finally, the bacteria in the environment diminish in two ways. Either, they are picked up by cows during the feeding process on grass (ρ_b), or they die off (μ_b) the rate of which depends on environmental factors, both abiotic (weather) and biotic (scavengers, wildlife) and human interference.

The use of infected semen in artificial insemination has been mentioned as a vehicle for the spread of the bacterium. The dynamics of this element were modelled as the proportion of *Brucella*-infected semen straws (Δ) and the proportion of cows inseminated (η). In the same way, the conception rate under artificial insemination was set to be from 2 to 4 services for a cow pregnancy every year (Norman et al., 2010). ε represents the net infection rate through this route. Similarly, though the possibility of transmission through sexual contact is low, venereal transmission is still a potential infectious contact route (Uhring et al., 2013). Therefore we incorporate

this rate in a very reduced value ($\varphi_{mf} = 10^{-8}$) and we assumed that the rate from female to male is ten times higher ($\varphi_{fm} = 10^{-7}$).

- **Losses and control measures**

Losses were estimated in terms of the number of abortions, time estimated to detect an outbreak, losses in milk production and the number of calves having "heifer syndrome". If control measures were applied, the number of test-positive animals slaughtered, the number of animals vaccinated and the final prevalence and population size are also given. The proportion of animals vaccinated (ν) may vary from 0 to 1 and additionally the vaccine efficacy (χ) was established at 0.7 (Olsen and Stoffregen, 2005). Thus, the proportion $1 - \nu\chi$ represents the proportion of animals that (i) have not been vaccinated and (ii) those where the vaccine did not protect, and that thus remain in susceptible classes S and M . The proportion $\nu\chi$ represents those animals that are effectively vaccinated and therefore are moved from the susceptible class to the permanent immunised classes R_f or R_m . Culling of positive animals has been represented by the rate ζ , and it is applied to those animals showing a positive serological reaction to the diagnosis test. This parameter may vary from 0 in the case of culling is not applied to 2.99 with means that every month almost 0.96 of animals are removed. In the model there exist the possibility to apply periodic testing and remove positive animals every two years. The model accounts for the sensitivity (Se) and the specificity (Sp) of diagnostic tests, and therefore, $1 - Sp$ is the proportion of uninfected animals showing serological reaction that will be slaughtered as false positives.

The cumulative number of abortions was estimated through the mean and the annual cumulative number of animals in the I_b compartment until the tenth year. The cumulative number of heifers infected through vertical transmission was estimated from the I_f compartment producing infected calves (δ), considering those surviving until eighteen months. Milk losses were estimated according to the average yield per lactation per cow, where this parameter may vary from 500 to 10000kg. Milk losses were estimated considering that $\frac{9}{12}$ of the time cows are producing milk when the population is on carrying capacity. In the case of culling as control measure, seropositive animals ($Se[P_1 + I_b + I_f + P_n + I_m] + (1 - Sp)[S + M + R_f + R_m]$) were involved using to the net culling rate (ζ_f or ζ_m). Time to detect the first abortion storm was estimated when the proportion of abortions I_b was superior to the 20% of the normal number of calves expected. Table 5.1 describes the rest of model parameters, their possible values, and the references from which they were obtained.

5.4 Simulations and scenarios

We developed a simulation tool for managers and decision makers, so that the consequences of control measures and changes in models parameters may be evaluated directly through a `shiny` application. The user manual is to be found in section 9.1 and the listing of the program is given in section 9.2. The model inputs can be changed by the user. In the same way, different control strategies may be evaluated and also the running time can be changed to evaluate strategies at long-term.

Table 5.2 presents the effects of different initial conditions in the system without control measures. The losses in milk production were estimated for an average lactation yield of 5000 kg and the initial susceptible population was 150 cows and 4 bulls. In the top half of the table, the effects of the introduction of one infected individual from different infectious and infective classes are presented (first and second columns). In the bottom half of the table, different proportions of artificial insemination and levels of infected semen straws are used as reproduction system and their effects are also shown. In general, the spread of the disease and its effects were worst when an abortive stage (I_b) was introduced in the system, followed by the introduction of an infected primiparous cow (P_1). The times expected (in months) until the abortion storm is apparent were ten and fourteen months respectively for the introduction of one I_b and one P_1 . The time increased to 77 months when an infected male was introduced into the herd. When A.I. was used the time to an outbreak of abortions took between 32 to 42 months in function of the proportion of inseminated cows and the quantity infected semen.

Table 5.2: Time estimated to a storm of abortions, cumulative number of abortions, infected heifers, and milk losses in dairy cattle due to *B. abortus*

| Initial condition | Time (months) | Cumulative abortions | | | Infected Heifers | | | Milk losses (kg) | | | | |
|-------------------|---------------|----------------------|--------|---------|------------------|--------|---------|------------------|--------|--------|---------|---------|
| | | year 2 | year 5 | year 10 | year 2 | year 5 | year 10 | year1 | year 2 | year 5 | year 10 | |
| 1 I_b | 10 | 52.3 | 197.6 | 337.8 | 0.1 | 13.3 | 29.6 | 33020 | 261234 | 987830 | 1689120 | |
| 1 P_1 | 14 | 31.0 | 190.0 | 334.0 | 0.7 | 12.7 | 29.0 | 14288 | 155096 | 949426 | 1668283 | |
| 1 P_n | 22 | 7.7 | 172.3 | 322.6 | 0.2 | 9.4 | 27.7 | 2510 | 38456 | 861718 | 1612993 | |
| 1 I_f | 23 | 6.4 | 170.2 | 321.3 | 0.2 | 9.7 | 27.6 | 2010 | 32105 | 850981 | 1606255 | |
| 1 I_m | 77 | 0.0 | 0.1 | 193.1 | 0.0 | 0.0 | 12.2 | 0 | 0 | 549 | 965670 | |
| η | Δ | | | | | | | | | | | |
| 0.20 | 0.05 | 42 | 0.1 | 92.3 | 282.9 | 0.0 | 2.3 | 23.0 | 24 | 465 | 461470 | 1414334 |
| 0.20 | 0.10 | 39 | 0.2 | 111.0 | 289.7 | 0.0 | 3.8 | 23.8 | 49 | 929 | 555091 | 1448538 |
| 0.50 | 0.05 | 38 | 0.2 | 116.5 | 292.0 | 0.0 | 4.2 | 24.0 | 61 | 1161 | 582015 | 1459326 |
| 0.50 | 0.10 | 35 | 0.5 | 131.2 | 298.4 | 0.0 | 5.3 | 25.0 | 122 | 2316 | 655907 | 1492094 |
| 1.00 | 0.05 | 35 | 0.5 | 131.2 | 298.4 | 0.0 | 5.3 | 25.0 | 122 | 2316 | 655907 | 1492094 |
| 1.00 | 0.10 | 32 | 0.9 | 143.5 | 304.7 | 0.0 | 6.4 | 25.5 | 242 | 4611 | 717288 | 1523630 |

I_b = infected abortion; P_1 = Primiparous infected cow; P_n = multiparous infected cow; I_f = infected full-term delivery; I_m = infected bull; η = proportion of cows inseminated; Δ = proportion of infected semen straws

5.4. SIMULATIONS AND SCENARIOS

Table 5.2 also presents the cumulative number of abortions expected, the number of infected heifers carrying the disease and the milk losses since the introduction of one infectious case. When infective females were introduced the cumulative number of abortions after 5 years varied between 170 and 197 and the cumulative expected number increased from 321 to 338 after 10 years. In the case of sexual infections this cumulative number changed and varied between 193 to 304, respectively when only natural mating and only artificial insemination were used as reproductive strategies. In general, the number of heifers carrying infection was similar according to the initial condition, and they were between 23 to 30, with the exception of introduction of an infected male, which produced an expected number of 12 infected heifers after 10 years.

In the same way, the last four columns of the table present the cumulative number of milk losses. During the first year, significant losses were expected when infective cows were introduced; in the fifth year similar losses were expected according to the initial condition and even when AI was used. Losses in milk production were equivalent to a loss of 2 to 4 kg milk per cow per day. A similar quantity was found when the system was carried out at 10 years of the initial infection when the losses varied from 2.1 to 3.7 kg per cow and per day.

Table 5.3: Control strategies and results after 10 years of applying the control measures under natural mating

| Strategy | Parameters | Cumulative abortions | Cumulative milk losses | Slaughtered animals (cumulative) | Final population | Susceptible cows | Prevalence % |
|---------------------------------|---|----------------------|------------------------|----------------------------------|------------------|------------------|--------------|
| <i>No control</i> | | 338 | 1689120 | 0 | 98 | 24 | 74 |
| Vaccination | $\nu = 100\%$ $\chi = 100\%$ | 77 | 386224 | 0 | 179 | 0 | 2 |
| Vaccination | $\nu = 100\%$ $\chi = 70\%$ | 159 | 799695 | 0 | 173 | 27 | 15 |
| Culling (monthly) | $\zeta = 2.99$ $Se=1; Sp = 1$ | 0 | 599 | 2 | 189 | 179 | 0 |
| Culling (monthly) | $\zeta = 2.99$ $Se = 0.90; Sp = 0.95$ | 0 | 649 | 161 | 0 | 0 | 0 |
| Periodic testing(bianual) | $\zeta = 2.99$ $Se = 1.0; Sp = 1.0$ | 83 | 416274 | 144 | 46 | 45 | 0 |
| Periodic testing(binannual*) | $\zeta = 2.99$ $Se = 0.90; Sp = 0.95$ | 72 | 361452 | 160 | 32 | 31 | 0 |
| Vaccination+ culling (bianual) | $\zeta = 2.99; \nu = 100\%$ $\chi = 70\%;$ $Se = 1.0; Sp = 1.0$ | 25 | 127126 | 45 | 180 | 53 | 0 |
| Vaccination+ culling (bianual) | $\zeta = 2.99; \nu = 100\%$ $\chi = 70\%;$ $Se = 0.90; Sp = 0.95$ | 21 | 106305 | 131 | 158 | 46 | 0 |
| Vaccination+ culling (bianual) | $\zeta = 2.1; \nu = 100\%$ $\chi = 70\%;$ $Se = 0.90; Sp = 0.90$ | 0 | 0 | 142 | 152 | 46 | 0 |
| Vaccination+ culling (bianual*) | $\zeta = 0.7; \nu = 100\%$ $\chi = 70\%;$ $Se = 0.90; Sp = 0.90$ | 0 | 14 | 52 | 172 | 54 | 0 |

ν = proportion animals vaccinated; χ = vaccine efficacy; ζ = instantaneous culling rate; Se = test sensitivity; Sp = test specificity
* = additional outbreak occurs after initial period

Table 5.3 presents the effects of different control measures after 10 years of introduction of an infected abortive cow into the herd for a system with natural mating and Table 5.4

presents the effects for a system with AI, where a proportion of semen-straws are infected. The results are given in terms of the cumulative number of abortions, cumulative milk losses, animals slaughtered because of their serological reaction, final population, number of susceptible animals persisting in the system and the final prevalence reached.

Table 5.4: Control strategies and results reached after 10 years of application under A.I. with 5% of infected straws

| Strategy | Parameters | Cumulative abortions | Cumulative milk losses | Slaughtered animals (cumulative) | Final population | Susceptible cows | Prevalence % |
|----------------------------------|--|----------------------|------------------------|----------------------------------|------------------|------------------|--------------|
| Vaccination+ culling (bianaual) | $\zeta = 2.99$; $\nu = 100\%$ $\chi = 70\%$; $Se = 0.90$; $Sp = 0.90$ | 0.5 | 2552 | 177 | 166 | 35 | 0 |
| Vaccination+ culling (bianaual*) | $\zeta = 0.7$; $\nu = 100\%$ $\chi = 70\%$; $Se = 0.90$; $Sp = 0.90$ | 25 | 126490 | 63 | 166 | 40 | 4 |

ν = proportion animals vaccinated; χ = vaccine efficacy; ζ = instantaneous culling rate; Se = test sensitivity; Sp = test specificity
* = additional outbreak occurs after initial period

Vaccination applied alone as control measure proves inefficient although the prevalence decreases. Our results show that, even though all the female calves were vaccinated with a 100% vaccine efficacy, after 10 years 2% prevalence persists in the animals and a considerable number of abortions and losses of 0.85 kg of milk per cow per day are expected. If vaccine efficacy is reduced to 70% the number of abortions increases, milk losses increase to 1.75 kg per cow per day and the final prevalence increases to 15%. Culling was applied in two ways: monthly (rows 4 and 5 of Table 5.3) and biannually (rows 5 and 7 of Table 5.3). Permanent culling (monthly) with 100% of Se and 100% of Sp applied to the total population allows the identification of infective cases and they are removed as soon as they are detected so that two animals are slaughtered and milk losses are minimal. Permanent culling with $Se = 0.9$ and $Sp = 0.95$ destroys the population, as expected, because of the imperfect test characteristics. Biannual testing, assuming perfect characteristics reduces the prevalence of the disease to 0% but also reduces the final population to 46 animals and losses are quantified in 83 abortions, 144 slaughtered animals and 0.91 kg milk per cow per day at the end of the 10 years. Biannual testing, with imperfect characteristics (i.e. $Se = 0.9$ and $Sp = 0.95$), reduces the prevalence to 0% but the population is reduced substantially to 32 individuals and losses are 72 abortions, 160 animals slaughtered and 0.79 kg of milk per cow per day; additionally, other outbreaks may appear in the future.

The application of vaccination and culling may be evaluated in the last four rows of Table 5.3. Strict culling every two years plus vaccination with a vaccine efficacy of 70% using perfect or imperfect tests will produce the disease elimination at the end of this period, but losses in slaughtered animals (45 to 131) and milk yield (0.23 to 0.27 kg per cow per day) are expected. However, if the culling rate is reduced, losses in milk are almost negligible although between 52 to 152 animals are lost because of the slaughtering. The last strategy, which implies the testing of the half the population every two years and

the application of strict vaccination and imperfect tests, is the most plausible scenario for the brucellosis control and eradication without major losses, although as mentioned it will require permanent surveillance through time because of the possibility of outbreaks in future.

For systems under artificial insemination (AI), (see Table 5.4) the application of strict vaccination and the testing of the whole herd is the unique measure that can eradicate the disease, although losses can be considerable because of the elimination of seropositive animals (i.e. 177 for our case during time of application). The application of half-testing for these systems does not produce the disease elimination (prevalence 4%) and losses of 0.27 kg of milk per cow and day and new outbreaks are still expected. This measure makes systems under AI that do not prove that is semen free of brucellosis more risky than systems with natural mating.

Figures resulting from the standard simulation are shown in Figures 9.1 – 9.6.

5.5 Discussion

The dynamics of bovine brucellosis transmission are highly influenced by the cattle's reproductive cycle and its parameters. Bovine brucellosis is considered an economically devastating zoonosis (England et al., 2004). In economic terms, losses due to abortions and the consequent lactation delays make this disease a serious problem for dairy farmers and for cattle production in general because of a considerable reduction in animal replacements and therefore a reduction in the production efficiency. Furthermore, brucellosis is of the importance due to its zoonotic risk and the potential transmission to other livestock species and wildlife (Godfroid, 2004; Zinsstag et al., 2005; Li et al., 2014). For instance, in Ecuador brucellosis in cattle apparently is the main source for human infections (Ron-Román et al., 2014; Rodríguez-Hidalgo et al., 2015), so that the elimination of the disease in this animal reservoir will help to improve the status of public and animal health sectors.

In this article, we develop a model that emphasises the split of the susceptible population according to the sex and infectious stage. In females, because of *Brucella* affinity for reproductive tissues the distinction between abortive cows and the ones having a full-term parturition is of real importance in brucellosis transmission dynamics, because of the large quantities of bacteria discharged after abortion (Yamamoto et al., 2008; England et al., 2004). Similarly, the distinction between infectious primiparous and infectious multiparous cows is of relevance due to the high abortion probability presented in the first group in comparison to those in subsequent pregnancies after a first abortion. In males only two disease categories are necessary to describe their participation in the disease cycle: susceptible and infective-infectious males who may introduce the disease through sexual contact.

With respect to cattle demography of, we argue that in healthy herds without repro-

ductive problems, a proportion near to $2/3$ of the female calves have to be preserved as future replacements. This means that $1/3$ of female calves must be sold in order to maintain the population near to the carrying capacity of the system. Carrying capacity of the systems may be calculated from the pasture stocking rate in field. If there is brucellosis infection in the herd, a deficit of female calves may become evident and therefore milk production losses may start to appear.

In the model, direct contact between females is one of the most important transmission routes; however, the contributions sexual transmission, artificial insemination, vertical transmission and environmental reservoirs are also important and have to be taken into account. Calves infected through vertical transmission have been mentioned to be negative to diagnostic serological tests, but they tend to abort during their first pregnancy. Such animals pose a serious threat to brucellosis control and eradication (Díaz-Aparicio, 2013; Cheville et al., 1998).

Environmental reservoirs may accumulate important quantities of bacteria. In grass-based production systems may become a source of infection because of the cycles in pasture rotation. The contribution of this route becomes evident after a first abortion storm when the levels of bacteria in the environment are relatively high. Under favourable external conditions, where *Brucella* may survive for long periods, an important source of risk is created, given that the resting-time for pastures is less than 40 days in many places (FAO/WHO/OIE, 2006; Cheville et al., 1998). In this sense, the use calving areas will decrease close contact between animals and external bacteria; however, there is not any specific strategy to reduce this contact in case of an abortion.

Although the venereal route has not been considered to be epidemiologically important in transmitting brucellosis in cattle, infected semen used in artificial insemination is a proven important route (Díaz-Aparicio, 2013), and maybe responsible for the spread of the disease in different regions and countries (Eaglestone and Garcia, 2000). Simulations of the model predict for this route that the time for an abortion storm is longer (from 30 to 40 months) in comparison to the introduction of an infective female (from 10 to 25 months). Natural mating may also be a source of infection and the time required to generate a abortion storm will take nearly 70 months. The times to detect a brucellosis outbreak (10-70 months) in our model are similar to those reported by Yamamoto et al. (2008) when abortions were used as a surveillance strategy to detect brucellosis outbreaks. In similar way, AI as a system of reproduction, according to the our results is more risky for brucellosis transmission and its use must be allowed only when brucellosis free-semen is guaranteed.

According to the brucellosis parameters used in our model, and given the available control and eradication tools, brucellosis in cattle is definitely an eradicable disease. However, a combination of measures must be implemented. For example in endemic zones, strict vaccination for female calves and the biannually serological testing of part (at least half)

of the herd may reduce the disease prevalence to a negligible proportion in less than ten years. In production systems under artificial insemination the culling rate must be higher in comparison to natural mating systems, but strict epidemiological surveillance is also required because the possibility of future outbreaks, as was demonstrated in the simulations. The model, developed in this work, might also help to evaluate the consequences of not controlling the disease in more economic terms.

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Chapter 6

APPLICATION OF DISCRETE-COUNT DISTRIBUTIONS TO ESTIMATE EFFECTIVE POPULATION SIZE IN CATTLE

Modified from "Estimation of effective population size using bivariate discrete distributions for modeling family size in beef cattle". (2008) L. Ron Garrido, A.N. Birchmeier, S. Munilla, R.J.C. Cantet. *Livestock Science*, 117:43-51

Estimation of effective population size using bivariate discrete distributions for modeling family size in beef cattle

L. Ron Garrido^a, A.N. Birchmeier^b, S. Munilla^b, R.J.C. Cantet^{b,c,*}

^a *Facultad de Ingeniería en Biotecnología, Escuela Politécnica del Ejército, Ecuador*

^b *Departamento de Producción animal, Facultad de Agronomía, Universidad de Buenos Aires, Argentina*

^c *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

Received 3 January 2007; received in revised form 5 November 2007; accepted 8 November 2007

Abstract

Pedigree records of 72,808 animals (45,668 females and 27,140 males) from the genetic evaluation program of the Argentine Brangus Association were used to estimate effective number of founders (N_f), effective number of ancestors (N_a), and effective population size under random mating (N_e) or selection (N_{eS}), in order to assess genetic variability. The average level of completeness of the pedigree was low (0.17) and the average level of inbreeding (F) calculated from the pedigree was equal to 0.24%. Animals in the reference population were 21,662 calves born from 2001 to 2005. The estimated measures of variability were $N_f=765.7$ and $N_a=387.5$. The numbers of ancestors responsible for 100%, 50%, or 20% of the genes in the reference group, were equal to 12,471, 273, and 22, respectively. Direct estimates of N_e and N_{eS} were calculated using the variances and covariances of family sizes, i.e. male and female progeny numbers for bulls and cows. Estimates of the dispersion parameters were from the Bivariate Poisson model for the cows, and from the Generalized Bivariate Negative Binomial (GBIVARNB) distribution for the bulls. The latter probability mass function accounted for overdispersion, a characteristic present in the sampling distribution of family size of bulls. The estimated variances of male and female progeny and the covariance between them for the bulls were 5.70, 271.28, and 30.15, respectively, and 1.15, 2.10, and 1.06 for the cows. Generation intervals (in years) were: sires of bulls=5.0, sires of cows=5.7, dams of bulls=4.4, and dams of cows=5.2. The estimated N_e was 274, which corresponds to a rate of inbreeding (F) of 0.18%, whereas $N_{eS}=125$ and $F=0.40\%$. As a check of the proposed methodology, all analyses were also performed using the pedigree records of 10,483 Angus animals from a herd with an average level of completeness of 0.68. Using the GBIVARNB model for both bulls and cows the estimated $N_e=95.4$, thus $F=0.5\%$ in perfect agreement with the calculated average inbreeding from pedigree records. Under selection, $N_{eS}=79.3$ and $F=0.6\%$. The larger difference between estimated N_e and N_{eS} in the Brangus was related to the smaller bull to cow ratio in the breed. Therefore, it seems desirable to continue monitoring the effective size of the Argentine Brangus to prevent problems of inbreeding and lack of variability in the future.

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Keywords: Brangus; Effective population size; Family size; Bivariate discrete distributions; Overdispersion; selection

1. Introduction

In order for a composite breed not to dissipate the initial advantage of increased heterozygosity by becoming inbred, it is essential that heterozygosity (heterosis)

* Corresponding author. Av. San Martín 4453, C1417DSE, Buenos Aires, Argentina. Tel.: +54 11 4524 8000x8184; fax: +54 11 4514 8737.

E-mail address: rcantet@agro.uba.ar (R.J.C. Cantet).

be retained by maintaining an effective population size (N_e) sufficiently large (Gregory et al., 1993). Also, Hill (2000) observed that for a trait with heritability 1/3, (for example weaning weight in beef cattle), a value of $N_e=250$ is required to maintain the additive variance at its initial value. The Brangus breed is the largest composite population of beef cattle in the subtropics of Argentina. Since its creation in 1978, the Argentinean Brangus Breeders Association (AAB) has kept an open registry policy to maintain high levels of variability and to retain a high level of heterosis. The initial motivation for the research presented here was to quantify what is the current level of genetic variability, and to evaluate the pedigree structure of Brangus in Argentina.

Estimation of the N_e when pedigree records are available, can be accomplished indirectly by calculating the change in inbreeding (ΔF) since the breed formation, and then solving the expression $\Delta F=1/(2N_e)$ (Wright, 1931). However, this estimate is affected by the level of completeness in the pedigree, which causes underestimation of inbreeding (Miglior and Burnside, 1995; Lutaaya et al., 1999; Cassell et al., 2003). There is an extensive literature on direct calculation of N_e (see the review by Caballero, 1994) and several formulae are available depending on the assumptions of the data at hand. Hill (1979) obtained an expression for N_e that takes into account overlapping generations and the structure of the mating system through the variances and covariances of progeny numbers, or family sizes. In case of selection, the expression by Hill (1979) does not account for inherited selective advantage, i.e. the process by which the progeny from selected parents tend to have larger family size than those offspring from parents with smaller family size. Based on Santiago and Caballero (1995), Nomura (1996) derived an expression for N_e in selected populations with overlapping generations.

It is frequently assumed that the random variable family size in different animal species follows a Poisson distribution (Harris and Allenford, 1989; Goddard and Smith, 1990; Caballero, 1994; Joshi et al., 1999) in which mean and variance are equal. This assumption is unlikely to be fulfilled for most farm animal species where a small group of sires have a large contribution to the progeny pool. This, in turn, may induce overdispersion from a Poisson probability mass function. In addition, the family sizes of sires and dams in the formulae of Hill (1979) and Nomura (1996) require a bivariate specification, so that the covariance of male and female progeny numbers for both bulls and cows can be calculated. Possible discrete bivariate distributions for family sizes are the Bivariate Poisson (BP) and the Generalized Bivariate Negative Binomial (GBI-

VARNB, Gurmu and Elder, 2000). Both distributions account for correlations between male and female progeny numbers, but only the GBIVARNB takes overdispersion into account. The goals of this research are threefold: 1) to evaluate the pedigree structure through the effective number of founders (N_f), and the effective number of ancestors (N_a); 2) to estimate the (co) variances of family sizes of bulls and cows using either the BP or the GBIVARNB distribution to account for overdispersion, and 3) to estimate N_e using the (co) variances from the previous step. In doing so we used the data from purebreds and grades from the genetic evaluation program of the Brangus breed in Argentina. As a control population for the proposed methodology, we analyzed an Angus herd that has a more complete pedigree information than the Brangus breed.

2. Methods

2.1. Data

Data used for the study consisted of the pedigree records from 72,808 animals (45,668 females and 27,140 males) supplying records to ERBra, the genetic evaluation program of AAB. The animals were born from 1959 to 2005, most of them in Argentina but with some individuals originated in USA, Brazil or Bolivia. A large number of Brangus animals included in the ERBra are grades, which usually lack either sire or dam identification. The number of participating herds in 2005 was 56, from which 17 produce both purebred and grade animals whereas the rest raise grade cattle only. It is estimated that more than 75% of all purebred and grade Brangus animals participate of the ERBra. Most herds register both purebreds and grades, with a ratio of about 1:5. The AAB keeps the registry for the grades, whereas the national association of cattlemen keeps the herdbook for the purebreds of all breeds including the Brangus. Since its beginning in 1978, the AAB has kept an open policy of registering grade animals in order to maintain a high level of variability, and to retain the maximum possible levels of heterosis between Angus and Zebu cattle. Selection policies in the breed have resulted in the most popular bull sires being born in Argentina. Two herds are involved in an active embryo transfer program from a US based cattle company and register animals born locally but out of US parents. The total fraction of calves that are born to US bulls, either by embryo transfer or by artificial insemination, is about 3%.

The control purebred Angus herd consisted on records of 10,483 animals (4700 males and 5783 females) born between 1938 and 2005. The herd is located in Pasteur, western Buenos Aires province. The number of cows at any given year was about 250 up to 1990, moment at which was reduced to its actual size of about 100 females. A large proportion of all matings were to popular US bulls through artificial insemination. Since 1990, embryo transfer has become a common management practice with the 10% superior cows.

2.2. Pedigree structure and inbreeding

Most analyses to assess pedigree structure and to calculate F were performed using the program ENDOG (Gutiérrez and Goyache, 2005). The level of completeness of the Brangus and Angus pedigrees are seen in Fig. 1. In Brangus, almost 40% of the animals have either parent unknown due to the open policy of registration and genetic evaluation. Going further back, the amount of information on ancestors dramatically decreases. A totally different picture is observed in the Angus herd, with a reasonably informative pedigree. A global measure of pedigree completeness is the index proposed by MacCluer et al. (1983). The coefficient is defined in the 0–1 range, and is interpreted as the ability of the pedigree to measure the inbreeding of the animal. For any individual, the measure is computed as $\frac{2C_{\text{sire}}C_{\text{dam}}}{C_{\text{sire}}+C_{\text{dam}}}$, and $C = \frac{1}{d} \sum_{i=1}^d a_i$. The value a_i is the proportion of ancestors in generation i that are known, whereas d is the number of generations traced backward (Sørensen et al., 2005). We set $d=5$ generations, and averaged the coefficients of all individuals. Compared with the values reported for dairy cattle

breeds (0.85–0.93 in UK Holsteins, Kearney et al., 2004; 0.93–0.95 in Danish Holstein, Danish Jersey and Danish Red, Sørensen et al., 2005; 0.79 to 0.99 for Holsteins of 12 countries associated to Interbull, VanRaden, 2005), the level of completeness of the Argentinean Brangus was low: 0.17. In comparison, the Angus herd presented a completeness level equal to 0.68.

The effective number of founders (N_f), and the effective number of ancestors (N_a), were calculated to get further insight into the pedigree structure under such a loss of parent identification. A founder is an animal without known parents. As founders usually have unbalanced contributions, Lacy (1989) defined N_f to be the theoretical number of founders with balanced contributions that would be expected to produce the same genetic diversity as in the population under study. In case all founders have equal contributions, the actual number and the N_f are equal. The N_f is calculated as $N_f = \left(\sum_{i=1}^f p_i^2 \right)^{-1}$ where p_i is the fractional contribution of the genes of founder i to a reference population, and f is the total number of founders. However, the measure does not take into account events that limit the genetic

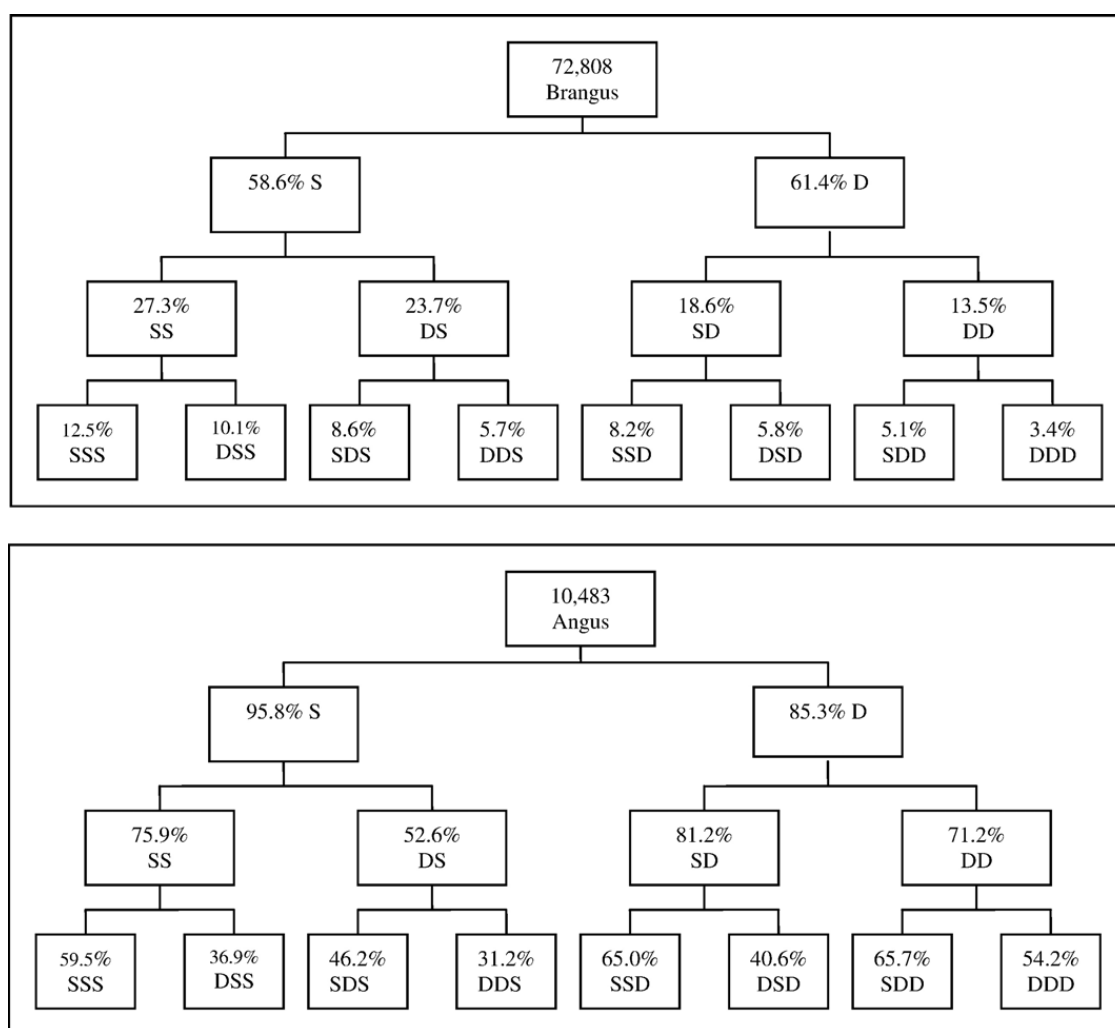


Fig. 1. Level of completeness of the Brangus and Angus pedigrees. S stands for Sire and D for Dam, so that SS means Sire of Sire; DSD dam of the Sire's dam, etc.

Table 1
Age distribution of Brangus cows from 2000 to 2004

| Age (in years) | 2000 | 2001 | 2002 | 2003 | 2004 |
|----------------|-------|-------|-------|-------|-------|
| 2 | 0.028 | 0.031 | 0.030 | 0.029 | 0.031 |
| 3 | 0.245 | 0.225 | 0.215 | 0.213 | 0.216 |
| 4 | 0.145 | 0.148 | 0.206 | 0.187 | 0.185 |
| 5 to 8 | 0.478 | 0.471 | 0.435 | 0.458 | 0.452 |
| 9 or more | 0.103 | 0.123 | 0.114 | 0.113 | 0.115 |

variation in the population such as drift and bottlenecks (for example, due to breed formation, or differential use of sire and dams). Boichard et al. (1997) suggested calculating the *effective number of ancestors* (N_a): “the minimum number of ancestors (founders or not) necessary to explain the genetic diversity under study” (Caballero and Toro, 2000, page 339). Similar to N_f the calculus of N_a is $N_a = \left(\sum_{i=1}^a q_i^2\right)^{-1}$ with q_i being now the marginal (or non-redundant) contribution of i to the pool of a ancestors. However, this measure does not account for additional losses of genes due to drift (Boichard et al., 1997; Caballero and Toro, 2000). Both N_f and N_a were calculated with respect to the contributions to the calves born in the reference population, which consisted of the calves born from 2001 to 2005: 21,662 in Brangus and 670 in the Angus herd.

The *effective number of herds* supplying fathers, grandfathers and great-grandfathers (Robertson, 1953) was computed for the Brangus using the inverse of the probability that two animals taken at random in the population have their sires (or grandsires, or great-grandfathers) in the same herd for each path (Gutiérrez and Goyache, 2005).

2.3. Effective population size

Boichard et al. (1997) observed that is difficult to assess drift using inbreeding measures when pedigree information is highly incomplete. Therefore, we attempted an alternative approach: the direct estimation of *effective population size* (N_e) using demographic parameters. In its original definition, Wright (1931) indicated that N_e is “the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration”. As in any other breed of cattle, the Brangus and Angus in Argentina have overlapping generations and the estimation of N_e as proposed by Hill (1972, 1979) was selected for the endeavor. Harris and Allenford (1989) compared several estimators of N_e and noted that the expression of Hill (1979) provided one of the most accurate estimates. Estimation by the approach of Hill (1979) results from solving for N_e in

$$\frac{1}{N_e} = \frac{1}{16ML} \left[2 + V_{mm} + 2 \left(\frac{M}{F}\right) C_{mm,mf} + \left(\frac{M}{F}\right)^2 V_{mf} \right] + \frac{1}{16FL} \left[2 + V_{ff} + 2 \left(\frac{F}{M}\right) C_{fm,ff} + \left(\frac{F}{M}\right)^2 V_{fm} \right]. \quad (1)$$

In expression (1), M and F are the number of bulls and cows that produce progeny during any given breeding season, and L

is the generation interval, or mean age of the parents when their reproductive progeny are born. Dispersion parameters of *family size* (or number of *reproductive progeny* from each sex) are the variance of male progeny among bulls (V_{mm}), the covariance between male and female progeny among bulls ($C_{mm,mf}$), the variance of female progeny among bulls (V_{mf}), the variance of male progeny among cows (V_{fm}), the covariance between male and female progeny among cows ($C_{fm,ff}$), and the variance of female progeny among cows (V_{ff}). All these demographic statistics carry information on the number of breeding individuals and their reproductive contributions, and allow assessing the genetic variability (de Rochambeau et al., 2000). In his derivation of (1), Hill (1979) assumed constant population size and a stable age distribution, so that the values of M and F are constant. A check of the age distribution of Brangus cows at different years suggests that the first assumption is correct for the females in these data (Table 1), but not for the bulls (Table 2). Besides, the requirement of a constant population size is not fulfilled in Argentine Brangus, as the number of evaluated animals has increased from 2001 to 2005.

To account for the possibility of selection, it was also computed the expression of N_e under selection (N_{eS}) given by Nomura (1996), which is equal to:

$$N_{eS} = \frac{4 N_M N_F}{N_M + N_F} \quad (2)$$

where

$$N_M = \frac{4 M L^2}{\left[\left(\frac{1}{\mu_{mm}} + \frac{1}{\mu_{mf}} \right) (1 - \alpha_m) + \left(\frac{V_{mm}}{\mu_{mm}^2} + \frac{V_{mf}}{\mu_{mf}^2} + \frac{2 C_{mm,mf}}{\mu_{mm}\mu_{mf}} + 4 Q^2 C_M^2 \right) (1 + \alpha_m) \right]}$$

and

$$N_F = \frac{4 F L^2}{\left[\left(\frac{1}{\mu_{fm}} + \frac{1}{\mu_{ff}} \right) (1 - \alpha_f) + \left(\frac{V_{fm}}{\mu_{fm}^2} + \frac{V_{ff}}{\mu_{ff}^2} + \frac{2 C_{fm,ff}}{\mu_{fm}\mu_{ff}} + 4 Q^2 C_F^2 \right) (1 + \alpha_f) \right]}$$

Formulae for N_M and N_F were different from Nomura's (1996) in the sense that they include the term for the covariance between male and female reproductive progeny numbers divided but their respective means. The scalars μ_{mm} , μ_{mf} , μ_{fm} , and μ_{ff} , are the expected family sizes of sires of males, sires of females, dams of males and dams of females, respectively. Besides, α_m and α_f are the respective deviations from Hardy–Weinberg proportions in male and female parents. The term Q^2 accounts for the cumulative effects of selection on an inherited trait, and C_m^2 and C_f^2 are the variances of relative selective

Table 2
Age distribution of Brangus bulls from 2000 to 2004

| Age (in years) | 2000 | 2001 | 2002 | 2003 | 2004 |
|----------------|------|------|------|------|------|
| 2 | 0.06 | 0.08 | 0.08 | 0.04 | 0.04 |
| 3 | 0.13 | 0.12 | 0.14 | 0.23 | 0.14 |
| 4 | 0.20 | 0.20 | 0.11 | 0.17 | 0.29 |
| 5 to 8 | 0.45 | 0.42 | 0.51 | 0.34 | 0.30 |
| 9 or more | 0.16 | 0.18 | 0.16 | 0.17 | 0.22 |

advantage of among families of males and females, respectively (Santiago and Caballero, 1995). The parameters Q^2 , C_m^2 and C_f^2 were estimated as suggested by Nomura (1996).

The generation interval L was calculated as the average age of parents when their replacing progeny was born (James, 1977) from the four selection pathways (sires of bulls mm, sires of cows mf, dams of bulls fm, and dams of cows ff): $L = \frac{1}{4}(L_{mm} + L_{mf} + L_{fm} + L_{ff})$.

2.4. Variances and covariances of family size

There seems to be no experimental estimates of the variance of family size in beef cattle. In this research, mean, variance and covariances of family sizes were calculated for sires of bulls (bull sires), dams of bulls (bull dams), sires of cows, and dams of cows. In theoretical models, family size is assumed to follow a Poisson distribution (Caballero, 1994), in which the expected value and the variance are equal. Estimates of the empirical sampling variances of family size and the covariance between female and male progenies were calculated using data on 80 bulls and 40 cows in Brangus and 178 bulls and 60 cows in Angus, as a large number of sires and dams do not produce both reproductive male and reproductive female progeny. The mean–variance equality assumption of the Poisson model was empirically challenged in this sample, and the results are displayed in Table 3.

With the exception of the statistics for the male progeny of cows, all variances were greater than their corresponding means, the largest difference being observed among the statistics of female progeny from bulls. These results suggest that family size of Brangus and Angus bulls displays overdispersion from a Poisson distribution. In cows, the results are less clear. There were also positive correlations of 0.45 and 0.16 between male and female progenies of Brangus bulls and cows, respectively. Corresponding sampling correlations in Angus were 0.27 and 0.03. To further collect evidence of overdispersion and of covariance in the numbers of male and

female progenies, we fitted two bivariate discrete distributions to the family sizes. The first one is the Bivariate Poisson (BP) model (Cameron and Trivedi, 1998, page 256). Let y_{1i} and y_{2i} be the number of progeny of bull or cow i for sex 1 (male) and 2 (female), respectively. Then, the BP model is obtained as

$$y_{1i} = x_{1i} + x_{3i} \quad y_{2i} = x_{2i} + x_{3i}$$

where x_{1i} , x_{2i} , and x_{3i} are independent Poisson random variables with parameter λ_1 , λ_2 , and λ_3 , respectively. First and second moments of this joint density are equal to:

$$E(y_{1i}) = \lambda_1 + \lambda_3 = \text{Var}(y_{2i}); \quad E(y_{2i}) = \lambda_2 + \lambda_3 = \text{Var}(y_{2i}); \\ \text{Cov}(y_{1i}, y_{2i}) = \lambda_3.$$

The parameters of the joint distribution were estimated by maximum likelihood with the EM algorithm by means of the program *bivpois* (Karlis and Ntzoufras, 2005), which is written in the software *R*. Two Bivariate Poisson models were fit: the BP₁ allowing λ_3 to be estimated from the data, i.e. no covariance between male and female progeny numbers is assumed, and the BP₂ with $\lambda_3 \neq 0$.

Another model fitted to the distribution of male and female progeny numbers was the Generalized Bivariate Negative Binomial (GBIVARNB; Gurmu and Elder, 2000), which unlike the BP model allows overdispersion. Parameters of the GBIVARNB are θ_1 , θ_2 , α , and ρ , and the variances and covariance among the variables are equal to

$$\text{Var}(y_{1i}) = V\theta_{1i}^2 - \theta_{1i}^2 - \theta_{1i}; \quad \text{Var}(y_{2i}) = V\theta_{2i}^2 - \theta_{2i}^2 - \theta_{2i}; \\ \text{Cov}(y_{1i}, y_{2i}) = \theta_{1i}\theta_{2i}(V - 1)(2).$$

Gurmu and Elder (2000) indicated that V is equal to

$$V = \frac{\alpha + 1}{[\lambda^2(1 + \rho^2)]} [\alpha - 4\rho\sqrt{\alpha} + \rho^2(\alpha + 6)]$$

where $\lambda = [\alpha - 2\rho\alpha^{1/2} + \rho^2(\alpha + 2)] / (1 + \rho^2)$. The parameters of the GBIVARNB model were estimated using the program *gbcount.prg* written in GAUSS by Gurmu and Elder (2000). The following form of the Akaike information criterion (AIC, Burnham and Anderson, 1998, page 46) was used to compare the fit from the different models

$$\text{AIC} = -2l + 2p$$

where l denotes the maximum of the log-likelihood and p is the number of parameters. The model with the minimum AIC is to be selected.

3. Results

There were 29,127.5 equivalent founders (23,096 founder plus 12,063 half-founders or animals with one unidentified parent) in the Brangus breed. The reference population consisted of 21,662 calves born from 2001 to 2005. The number of ancestors responsible for 100% of

Table 3
Sampling statistics of family size of bull sires and bull dams

| | | | Male progeny | Female progeny |
|----------------|-------|-------------|---------------------------|----------------|
| <i>Brangus</i> | | | | |
| Bulls | N=84 | \bar{m} | 2.12 | 17.81 |
| | | V_m | 3.96 | 428.35 |
| | | $C_{mm,mf}$ | 18.49 (0.45) ^a | |
| Dams | N=40 | \bar{f} | 1.14 | 2.10 |
| | | V_f | 0.23 | 3.27 |
| | | $C_{ff,fm}$ | 0.14 (0.16) ^a | |
| <i>Angus</i> | | | | |
| Bulls | N=178 | \bar{m} | 2.06 | 8.02 |
| | | V_m | 4.43 | 203.40 |
| | | $C_{mm,mf}$ | 8.16 (0.27) ^a | |
| Dams | N=60 | \bar{f} | 1.13 | 2.20 |
| | | V_f | 0.22 | 5.86 |
| | | $C_{ff,fm}$ | 0.04 (0.03) ^a | |

^a Correlation between male and female family sizes.

the genes in the reference group was 12,471. However, 50% of that variation was explained by 273 animals, and 20% by 22 ancestors. The animal with the largest individual contribution to the 2001–2005 calves was a bull responsible for 2.4% of the genetic variability. The effective number of founders was $N_f=765.7$, whereas the effective number of ancestors was $N_a=387.5$. The average value of F calculated from the entire pedigree was equal to 0.24%.

There were 992 equivalent founders (285 founders plus 1414 half-founders) in the Angus herd. For the 670 calves born in the period 2001–2005, the effective numbers of founders ($N_f=46.3$) and ancestors ($N_a=46$) were similar. The numbers of ancestors responsible for 100%, 50%, or 20% of the genes were respectively equal to 144, 17, and 4 animals. The individual with the largest individual contribution to the 2001–2005 Angus calves was a bull responsible for 4.9% of the genetic variability. The average inbreeding calculated with all pedigree information was 0.5%.

With respect to the differential contributions of bulls from different herds, Table 4 shows the actual and effective (Robertson, 1953) number of herds contributing sires, grandsires and great-grandsires to the Brangus population. The largest reduction from the actual to the effective number is for the sires (52 to 9.04). Interestingly enough, the 9 elite herds contributing most sires to the breed provided 50% of the animals with records in the ERBra. From these herds, those six that contribute most grandsires and those four that contribute most great-grandsires provided 44% and 40%, respectively, of the animals with records in the ERBra. Thus, although the nucleus is constituted by 16% (9 out of 56) of the herds, they contributed 50% of the animals to the breed.

The numbers of bulls (M) and cows (F) in any given year were estimated by the average number of reproductive males and females respectively that were parents of the calves born in the period 2001–2005 for both Brangus and Angus. The estimates in the Brangus breed were $M=210$ sires and $F=5415$ dams. Corresponding estimates in the Angus herd were $M=23$

Table 4

Actual and effective number of Brangus herds contributing sires, grandsires and great-grandsires

| Ancestral generation | Actual number of herds | Effective number of herds |
|----------------------|------------------------|---------------------------|
| Sires | 52 | 9.04 |
| Grandsires | 29 | 6.44 |
| Great-grandsires | 15 | 4.23 |

Table 5

Parameter estimates of the Bivariate Poisson (BP) models for family size

| Parameters | BP ₁ | | BP ₂ | |
|----------------|-----------------|---------|-----------------|---------|
| | Bulls | Cows | Bulls | Cows |
| <i>Brangus</i> | | | | |
| λ_1 | 2.12 | 1.14 | 1.29 | 0.09 |
| λ_2 | 17.81 | 2.10 | 16.98 | 1.04 |
| λ_3 | 0.00 | 0.00 | 0.83 | 1.06 |
| Log-likelihood | -1080.08 | -115.21 | -1.066.49 | -112.13 |
| AIC | 2164.16 | 234.43 | 2138.98 | 230.27 |
| <i>Angus</i> | | | | |
| λ_1 | 8.02 | 2.20 | 7.66 | 2.17 |
| λ_2 | 2.06 | 1.13 | 1.70 | 1.11 |
| λ_3 | 0.00 | 0.00 | 0.34 | 0.03 |
| Log-likelihood | -1906.45 | -187.97 | -1893.64 | -187.96 |
| AIC | 3816.90 | 379.95 | 3793.28 | 381.93 |

and $F=106$. Generation intervals (in years) were $L_{mm}=5.0$, $L_{mf}=5.7$, $L_{fm}=4.4$, and $L_{ff}=5.2$ for the Brangus, and $L_{mm}=5.2$, $L_{mf}=5.9$, $L_{fm}=3.1$, and $L_{ff}=3.9$ for the Angus. The smaller value of L_{fm} in both breeds may be due to the high level of embryo transfer used to produce bulls in the nucleus herds with selected young heifers. Average generation intervals were 5.1 years in Brangus and 4.8 years in Angus.

Estimates of the parameters for family sizes using the BP models are presented in Table 5. The smaller values of AIC for the BP₂ model in Brangus suggest that family numbers of male and female progeny of both bulls and cows are not independent random variables. The same was observed for the Angus bulls, but the BP₁ model had a slightly smaller (i.e. better) AIC than the BP₂ model for the cows. This later result was consistent with the findings from the GBIVARNB model where the estimated covariance of male and female family size of the cows was zero.

The estimates of the parameters for the distributions of family sizes of bulls and cows under the GBIVARNB

Table 6

Parameter estimates of the Generalized Bivariate Negative Binomial (GBIVARNB) model for family size

| Parameters | Brangus | | Angus | |
|----------------|---------|---------|---------|-----------|
| | Bulls | Cows | Bulls | Cows |
| θ_1 | 2.12 | 1.14 | 1.58 | 1.00 |
| θ_2 | 17.81 | 2.10 | 4.56 | 1.38 |
| α | 1.25 | 19.18 | 0.86 | 453613.14 |
| ρ | 0.00 | 0.98 | 0.00 | 2.46 |
| Log-likelihood | -498.22 | -114.42 | -833.01 | -145.38 |
| AIC | 1004.45 | 236.84 | 1674.03 | 298.75 |

model are shown in Table 6. In Brangus, the AIC values for comparing the fit of different distributions favored the GBIVARNB model for the bulls. On the contrary, the BP₂ model provided a slightly better fit for the cows. Thus, the distribution of reproductive male and female progeny numbers for the bulls displayed overdispersion with respect to the Bivariate Poisson model in which mean and variance are equal (see expression (2)), and there was evidence for positive covariances among male and female progenies of bulls and cows. On the other hand, the GBIVARNB model produced the best fit for bulls and cows in the Angus.

Variances and covariances of family sizes were then calculated assuming the GBIVARNB model for the Brangus bulls, the BP₂ model for the Brangus cows, and the GBIVARNB model for the Angus bulls and cows. The results are shown in Table 7. In both breeds variances of male and female progeny numbers, as well as the covariance between family sizes, were larger for bulls than for cows, with the largest difference being in the variance between female progenies. Comparison of the estimated parameters under the GBIVARNB model and those displayed in Table 3, in which the implicit sampling distribution is the Bivariate Normal, shows that means are similar but variances and covariances are different.

When calculating the formula of Hill (1979) in Brangus, by using the estimates in Table 7, the number M of bulls and F of cows, and the generation interval as calculated above, resulted in $N_e=273.9$, which then produces $\Delta F=1/(2 N_e)=0.0018$, or 0.18%. This latter value is smaller than the observed average F which was

equal to 0.24%. When attempting to account for selection, the estimate of N_{eS} using the expression of Nomura (1996), resulted in a value of 124.8 so that $\Delta F=0.40\%$. For Angus, with a more complete pedigree, $N_e=95.4$, and $\Delta F=0.5\%$ in perfect agreement with the observed average inbreeding of all 10,483 animals. The effect of selection was less pronounced in the Angus herd than in Brangus breed as $N_{eS}=79.3$ and $\Delta F=0.63\%$.

4. Discussion

The indexes calculated using the Brangus calves born in the period 2001–2005 as the reference population were $N_f=765.7$ and $N_a=387.5$. In Austrian breeds, Sölkner et al. (1998) reported $N_f=221$ and $N_a=114$ for Simmental, $N_f=97$ and $N_a=52$ for Braunvieh, and $N_f=113$ and $N_a=39$ for Pinzgauer. Gutiérrez et al. (2003) obtained values of N_f ranging from 48 to 265 and N_a ranging from 25 to 163, in eight Spanish breeds. For the Italian breeds Chianina, Marchigiana and Romagnola, Bozzi et al. (2006) estimated N_f to be 152.1, 70.9 and 89.8; corresponding values of N_a were 73.6, 48.0 and 59.5. In the Mexican composite breed Tropicarne, Ruíz-Flores et al. (2006) found $N_f=48$ and $N_a=20$. In Brazil, Vozzi et al. (2006) reported $N_f=87.2$ and $N_a=59.8$ for Nellore, and $N_f=107.9$ and $N_a=61.5$ for Polled Nellore. In Ireland, Mc Parland et al. (2006) observed values of N_f varying from 55 (Simmental) to 357 (Charolais), whereas N_a ranged from 35 (Simmental and Hereford) to 82 (Limousin). Hence, the values of N_f and N_a in Brangus are higher than most reported estimates for beef cattle. Difference in population size, production systems for beef cattle, and above all in the policy of registration, may explain the reasons for the larger figures in Brangus. Sørensen et al. (2005) observed that the total number of founders contains limited information on the genetic basis for the population as founders are assumed to be unrelated, because their parentage is unknown. The largest source of founders in Argentinean Brangus is from grade dams. These are cows bringing grade calves with records to ERBra. Most of these are born following natural mating, so that groups of contemporary cows may be paternal half-sibs, and this may have induced a slight overestimation of N_f in the Brangus population.

The amount of inbreeding in populations with incomplete parent identification is underestimated (Miglior and Burnside, 1995; Lutaaya et al., 1999; Cassell et al., 2003). Rather than trying to amend the lack of information by using the average F of animals born in the same year as proposed by VanRaden (1992), we attempted an indirect method by calculating the N_e under the assumption of

Table 7
Estimates of the variances and covariances for family sizes assuming different distributions for bulls and cows

| Family size | Model | Parameters | Estimate |
|----------------|-----------------|---------------|----------|
| <i>Brangus</i> | | | |
| Bulls | GBIVARNB | V_{mm} | 5.70 |
| | | V_{mf} | 271.28 |
| | | $Cov_{mm,mf}$ | 30.15 |
| Cows | BP ₂ | V_{fm} | 1.15 |
| | | V_{ff} | 2.10 |
| | | $Cov_{fm,ff}$ | 1.06 |
| <i>Angus</i> | | | |
| Bulls | GBIVARNB | V_{mm} | 4.48 |
| | | V_{mf} | 28.67 |
| | | $Cov_{mm,mf}$ | 8.36 |
| Cows | GBIVARNB | V_{fm} | 1.00 |
| | | V_{ff} | 1.38 |
| | | $Cov_{fm,ff}$ | 0.00 |

overlapping generations (Hill, 1979) and selection (Nomura, 1996). These methodologies are based on the assumptions of equal age distribution for bulls and cows and constant population size. The first assumption was verified in cows (see Table 1) but not in bulls (Table 2), and the changing herd size with time in both Brangus and Angus do not comply with the second assumption. It is unlikely to find beef cattle populations of constant size over extended periods of time. The consequences of those violations on the estimates of N_e are unknown. Key elements of the formulae by Hill (1979) and Nomura (1996) are the variances and covariances of family sizes (number of reproductive progeny) of bulls and cows, parameters that convey information on the mating system. A weakness of the approach is that a lot of information is discarded in the process of estimating the (co)variances of family size in beef cattle, as only data from bulls and cows that have reproductive progeny of both sexes (i.e. bull sires and bull dams) are included, and these constitute a relatively small fraction of all breeding individuals. This may induce an overestimation of N_e , as the information from a portion of sires of cows and most dams of cows is not used in the estimation process. Family size of dairy bulls has been considered to follow a Poisson distribution (Goddard and Smith, 1990), and there was experimental evidence for a Poisson male family size in *Drosophila melanogaster* (Joshi et al., 1999). However, our analysis shows that this was not the case for the family sizes of beef bulls as there was considerable overdispersion, i.e. the variance was higher than the mean. In addition, covariances of male and female family sizes of bulls and cows are needed in Hill's (1979) formula. Therefore, a proper specification of these discrete random variables necessarily involves a bivariate distribution such as the BP or GBIVARNB. The latter probability mass function also accounts for overdispersion (Gurmu and Elder, 2000). The methodology used here (i.e. estimating inbreeding by means of the formula of Hill (1979) for N_e , using variances and covariances of family sizes under a GBIVARNB model) seems to be an able procedure when pedigree information is weak, and gives similar results to the calculated inbreeding when pedigree information is more complete as in the Angus data.

The direct estimate of N_e under the assumption of no selection is above the value of $N_e=250$ mentioned by Hill (2000) to keep the additive variance at its initial value for a trait with $h^2=1/3$. However, the estimate from a selection model (N_{eS}) is strikingly different (less than half) than the value of N_e . A much smaller difference between estimated N_e and N_{eS} was obtained in Angus where N_{eS} was about 83% of N_e . When comparing all elements in formula (2), the ratio of bulls

to cows ($M:F$) was markedly different in both populations: 0.038 in Brangus vs. 0.216 in Angus. Had it been $M:F$ in the Brangus equal to the one observed in the Angus herd while keeping all remaining parameters the same, would have resulted in an increase in N_{eS} to 798. Therefore, the heavier bull usage in the Brangus resulted in a decrease in effective population size. There seems to be no reported direct estimates of N_e (rather than equating it to the change in F) for composite cattle breeds. However, the estimated N_{eS} is in the range of 50 to 150 in which most estimates of the parameter are reported for beef cattle breeds (Boichard et al., 1997; Nomura et al., 2001; Gutiérrez et al., 2003; Bozzi et al., 2006; Mc Parland et al., 2006; Vozzi et al., 2006). Notwithstanding this, and as it would have been expected a larger effective population size in a composite breed with an open policy registration, it seems desirable to continue monitoring the effective size of the Brangus to prevent problems of inbreeding and lack of variability in the future.

Acknowledgments

This research was made possible by grants UBACyT G018-Program 2004–2007 of Universidad de Buenos Aires, and CONICET PIP 5338 2005–2006 from Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina. The authors would like to thank the associated editor and to an unknown reviewer for their insightful comments which largely helped to improve an earlier version of the present paper.

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Chapter 7

GENERAL DISCUSSION

The potential of decision-support tools in public health has not been fully explored in developing countries (Nsubuga et al., 2006). The lack and/or weakness of supporting information when contemplating decisions becomes even more evident in the case of NZD. This is because, on the one hand, in many cases the relationships, connections and the risk between the health status of livestock and human population remain unknown and on the other hand, because the philosophy of One Health has not been fully embraced by decision makers on the public health side. In this document, we analysed the Ecuadorian case of two livestock-related zoonoses (brucellosis and cysticercosis) and we tried to find and evaluate the relationships with the various components of food security (also including adequate sanitation), and the biological variability within livestock species (WHO, 2015) (<http://www.who.int/trade/glossary/story005/en/>). Just like in other developing countries, the real burden of livestock-related NZD such as brucellosis and cysticercosis in humans and livestock have not been estimated in Ecuador. This work attempts to elucidate the links between human cases, sanitary conditions and the presence of livestock through the application of different statistical and risk analysis models and through the visualisation of the geographical human incidence of those diseases, using hospital records (Ron-Garrido et al., 2013, 2015).

Effective prevention of diseases and health problems associated with animal husbandry requires modern techniques of risk management (Pfeiffer, 2004; Salman, 2003; Kulldorff, 1997). Evaluation, monitoring and surveillance of health problems are the cornerstones for control and eradication of diseases and production problems (Salman, 2003). On the other hand, the application of corrective measures requires in most cases suitable measures of the risk implied and in the case of livestock an estimation of the economic losses. Thus, technical data collection such as identification of animals and use of pedigree registers, animal-movement information and traceability of nutritional compounds either for

human or animal consumption have become a fundamental tool in the prevention, evaluation and the control of many livestock-related problems and subsequent food security. However, although these strategies have been well implemented in developed countries and in highly industrialised production systems, in general in extensive and small-holder livestock systems in developing countries, there is a degree of carelessness when it comes to the evaluation and assessment of risks related to health problems in animal husbandry and consequently in the prevention of health problems in livestock and human populations.

The important role of active surveillance systems in the disease control programs highlights the need for cooperation between public health officials, veterinary officers and farmers (Russo et al., 2009). An active surveillance approach enhances disease detection and provides additional epidemiological data that can be used to apply interventions in order to reduce disease rates. However, in the case of NZD active surveillance has hardly been implemented in developing countries. Thus passive surveillance, although sometimes ineffective, may help to prevent future events and prioritise areas of intervention (Sanodze et al., 2015; O'Neal et al., 2011), especially when repeated cases appear in similar zones, but (for example) in different years. Passive surveillance is a relatively inexpensive strategy to cover large areas and it provides critical information for monitoring the community health status. However, information provided by passive surveillance very often depends on people in different institutions, so data quality and timeliness are difficult to control (Nsubuga et al., 2006). Thus, surveillance of NZD may include both active or passive gathering and collection of events in humans. The passive collection of data involves the reporting of clinical cases to the health authorities by hospitals. Such is yet the case of NZD in Ecuador, where due to lack of synchronisation among the different health care systems and other institutions, active surveillance proves unreliable (MSP, 2015; INEC, 2008; Cartelle et al., 2015). Due to the fact that at acute and chronic phases, some NZD such as human brucellosis and NCC require hospitalisation and also given the data structure of ICD codes developed by the Ecuadorian office of statistics INEC (2008), it is possible to trace back the origin of human cases to the possible places, where the disease was contracted or at least initially reported. This information provided a data structure, where the province, municipality and parish of patient origin is reported. Furthermore, information such as the patient's characteristics like age, gender and convalescence time in the hospital can be found. On the side of the hospital, information about the health-care system and the health level can be found. To the best of our knowledge, before the current work, this kind of information had not been used to study risk factors for human disease rates for livestock-related neglected zoonoses like brucellosis, cysticercosis and epilepsy. In this way, 163, 5865 and 19327 hospitalised patients suffering respectively of human brucellosis, NCC and epilepsy were registered between 1996 and 2008. There were considerable differences with the official data reported and the data collected in hospitals (Cartelle et al., 2015; Ron-Garrido et al., 2015), the hospital system being the

one wherein more information was collected. As with other neglected diseases, livestock-related NZD are difficult to diagnose due to the lack of specific symptoms or signs, being sometimes even asymptomatic (Maudlin et al., 2009; Halliday et al., 2012; Celebi et al., 2007; Pawlowski et al., 2005). Furthermore, collected information may not describe the real situation realistically, due to the poor capacities implemented in healthcare centres when it comes to the application of more performant diagnostic techniques, because of unattainable poor communities or rural communities living in remote areas, not able to afford the costs involved in the diagnosis and treatment (Halliday et al., 2015; Bhattarai et al., 2012). Despite the drawbacks in the data collection, this information is still a valuable utility, given that it constitutes a collection of several years since the electronic data were available and also because several other socio-economic, agricultural and climatological variables were measured at the same time. Thus, this methodology for measuring and monitoring events constitutes a new approach to work out some of the problems related to the identification of risk zones, elucidation of relative importance of potential risk factors and target areas of intervention. One of the principal advantages of this methodology is the relative low cost of implementation in developing countries.

Through the application of scanning tools and regression models in the surveillance of livestock-related neglected diseases (brucellosis, NCC and epilepsy) several findings were reported. First at all, as expected in Ecuador, the presence of these zoonoses were related to livestock populations. In the case of the human brucellosis in hospitals, the presence of cattle population and cattle density were the main factors for the disease occurrence and for the increasing rate in incidence noted in hospitals. In this analysis, this was confirmed by means of Poisson regression models and decision tree regression. Additionally, and in order to verify our predictions, Figure 7.1 presents the rates of human cases identified at municipal level during the study period. In the figure, the overlaid large red circles represent the human brucellosis clusters of patients (significant and not significant) identified by the scanning statistics and the points represent the health status of cattle herds (red points present positive herds) evaluated in a national survey. Clearly, possibly apart from the south-eastern part of the country, there exist a high relationship between cattle brucellosis status and human cases in hospitals as predicted by the models. Similar findings were further tested using bacteria isolations from human and cattle samples (Rodríguez-Hidalgo et al., 2015; Ron-Román et al., 2014, 2012). In human brucellosis case the use of hospital reports were, and still it is, more reliable than official reports. Table 7.1 presents a comparison of both systems since 1996 until 2014.

Table 7.1: Human brucellosis cases reported through the official and hospital systems in Ecuador from 1996 to 2014

| Year | Official report | Hospital registers |
|------|-----------------|--------------------|
| 1996 | 9 | 17 |
| 1997 | 5 | 9 |
| 1998 | 1 | 8 |
| 1999 | 5 | 12 |
| 2000 | 3 | 16 |
| 2001 | 5 | 12 |
| 2002 | 2 | 7 |
| 2003 | 1 | 8 |
| 2004 | 4 | 13 |
| 2005 | 6 | 13 |
| 2006 | 5 | 7 |
| 2007 | 9 | 20 |
| 2008 | 0 | 12 |
| 2009 | 0 | 11 |
| 2010 | 0 | 11 |
| 2011 | 0 | 8 |
| 2012 | 2 | 11 |
| 2013 | 4 | 20 |
| 2014 | 11 | 10 |

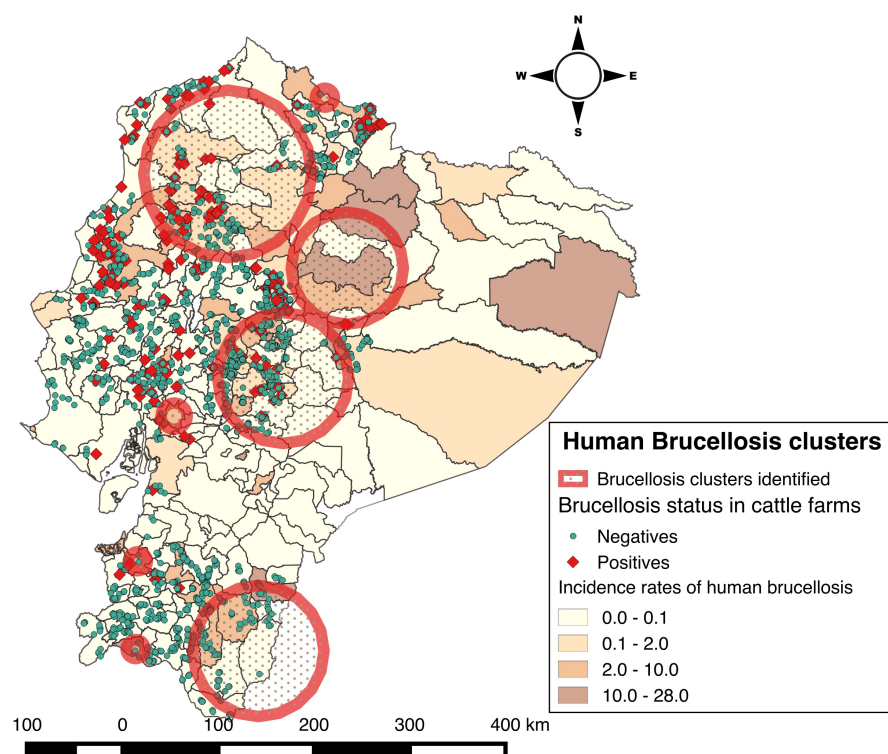


Figure 7.1: Human brucellosis clusters identified and positive brucellosis herds. Space clusters of human brucellosis cases with a maximum of 25% of the total centroids used in the circular scanning window between 1996 and 2008.

According to the Figure 7.1 and Figure 3.4, several human brucellosis clusters were found at the time and later on several of our assumptions were confirmed (CIZ National Brucellosis, Bovine Tuberculosis and Ticks Survey) especially in the North, centre and some regions in the coastal parts of the country. Although, at national level the herd seroprevalence of brucellosis was estimated to be 8.3% ($CI_{95\%}$: 7.3%-9.5%; 198/2374 farms) in 2014, in the northern part of the country this prevalence increases to 16.8% ($CI_{95\%}$: 13.2%-21.0%; 63/376). In the southern half of the country the herd prevalence decreases to 1.2% ($CI_{95\%}$: 0.4%-2.7%; 6/505), but the rate of brucellosis human cases apparently was high. A possible explanation for this cluster could be that either brucellosis as a transboundary disease in the zone, food products can be transported from Northern Perú where *B. abortus* has been reported in recent years in Cajamarca region (OIE, 2005). Likewise, dairy products from other endemic zones may be traded and consumed in this area. In any case, the areas of concern identified through of this study can provide a starting point for implementing targeted intervention efforts. However, due to asymptomatic forms of the disease more data would be necessary to pinpoint potential target zones for interventions and, additionally, trade networks of animal movements and dairy products would be necessary to be incorporated in the analysis to map the spread of human cases.

In the case of cattle brucellosis, the rationale for this and another livestock disease eradication is often founded in benefit-cost calculation for farmers (Wolf, 2006). However, in the case of diseases where human health is at risk additional economic and social effects should be also taken into account. In the current work, we evaluated at farm level the incentives needed for prevention and/or eradication of brucellosis. It is important to mention that to accomplish eradication in the human population the disease reservoir must be eliminated. In this way, the application of disease dynamics models allowed us to estimate losses in cattle farms due to brucellosis. These losses were estimated in terms of abortions and milk yield losses that can result from a reduction in the cattle farm population or from delays in production due to abortions. Additionally, in absence of control measures cattle brucellosis is an economically catastrophic problem because a lack of female replacements will be noted and therefore the production systems become inefficient. Economic losses in monetary terms have been estimated in India, where for example the loss per animal was US\$6.8 (Singh et al., 2015). In our case, *i.e.* in a system where a cow may yield 15 kg of milk per day, we predict that for the total herd losses can range from 2 to 4 kg milk per cow per day and an equivalent number of 2 abortions per cow in a period of 10 years. This estimation, substantially increases the losses. An additional concern is the infected male calves that are usually are sold immediately after parturition. Therefore, responses from private farmers and from public sectors must promote the eradication of the disease. The simulation model, presented in this work, allows the estimation of the number of animals to be vaccinated and the number of animals to be slaughtered when these control methods are envisaged on a

farm. Additionally, the predicted final prevalence of the disease can be calculated, so that different strategies can be studied and evaluated. The model clearly shows in endemic zones, because of the on-farm economic losses due to brucellosis, that the advantages of applying vaccination are evident as a preventive measure, but also as a control measure even with the current vaccination parameters known for brucellosis (vaccine efficacy nearly to 70% (Olsen and Stoffregen, 2005)). Considering that vaccination alone is not enough to control and eradicate the disease, it should be associated with continuous elimination of infected animals and hygienic measures.

Most importantly, the model predicts that brucellosis in a dairy herd is an eradicable disease. Strict vaccination of female calves and a biannual testing of half the population and culling of the seropositive animals may reduce the true prevalence in cattle to negligible values with minimum economic losses. Additionally in brucellosis eradication programs, the application of effective surveillance is critical and probably the first step. Thus, a major factor in the success of the program is the acceptance of the eradication program procedures by livestock owners in spite of the inconvenience, cost and additional work required (Ragan, 2002). The incentives described before must convince to farmers and veterinary public health officers to apply control and eradication programs in endemic zones. Additionally, active surveillance for detecting brucellosis in cattle markets through the use of milk-ring test may assist to increase the eradication rate. However, a critical component of a successful surveillance program remains a permanent animal identification. When brucellosis can be identified, contained and eliminated before the spread occurs, then eradication can be achieved (Ragan, 2002), as it was the experience in eradicating the disease in United States, which took however at least 50 years. Also important (as shown by the model) are adequate serological tests and proper laboratory facilities and capacities, otherwise unacceptable and unnecessary animal losses are experienced.

Also in Ecuador, the relationship between acquired epilepsy and neuro-cysticercosis remains unclear because of different factors, including the lack of specialised healthcare personnel, appropriate diagnostic techniques and the fact that acquired epilepsy is characteristic of many other infectious and non-infectious diseases in the endemic zones of the country. The application of geographical scanning tools and the use of statistical models to the rates of NCC and epilepsy hospitalised cases allowed us to identify several groups of municipalities where both problems had a rate above the average of the country. Figure 7.2 presents the significant municipality clusters when up to a maximum of 25% and 10% of the municipalities were included in the scanning window. In general, it can be stated that epilepsy clusters (red circles) were wider than NCC clusters and in some cases epilepsy clusters contained NCC clusters. This statement was more evident in the Sierra and Amazonia regions of the country. However, in the coastal region at least two epilepsy clusters were identified where apparently none or few cases of NCC have been diagnosed in hospitals. As in other developing countries, most people with epilepsy do not have

access to appropriate management (Pal et al., 2000). Furthermore, epilepsy clusters had a more recent appearance or are newer than NCC clusters. This finding is a concern and a new contribution to the epidemiology of NCC and epilepsy in Ecuador given the fact that in endemic areas a late onset of epilepsy is a strong predictor of neuro-cysticercosis (Pawlowski et al., 2005; García et al., 1995). Likewise, traditional endemic zones for NCC and epilepsy, recognised in other studies, were confirmed (Del Brutto et al., 2005; Rodríguez-Hidalgo et al., 2003; Cruz et al., 1999, 1995). However, appropriate discrimination of the type of epilepsy is necessary and field studies in human and veterinary sectors are of priority. Different models were applied to study the potential effect of different economic and landscape factors. Using different methodological approaches, it was found that around the country rates of epilepsy and NCC were related to the presence/absence of facilities for eliminating excreta. Overall, an increasing trend of the incidence of hospitalised cases (IHC) of epilepsy and a decreasing trend of the IHC of NCC were observed over time, but, oddly enough in contrast, within municipalities a positive linear relationship between both disorders was found as confirmed by the Figure 7.2. Figure 7.3 presents the updated data of both disorders, where the trends still remain.

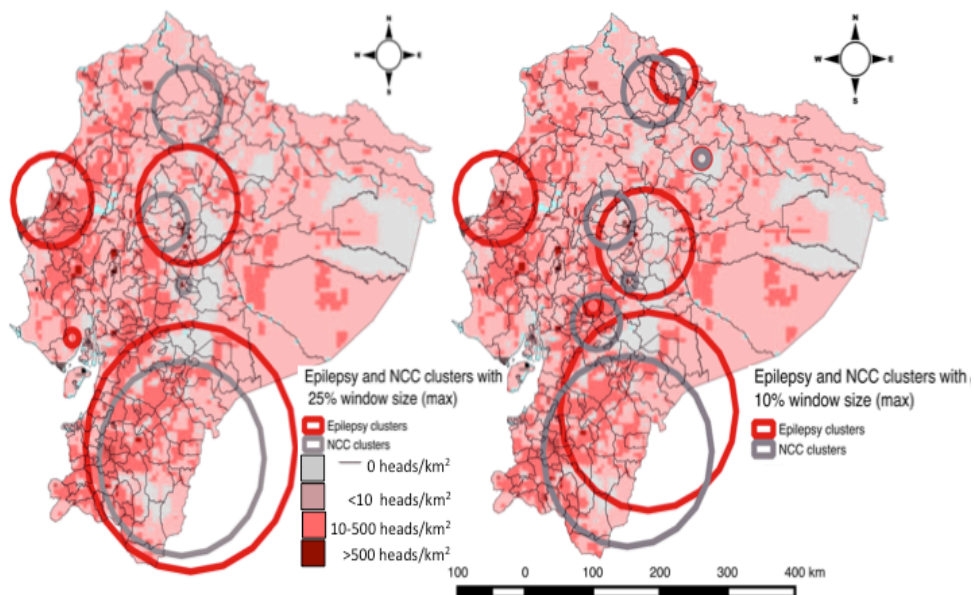


Figure 7.2: Significant space-time clusters of hospitalized Epilepsy and NCC cases with up to a maximum 25% and 10% of the total centroids included in the scanning window between 1996 and 2008. The base map represents the pig population density.

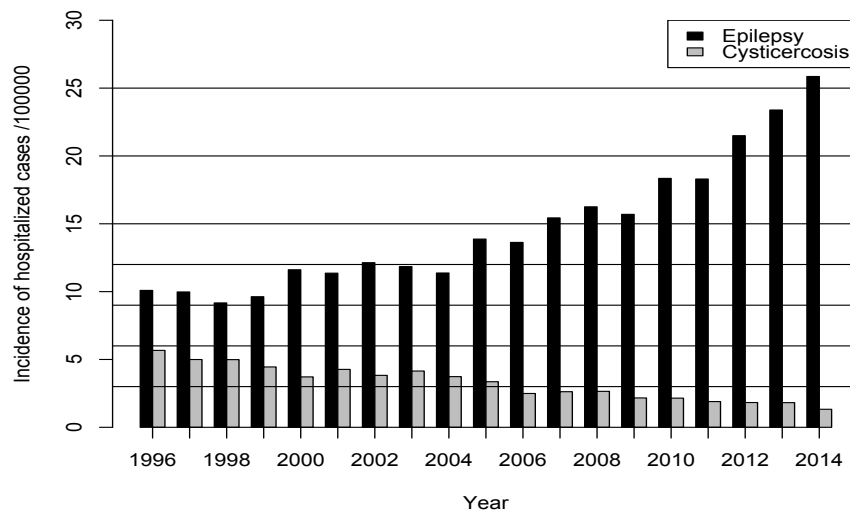


Figure 7.3: Incidence of hospitalized neurocysticercosis and epilepsy cases per 100.000 inhabitants in Ecuador between 1996 and 2014.

Taeniasis and cysticercosis in humans and pigs was declared a potentially eradicable problem by the International Task Force for Disease Eradication in 1993, using interventions (Health Organization, 2015). Theoretically, breaking the lifecycle seems easy by doing intervention strategies in the lifecycle (Pawlowski et al., 2005), but identification of tapeworm carriers in a population is far from easy. Human carriers living in rural areas are involved with perpetuation of the *T. solium* life cycle (Pawlowski et al., 2005). Emphasis has been placed on control through mass chemotherapy of human populations, but this strategy does not control the source of infections, which remains cysticercosis in pigs (Eddi et al., 2003). Thus, farmers need to improve pig management practices, where the more developed agricultural systems are not sustainable so that it is easier to find roaming pigs scavenging for food.

On the other hand, but in the same vein, the increasing demand for food of animal origin has tended to favour international high-output breeds over local breeds. Consequently, there has been a worldwide tendency to reduce the effective size of populations under selection (Hoffmann, 2010; Hill, 2010; Brotherstone and Goddard, 2005). In fact, there is a concern nowadays over the accelerating rate of loss of genetic variation in livestock populations and consequently about the reduced capacity for adaptive change and future evolution of them (Cervantez et al., 2011; Brotherstone and Goddard, 2005). Animal genetic diversity is critical for food security and rural development. It also allows farmers to select stock or develop new breeds in response to changing conditions, including climate change, new or resurgent disease threats, new knowledge of human nutritional requirements and changing market conditions (Hoffmann, 2010). For example, variability among individuals in terms of response to infection to the exposure to *Mycobacterium bo-*

vis has been evaluated and the use of genomic selection (single nucleotide polymorphism data) has given a potential approach and feasibility of developing animals genetically more resistant to bovine tuberculosis, to enable breeding of animals with enhanced resistance (Tsairidou et al., 2014). This approach has not been yet used in other livestock zoonoses, such as bovine brucellosis and porcine cysticercosis, but it may prove to be a useful control tool. Likewise, the utility and the use of performance records in animals is a necessary pre-requisite to effective decision making on breeding policy (Mason and Buvanendran, 1982). In this document a methodology to estimate the effective population size (N_e) is given, when partial information of the family sizes is known in a beef cattle population (Ron-Garrido et al., 2008). It must be noted however, that the choice of recording systems and type of records at farm level or higher hardly ever take monitoring of effective population size in consideration. The major constraint is that a large proportion of livestock species (dairy cattle in particular) in the tropics are owned by small farmers with poor communication, low education level, lack of qualified recorders and lack of incentives for recording. Identification of animals and keeping of pedigree records should be performed in order to monitor genetic variation within livestock breeds and avoid excess of inbreeding or variability in the future (Mason and Buvanendran, 1982). Furthermore, as mentioned in the text, genetic structure of the population results in an important factor for statistical evaluations when the genetic basis of the features under study has been proved because of the genetic resemblance. This latter aspect also never receives any attention and sampling frames continue to ignore this form of reduced within-herd variability and resulting underestimation of population variance.

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Chapter 8

CONCLUSIONS AND PERSPECTIVES

8.1 Conclusions

Brucellosis and cysticercosis have remained livestock-related neglected zoonoses as far as the public health and veterinary sectors in Ecuador. In this document, we tried to unearth and evaluate the relationship between livestock diseases and various potential risk factors in food security such as livestock populations, adequate sanitation and the biological variability within livestock species. Data provided by passive surveillance system generated in Ecuadorian hospitals was used for this analysis and non-specific epilepsies were used additionally to support neuro-cysticercosis data. We stated that this kind of surveillance provides critical information for monitoring the community and livestock health statuses with relation to the above zoonoses in a relatively cheap way.

For farmers, the rationale for the elimination of brucellosis is often founded in benefit-cost calculation and the incentives for preventing and to eradicate the disease. A mathematical model for the dynamics of brucellosis in cattle was developed. Thus, the model allows estimating losses in cattle farms due to brucellosis. These losses were estimated in terms of abortions, and milk yield losses that proceed from a reduction in the cattle farm population or from delays in the production due to abortions. It was estimated that, in the absence of control measures, brucellosis in cattle is an economically catastrophic problem because of a lack of female replacements. The model predicted that brucellosis in a dairy herd is an eradicable disease. Strict vaccination of female calves and biannual testing and culling of half the seropositive population may reduce real prevalence in cattle to negligible values with minimum economic losses. Animal genetic diversity is a critical factor for food security, and also it may reduce the risk of disease threats in livestock. In

this document, a methodology to estimate the effective population size is given. It can be applied when partial information of the family sizes is known in a beef cattle population. However, due to weakness in identification of animals and pedigree, the monitoring of genetic variation within livestock may still cause problems in inbreeding or variability in the future. Additionally, this genetic structure of livestock populations must be taken into account when statistical data analyses are performed for features with genetic background because of genetic resemblance. The use of statistical and modelling tools in the epidemiology of livestock and neglected zoonoses diseases is still playing an important role to advice decision makers in public health.

8.2 Implications and Perspectives

The application of statistical models and transmission dynamics models in the epidemiology of livestock diseases still have a lot of development in front of them. We introduce a new point of view in the treatment of mathematical models. The idea is to provide decision makers and livestock farmers a tool that allows them to evaluate the risk involved when a disease like brucellosis is introduced the herd, so that economic losses may be predicted. Similar studies and estimations may prove useful in many other diseases and husbandry problems. An important point here is to ensure dissemination of the necessary information and training material by the extension services. The fact that the presently developed model can be run on various types of electronic devices is in this respect certainly a bonus.

Additionally, the main data used for the zoonoses analysed here were provided by the Ecuadorian office of statistics and census (INEC, 2008), which collects the information on morbidity in hospitals. This kind of information helped to study the human and livestock conditions in the Ecuadorian case, and links were established, so that in future this kind of information with similar data structure might be used for other livestock diseases and also in other countries. However, due to asymptomatic forms of neglected tropical diseases, more and more accurate data will be necessary in order to identify target zones for interventions, for example information on trade networks of animals and dairy products would be necessary to incorporate in the forecasting models to map spread of diseases and predict possible human cases in new areas.

On the other hand, and because we did not find data about agricultural systems and productivity levels in the places where disease hotspots where localised, there is a necessity to additionally study these types of variables and their implication in zoonoses in Ecuador. This is important for example in the epidemiology of cysticercosis, where farmers need to improve pig management practices and where the required baseline information is not available, because the standard agricultural systems are not sustainable under the given circumstances (*e.g.* housing of animals, individual identification of animals, abstention

from using human excreta as crop fertiliser).

Finally, variability among individuals and livestock species in terms of response to infections and to the exposure has not been evaluated properly. The use of genomic selection (single nucleotide polymorphism data) has given a potential approach and feasibility of developing animals genetically more resistant to bovine tuberculosis to enable breeding of animals with enhanced resistance. This approach also can be used for bovine brucellosis and porcine cysticercosis.

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Chapter 9

APPENDICES

9.1 APPENDIX A: *Brucella abortus* user manual

Dynamics of *Brucella abortus* infection in cattle: decision support tool for managers

User manual – version 0.5

Lenin Ron-Garrido, Famke Jansen, Emmanuel Abatih, Claude Saegerman,
Norberto Bartoloni, Dirk Berkvens

9.1.1 Installation

1. Obtain and install the most recent versions of **R**[™] and **RStudio**[™]
2. Ensure the latest version of each of the following packages is downloaded and installed:
 - shiny
 - deSolve
 - rootSolve
3. Ensure the following files are present, all in the same directory/folder:
 - ui.R
 - server.R
 - BrucellaFunction.R

Attention!

Any name, allowed by the respective systems, can be used for the enclosing directory/folder. However, it is essential **not to change the names** of the three individual files. An error will occur when any of the three file names are changed. An error also occurs when the three files are **not in the same directory/folder**.

9.1.2 Starting the program

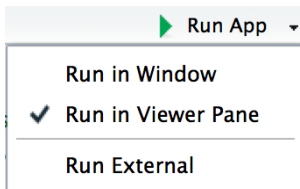
1. Open either `ui.R` or `server.R` or both in **RStudio** (`BrucellaFunction.R` may also be opened, but this is not necessary)
2. Select either `ui.R` or `server.R`: the top right of the script pane (the top left pane) should show a green arrow with the text **Run App**



Attention!

If **Run App** does not show, it may be due to either the package `shiny` not being installed, to the name of either `ui.R` or `server.R` having been changed or to the fact that both files are not in the same directory/folder.

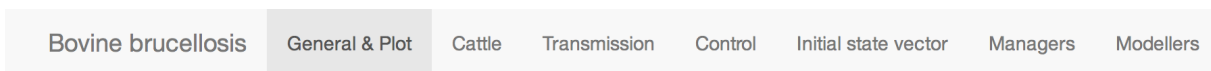
3. Click the small triangle to the right of **Run App** and select **Run in Viewer Pane** from the drop-down menu



4. Click the **Viewer** tab in the right bottom window pane and maximise its size by moving the horizontal divider to the top and the vertical divider as far left as possible, making sure the green arrow in the script pane remains visible (once the program runs, the divider can be moved further left to increase the size of the Viewer pane even more)
5. Click the green arrow in the script pane to start the program

9.1.3 Setting up the simulation

1. The individual tab panes can be selected from the tab menu at the top of the Viewer Pane.



Parameter and initial population values can be defined by means of individual sliders, divided over the different tab panes. Sliders can be moved by selecting the slider knob with the cursor and moving it. When the slider knob has been selected it can also be moved by means of the left and right arrows on the keyboard

2. The different tab panes are:

- (a) **General and plot:** General parameters
- Length of simulation in years
 - Number of game animals
 - *Brucella* prevalence in game animals
 - Number of humans
- (b) **Cattle:** (re)production and mortality rates (per month) of the various compartments of the cattle population
- Calving rate
 - Proportion of cows inseminated artificially
 - Synchronised insemination (checkbox)
 - Mortality rate susceptible females
 - Mortality rate infected primiparous cows during gestation
 - Average milk production per lactation
 - Mortality rate infected dry cows with gestations ended in full-term calving
 - Mortality rate infected dry cows with gestations ended in abortion
 - Mortality rate infected multiparous cows during gestation
 - Mortality rate infected males
 - Sales rate bulls
 - Carrying capacity bovine population
- (c) **Transmission:** infection rates (per month) between the different compartments of the model
- Effective horizontal infectious rate
 - Effective game animals infectious rate
 - Effective sexual transmission rate male to female
 - Effective sexual transmission rate female to male
 - Effective infectious rate of bacteria in environment
 - Effective infectious rate of infected semen straws
 - Abortion rate first gestation after infection
 - Abortion rate subsequent gestations after infection
 - Average delay between infection and abortion
 - Vertical transmission rate
 - Infection rate during primiparous and multiparous gestations as proportion of infection rate at abortion (= 1)
 - Infection rate at full-term calving as proportion of infection rate at abortion (= 1)


- Bacterial discharge rate at abortion
 - Bacterial discharge rate at full-term calving
 - Bacterial discharge rate during primiparous gestation
 - Bacterial discharge rate during multiparous gestation
 - Bacterial discharge rate of wildlife
 - Bacterial pick-up rate from environment by cattle
 - Bacterial pick-up rate from environment by wildlife
 - Bacterial pick-up rate from environment by people
 - *Brucella* mortality rate in environment
- (d) **Control:** disease prevention and control parameters
- Proportion vaccinated newborn females
 - Proportion vaccinated newborn males
 - Vaccination efficacy
 - Period control measures (y/n, yes is periodic control, no is continuous control)
 - Proportion test-positive females that are culled
 - Proportion test-positive males that are culled
 - Sensitivity of diagnostic test
 - Specificity of diagnostic test
- (e) **Initial state vector:**
- Susceptible cows (**S**)
 - Susceptible bulls (**M**)
 - Primiparous cows (**P1**)
 - Infected full-term calvings (**IF**)
 - Infected abortions (**IB**)
 - Multiparous cows (**PN**)
 - Infected bulls (**IM**)
 - Vaccinated cows (**Rf**)
 - Vaccinated bulls (**Rm**)
 - Bacteria in environment (**B**)
 - AI straw infection prevalence
- (f) **Managers:** cumulative results of selected output parameters (respectively after one year, two years, three years, five years and ten years) as well as time to first abortion storm, the final population, the susceptible population and the prevalence
- (g) **Programmers:** eigenvalue output related to model stability

9.1.4 Running the simulation

1. Set up the parameters as required
2. Click the **Run simulation** button in the **General and plot** tab pane
3. The differential equation model runs for the required time period and creates five sets of graphs in the **Plots** tab pane of the **RStudio** output pane (the user is taken automatically to the **Plots** pane). The graphs are created in individual plot windows that can be accessed by means of left and right arrows at the top left of the **Plots** pane. The graphs that can be created by the simulation are the following:
 - (a) Susceptible cows, Infected gestating primiparous cows, Infected gestating multiparous cows
 - (b) Infected full-term calvings, Infected abortions
 - (c) Susceptible males, Infected males
 - (d) Vaccinated females, Vaccinated males
 - (e) Bacteria in environment

Which graphs are produced is selected by means of checkboxes in the **General and plot** tab pane.

Attention!

Although it is possible to remove the graphs in the **Plots** pane (by clicking ), this feature is apparently not available when the App is running. It can thus apparently happen that **RStudio** runs out of memory and hangs if ‘too’ many graph windows have been created. It is therefore recommended to leave the App every now and then and start it up again. It is also recommended to only plot the graphs that are of interest when comparing different scenarios (*e.g.* there is little point in plotting vaccinated animals unless there is effectively vaccination)

4. To go back to the input and output window click the **Viewer** tab.

9.1.5 Saving the graph output

9.1.6 Stopping the program

The program is stopped by clicking  either in the **Console** or in the output pane.

9.1.7 Example output

The following graphs and tables are the output obtained when the default values for the various parameters are used (apart from selecting production of all five graphs).

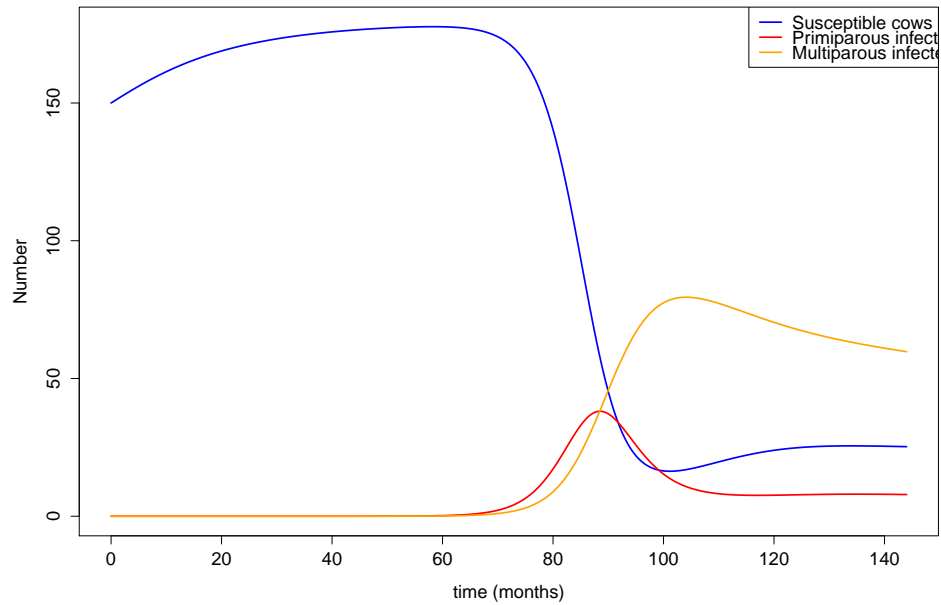


Figure 9.1: Susceptible cows, Infected gestating primiparous cows, Infected gestating multiparous cows

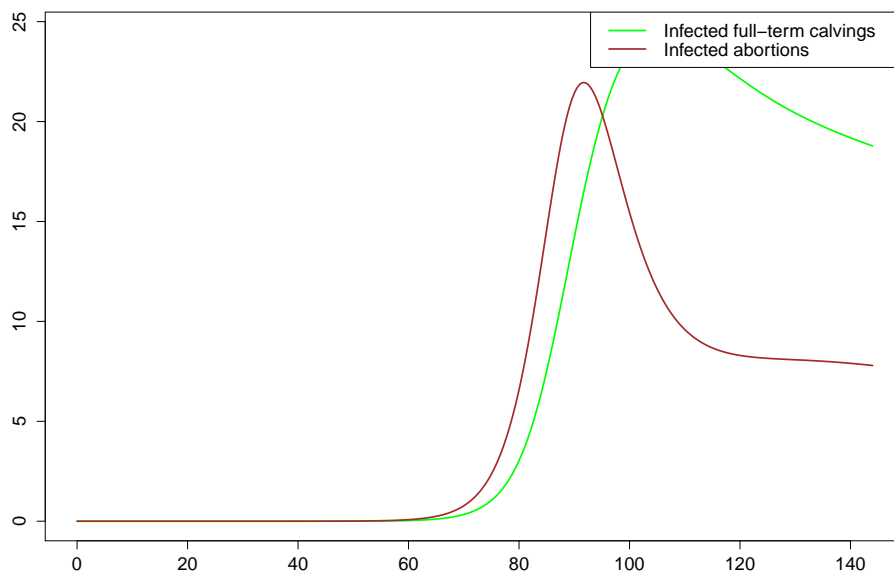


Figure 9.2: Infected full-term calvings, Infected abortions

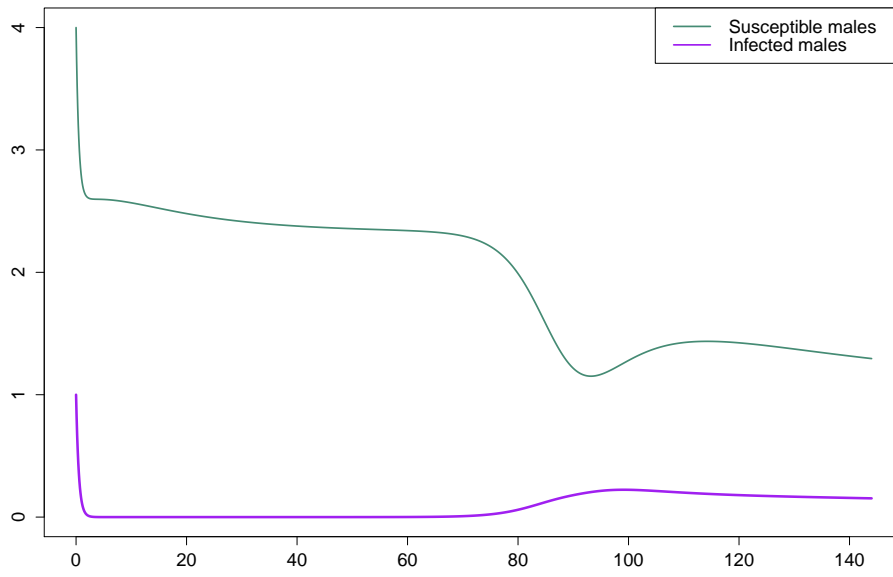


Figure 9.3: Susceptible males, Infected males

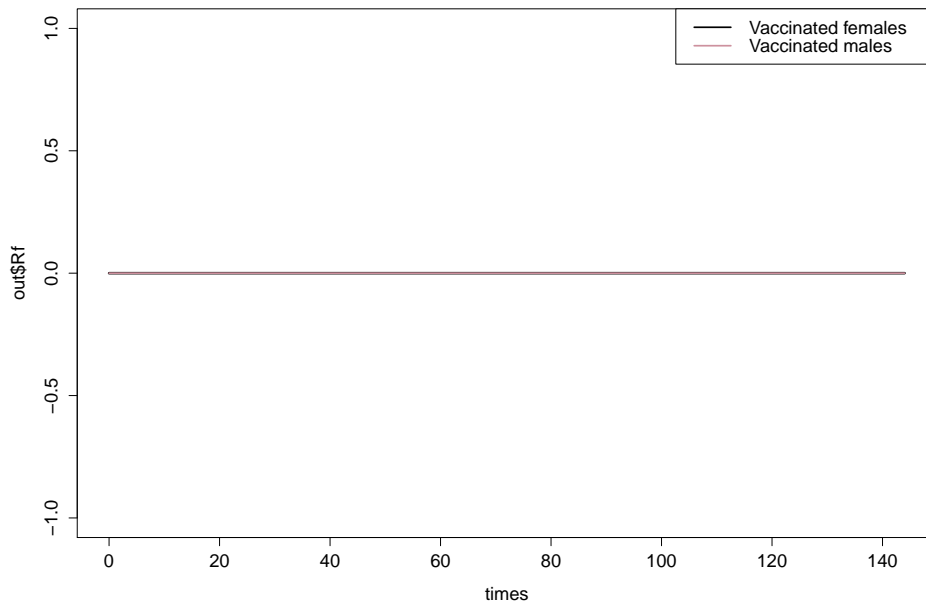


Figure 9.4: Vaccinated females, Vaccinated males

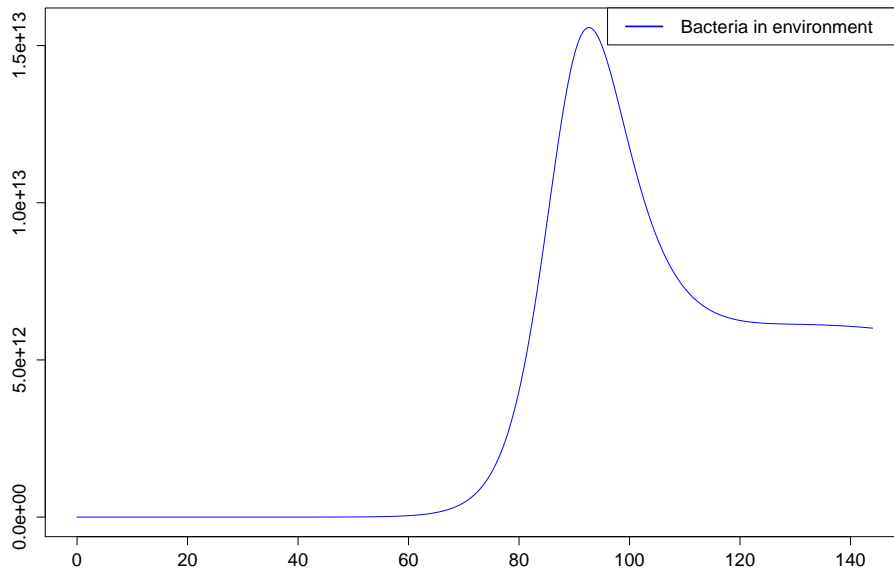


Figure 9.5: Bacteria in environment

Cumulative totals

| | Losses | Year01 | Year02 | Year03 | Year05 | Year10 |
|---|---|--------|--------|--------|--------|-----------|
| 1 | Number of abortions | 0.00 | 0.00 | 0.00 | 0.11 | 193.13 |
| 2 | Number of infected calves (Vertical transmission) | 0.00 | 0.00 | 0.00 | 0.00 | 24.37 |
| 3 | Milk yield (kg) | 0.01 | 0.15 | 2.20 | 549.02 | 965669.99 |
| 4 | Number of slaughtered animals after testing | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 5 | Number of vaccinated animals | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Time until first epidemic (months): 77
 Final Population: 133
 Final Susceptible Population: 23
 Final Prevalence %: 81

Figure 9.6: Managers tab pane

9.2 APPENDIX B: RStudio code listings

9.2.1 ui.R

Listing 9.1: Code listing: ui.R

```

1 library(shiny)
2 shinyUI(
3   navbarPage("Bovine brucellosis",
4     tabPanel("General & Plot",
5       mainPanel(
6         fluidRow(br()),
7         fluidRow(column(12, actionButton("graphIt", label = h2("Run
8           simulation")), align="center")),
9         fluidRow(br(), hr()),
10        fluidRow(
11          column(6,
12            sliderInput("time", "Time (in years)", min = 10,
13              max = 70, value = 12, step = 1),
14            sliderInput("w", "Number of game animals", min =
15              0, max = 10, value = 0, step = 1),
16            sliderInput("iw", "Prevalence infection in game
17              animals (%)", min = 0, max = 0.02, value =
18              0.00001, step = 0.00005),
19            sliderInput("h", "Human population", min = 0, max
20              = 10, value = 2, step = 1),
21            align="center"), # column
22          column(6,
23            checkboxInput("plotSusc", "Plot susceptible +
24              gestating cows", value = TRUE),
25            checkboxInput("plotAb", "Plot infected full-term
26              and abortions", value = TRUE),
27            checkboxInput("plotMale", "Plot males", value =
28              FALSE),
29            checkboxInput("plotVacc", "Plot vaccinated animals
30              ", value = FALSE),
31            checkboxInput("plotBact", "Plot bacteria", value =
32              TRUE)
33          ) # column
34        ) # fluidRow
35      ) # mainPanel
36    ), # tabPanel plot
37    tabPanel("Cattle",
38      mainPanel(
39        fluidRow(column(12, h4("Cattle population parameters"), align
40          = "center")), # fluidRow
41        fluidRow(column(6,
42          sliderInput("g", "Calving rate", min = 2/50, max
43            = 4/40, value = 3/40, step=0.005),
44          sliderInput("eta", "Proportion of cows
45            inseminated", min = 0, max = 1, value = 0 ,
46            step = 0.1),
47          checkboxInput("synchro", "Synchronized

```

Listing 9.1 (Cont.): Code listing: ui.R

```

33         reproduction", value = FALSE),
34         sliderInput("m_s", "Susceptibles mortality rate",
35           min = 0.001, max = 0.05, value = 1/38, step
36             =0.001),
37         sliderInput("m_pl", "Primopare mortality rate",
38           min = 0.001, max = 0.05, value = 1/38, step
39             =0.001),
40         sliderInput("milk", "Average milk yield per
41           lactation (kg)", min = 500, max = 10000,
42           value = 5000, step=100),
43         align = "center"), # column
44     column(6,
45         sliderInput("m_if", "Full-term infected mortality
46           rate", min = 0.001,max = 0.05,value = 1/38, step
47             =0.001),
48         sliderInput("m_ib", "Abortion infected mortality
49           rate", min = 0.001,max = 0.05,value = 1/38, step
50             =0.001),
51         sliderInput("m_pn", "Multiparous cows mortality rate
52           ", min = 0.001,max = 0.05,value = 1/32, step
53             =0.001),
54         sliderInput("m_im", "Infected bulls mortality rate",
55           min = 0.001, max = 0.05, value = 1/32, step =
56             0.001),
57         sliderInput("s_m", "Sales rate of males", min = 1.5,
58           max = 4,value = 2 ,step = 0.1),
59         sliderInput("K", "Bovine carrying capacity", min =
60           10, max = 300, value = 200, step = 5),
61         align = "center") # column
62     ) # fluidRow
63   ) # mainPanel
64 ), # tabPanel cattle
65 tabPanel("Transmission",
66   mainPanel(
67     fluidRow(
68       column(3,
69         sliderInput("f_c", "Effective Horizontal infectious
70           rate", min = 0.0001, max = 0.002, value =
71             0.0002, step = 0.0001),
72         sliderInput("f_w", "Effective infectious rate with
73           game animals", min = 0.00001, max= 0.002, value
74             = 0.0002, step = 0.0001),
75         sliderInput("f_mf", "Effective sexual infectious
76           rate m to f", min = 0, max = 1e-2, value = 1e-8,
77             step = 0.0001),
78         sliderInput("f_fm", "Effective sexual infectious
79           rate f to m (x10^9)", min = 0, max = 1e-2, value
80             = 1e-7, step = 1e-4),
81         sliderInput("f_e", "Environmental infection (
82           Effective rate)", min = 0, max = 1, value =
83             1.245e-2, step = 1e-4),
84         sliderInput("e", "Effective infectious rate AI", min
85             = 0.0001, max = 0.2, value = 0.0003, step =
86             0.0001),
87         align = "center"), # column

```

Listing 9.1 (Cont.): Code listing: ui.R

```

59     column(3,
60         sliderInput("l1", "aborting proportion", min = 0.1,
61                     max = 0.9, value = 0.6, step = 0.1),
62         sliderInput("ln", "Aborting proportion (2..)", min =
63                     0.01, max = 0.2, value = 0.05, step = 0.01),
64         sliderInput("x_b", "Average time between infection
65                     and abortion", min = 0.5, max = 8, value = 3,
66                     step = 0.5),
67         sliderInput("d", "Vertical transmission", min =
68                     0.05, max = 0.3, value = 0.1, step = 0.05),
69         sliderInput("a", "Infective proportion of P1,Pn",
70                     min = 0.00, max = 0.2, value = 0.05, step =
71                     0.001),
72         sliderInput("a1", "Infective proportion of If", min
73                     = 0.1, max = 1, value = 0.5, step = 0.1),
74     align = "center"), # column
75     column(3,
76         sliderInput("t_ib", "Discharge rate abortives:"
77                     , min = 1e8, max = 1e14, value = 1e12,
78                     step = 1e12),
79         sliderInput("t_if", "Discharge rate calvings:"
80                     , min = 1e8, max = 1e10, value = 1e9 ,
81                     step = 1e9),
82         sliderInput("t_p1", "Discharge rate in pregnancy (1)
83                     : " , min = 1e3, max = 1e5 , value = 1e3 , step
84                     = 1e3),
85         sliderInput("t_pn", "Discharge rate in pregnancy
86                     (2..):" , min = 1e2, max = 1e4 , value = 1e2 ,
87                     step = 1e2),
88         sliderInput("t_w" , "Discharge rate wildlife:"
89                     , min = 1e3, max = 1e5 , value = 1e3 ,
90                     step = 1e3),
91     align = "center"), # column
92     column(3,
93         sliderInput("r_b", "Pick up rate (pastures-cattle)",
94                     min = 0 , max = 0.5 , value = 0.0025, step =
95                     0.0001),
96         sliderInput("r_w", "Pick up rate (wildlife)" ,
97                     min = 1e-7, max = 1e-5, value = 1e-6 , step =
98                     1e-7),
99         sliderInput("r_h", "Pick up rate (humans)" ,
100                    min = 5e-6, max = 1e-4, value = 5e-5 , step =
101                    5e-6),
102         sliderInput("m_b", "Bacterial mortality rate" ,
103                    min = 0.01, max = 2 , value = 1 , step =
104                    0.01),
105     align = "center") # column
106 ) # fluidRow
107 ) # mainPanel
108 ), # tabPanel transmission
109 tabPanel("Control",
110     mainPanel(
111         fluidRow(
112             column(12,
113                 h4("Prevention and contol strategies"),

```


Listing 9.1 (Cont.): Code listing: ui.R

```

88     sliderInput("v", "Vaccination proportion females",
89               min = 0 , max = 1, value = 0.0 , step = 0.1),
90     sliderInput("v1", "Vaccination proportion males",
91               min = 0 , max = 1, value = 0.0 , step = 0.1),
92     sliderInput("ch", "Vaccination efficacy",
93               min = 0 , max = 1, value = 0.7 , step = 0.1),
94     checkboxInput("test", "Periodic testing", value =
95                 FALSE),
96     sliderInput("z_f", "Culling rate cows",
97               min = 0 , max = 2.99, value = 0.0 , step =
98                 0.01),
99     sliderInput("z_m", "Culling rate bulls",
100              min = 0 , max = 2.99, value = 0.0 , step =
101                0.01),
102     sliderInput("Se", "Test sensitivity",
103               min = 0.2, max = 1, value = 0.9 , step = 0.05),
104     sliderInput("Sp", "Test specificity",
105               min = 0.8, max = 1, value = 0.95 , step = 0.01),
106     align = "center" )
107   ) # fluidRow
108 ) # mainPanel
109 ), # tabPanel control
110 tabPanel("Initial state vector",
111         mainPanel(
112         fluidRow(
113         column(12,
114         h4("Initial population state vector"),
115         sliderInput("S" , "Number of susceptible cows",
116                   min = 0, max = 300, value = 150,
117                   step = 1),
118         sliderInput("M" , "Number of susceptible bulls",
119                   min = 0, max = 30, value = 4, step
120                   = 1),
121         sliderInput("P1", "Number of infected primiparous
122                   cows", min = 0, max = 300, value = 0, step
123                   = 1),
124         sliderInput("IF", "Number of infected full-term
125                   calvings", min = 0, max = 300, value = 0,
126                   step = 1),
127         sliderInput("IB", "Number of infected abortions",
128                   min = 0, max = 300, value = 0, step
129                   = 1),
130         sliderInput("PN", "Number of infected multiparous
131                   cows", min = 0, max = 300, value = 0, step
132                   = 1),
133         sliderInput("IM", "Number of infected males",
134                   min = 0, max = 30, value = 1,
135                   step = 1),
136         sliderInput("Rf", "Number of vaccinated cows",
137                   min = 0, max = 300, value = 0,
138                   step = 1),
139         sliderInput("Rm", "Number of vaccinated bulls",
140                   min = 0, max = 30, value = 0,
141                   step = 1),
142         sliderInput("B" , "Number of bacteria in environment

```

Listing 9.1 (Cont.): Code listing: ui.R

```

115         ",      min = 0, max = 1e15, value = 0,   step =
116         1e6),
117         hr(),
118         sliderInput("ai", "AI straw infection prevalence",
119                     min = 0, max = 1, value = 0, step =
120                     0.05),
121         align = "center")
122     ) # fluidRow
123 ) # mainPanel
124 ),
125 tabPanel("Managers",
126         mainPanel(h3("Cumulative totals"),
127                 tableOutput("manager"), br(), br(),
128                 textOutput("timeToEpi"),
129                 textOutput("Finalpop"),
130                 textOutput("Finalsuscl"),
131                 textOutput("FinalPrev")
132             ) # mainPanel
133         ), # tabPanel
134 tabPanel("Modellers",
135         mainPanel(
136             tableOutput("eigen")
137         ) # mainPanel
138     ) # tabPanel
139 ) # navbarPage
140 ) # shinyUI

```

9.2.2 server.R

Listing 9.2: Code listing: server.R

```

1 require("deSolve")
2 require("rootSolve")
3 require("shiny")
4 options(warn=-1)
5 shinyServer(function(input, output, session) {
6   source("BrucellaFunction.R", local=TRUE)
7 })
8 options(warn=0)

```

9.2.3 BrucellaFunction.R

Listing 9.3: Code listing: BrucellaFunction.R

```

1 observe({ if(input$graphIt > 0)

```

Listing 9.3 (Cont.): Code listing: BrucellaFunction.R

```

2 {
3 isolate({
4   f_e = input$f_e/1e12; f_c = input$f_c; a = input$a; a1 = input$a1;
5     f_w = input$f_w; iw = input$iw; w = input$w
6   eta = input$eta; f_mf = input$f_mf; e = input$e; ai = input$ai
7   f_fm = input$f_fm/1e9
8   m_b = input$m_b; r_b = input$r_b; r_w = input$r_w; r_h = input$r_h
9     ; h = input$h
10  g = input$g; th = input$th; K = input$K
11  d = input$d; o_f = 1/3
12  v = input$v; ch = input$ch; m_s = input$m_s; Sp = input$Sp; z_f =
13    input$z_f
14  s_m = input$s_m; z_m = input$z_m; perTest = input$test
15  l1 = input$l1; x_b = 1/input$x_b; x_f = 1/9; m_p1 = input$m_p1; Se
16    = input$Se
17  ln = input$ln; m_if = input$m_if
18  o_b = 1/3; m_ib = input$m_ib
19  m_pn = input$m_pn
20  m_im = input$m_im
21  t_ib = input$t_ib; t_if = input$t_if; t_p1 = input$t_p1; t_pn =
22    input$t_pn; t_w = input$t_w
23  milk = input$milk
24  time = input$time*12; synchro = input$synchro
25  plot1 = input$plotSusc
26  plot2 = input$plotAb
27  plot3 = input$plotMale
28  plot4 = input$plotVacc
29  plot5 = input$plotBact
30 })
31 parameters = c(f_e, f_c, a, a1, f_w, iw, w, eta, f_mf, e, ai, f_fm,
32   m_b, r_b, r_w, r_h, h,
33   g, th, K, d, o_f, v, ch, m_s, Sp, z_f, s_m, z_m, l1, x_b, x_f
34   , m_p1, Se,
35   ln, m_if, o_b, m_ib, m_pn, m_im, t_ib, t_if, t_p1, t_pn, t_w)
36 state = c(S=isolate(input$S), M=isolate(input$M), P1=isolate(input$
37   P1), IF=isolate(input$IF),
38   IB=isolate(input$IB), PN=isolate(input$PN), IM=isolate(input$IM)
39   , Rf=isolate(input$Rf),
40   Rm=isolate(input$Rm), B=isolate(input$B), XX=0)
41 BrucODE = function(t, state, parameters)
42 {
43   with(as.list(c(state,parameters)),
44     {
45     N      = S + M + IF + IB + P1 + PN + IM + Rf + Rm
46     m_c    = 0.00241+((0.03775-0.0022274)/((1+0.7*exp(-10*(N/K)
47       -1))^25000))
48     b1     = f_e*B + f_c*(a*IF + IB + a1*(P1 + PN) + f_w*iw*w)
49     b2     = (1-eta)*f_mf*IM + eta*e*ai
50     b3     = (1-eta)*f_fm*(IB+IF)
51     r      = m_b + r_b*N + r_w*w + r_h*h
52     b_adj  = ifelse(synchro, ifelse(t%%12>=11.6 | t%%12<=0.5,
53       0.5*12*g*(1-m_c)^(18), 0), 0.5*g*(1-m_c)^(18))
54     z_f    = ifelse(perTest, ifelse(t%%24>=23.6 | t%%24<=0.5, z_f,
55       0), z_f)
56     z_m    = ifelse(perTest, ifelse(t%%24>=23.6 | t%%24<=0.5, z_m,

```

Listing 9.3 (Cont.): Code listing: BrucellaFunction.R

```

    0), z_m)
45   t_vrt = (Rf+S+(1-d)*IF/o_f)
46   dS    = b_adj*t_vrt*(1-v*ch) - (b1 + b2 + m_s + (1-Sp)*z_f
    )*S
47   dM    = b_adj*t_vrt*(1-v*ch) - (b1 + b3 + m_s + s_m + (1-
    Sp)*z_m)*M
48   dP1   = (b1 + b2)*S - (l1*x_b + (1-l1)*x_f + m_p1 + Se*z_f
    )*P1
49   dIF   = (1-l1)*x_f*P1 + (1-ln)*x_f*PN - (o_f + m_if + Se*z
    _f)*IF
50   dIB   = b_adj*d*IF/o_f + l1*x_b*P1 + ln*x_b*PN - (o_b + m_
    ib + Se*z_f)*IB
51   dPN   = o_f*IF + o_b*IB - ((1-ln)*x_f + ln*x_b + m_pn + Se
    *z_f)*PN
52   dIM   = b_adj*d*IF/o_f + (b1+b3)*M - (m_im + s_m + Se*z_m)
    *IM
53   dRf   = b_adj*t_vrt*v*ch - (m_s + (1-Sp)*z_f)*Rf
54   dRm   = b_adj*t_vrt*v*ch - (m_s + s_m + (1-Sp)*z_m)*Rm
55   dB    = t_ib*IB + t_if*IF + t_p1*P1 + t_pn*PN + t_w*iw*w -
    r*B
56   dXX   = (1-Sp)*(z_f*(S+Rf) + z_m*(M+Rm)) + Se*(z_f*(P1+IF+
    IB+PN) + z_m*IM)
57   list(c(dS, dM, dP1, dIF, dIB, dPN, dIM, dRf, dRm, dB, dXX)
    )
58   })
59 }
60 by = 0.1; times = seq(0, time, by)
61 out = data.frame(rk4(y=state, times=times, func=BrucODE, parms=
    parameters))
62 par(xaxt="s", yaxt="s", bty="o")
63
64
65 if (plot1){
66   ymax = max(out$S, out$P1, out$PN)
67   plot(times, out$S, type="l", col="blue", lwd=2, ylim=c(0,ymax),
    ylab="Number", xlab="time (months)")
68   lines(times, out$P1, type="l", col="red", lwd=2)
69   lines(times, out$PN, type="l", col="orange", lwd=2)
70   legend("topright", c("Susceptible cows", "Primiparous infected
    cows", "Multiparous infected cows"), lty=c(1,1,1), lwd=c(2,2,2)
    , col=c("blue", "red", "orange"))
71   } # plot1
72 if (plot2){
73   ymax = max(out$IF, out$IB)
74   plot(times, out$IF, type="l", col="green", lwd=2, ylim=c(0,ymax),
    ylab="", xlab="")
75   lines(times, out$IB, type="l", col="brown", lwd=2)
76   legend("topright", c("Infected full-term calvings", "Infected
    abortions"), lty=c(1,1), lwd=c(2,2), col=c("green", "brown"))
77   } # plot2
78 if (plot3){
79   ymax = max(out$M, out$IM)
80   plot(times, out$M, type="l", col="aquamarine4", lwd=2, ylim=c(0,
    ymax), ylab="", xlab="")
81   lines(times, out$IM, type="l", col="purple", lwd=3)

```

Listing 9.3 (Cont.): Code listing: BrucellaFunction.R

```

82   legend("topright", c("Susceptible males", "Infected males"), lty=c
      (1,1), lwd=c(2,2), col=c("aquamarine4", "purple"))
83   } #plot3
84   if (plot4){
85     ymax = max(out$Rf, out$Rm)
86     plot(times, out$Rf, type="l", col="black", lwd=2)
87     lines(times, out$Rm, type="l", col="pink3", lwd=2)
88     legend("topright",c("Vaccinated females", "Vaccinated males"),lty=
      c(1,1),lwd=c(2,2) ,col=c("black","pink3"))
89   } #plot4
90   if (plot5){
91     ymax = max(out$B)
92     plot(times, out$B, type="l", col="blue", ylim=c(0,ymax), ylab="",
      xlab="")
93     legend("topright", "Bacteria in environment", lty=1, lwd=2, col="
      blue")
94   } # plot5
95
96   eigval = eigen(jacobian.full(state, BrucODE, time = 1e9, parms=
      parameters))$values
97   numEigVals = length(eigval)
98   eigval2 = array(0, c(numEigVals,2), dimnames=list(c(1:numEigVals),
      c("Real Part","Imaginary")))
99   eigval2[,1] = Re(eigval); eigval2[,2] = Im(eigval)
100  output$eigen = renderTable(formatC(eigval2, digits=4, format="f",
      width=9), align="rrr")
101  Losses = c("Number of abortions", "Number of infected calves (
      Vertical transmission)", "Milk yield (kg)", "Number of
      slaughtered animals after testing", "Number of vaccinated
      animals")
102  Year01 = Year02 = Year03 = Year05 = Year10 = rep(0, 5)
103  for(i in 1:12) Year01[1] = Year01[1] + o_b*mean(out$IB[((i-1)/by
      +1):(i/by)]); Year02[1] = Year01[1]
104  for(i in 13:24) Year02[1] = Year02[1] + o_b*mean(out$IB[((i-1)/by
      +1):(i/by)]); Year03[1] = Year02[1]
105  for(i in 25:36) Year03[1] = Year03[1] + o_b*mean(out$IB[((i-1)/by
      +1):(i/by)]); Year05[1] = Year03[1]
106  for(i in 37:60) Year05[1] = Year05[1] + o_b*mean(out$IB[((i-1)/by
      +1):(i/by)]); Year10[1] = Year05[1]
107  for(i in 61:120) Year10[1] = Year10[1] + o_b*mean(out$IB[((i-1)/by
      +1):(i/by)]);
108  for(i in 1:12) Year01[2] = Year01[2] + (1-0.00241)^18*d*o_f*mean(
      out$IF[((i-1)/by+1):(i/by)]); Year02[2] = Year01[2]
109  for(i in 13:24) Year02[2] = Year02[2] + (1-0.00241)^18*d*o_f*mean(
      out$IF[((i-1)/by+1):(i/by)]); Year03[2] = Year02[2]
110  for(i in 25:36) Year03[2] = Year03[2] + (1-0.00241)^18*d*o_f*mean(
      out$IF[((i-1)/by+1):(i/by)]); Year05[2] = Year03[2]
111  for(i in 37:60) Year05[2] = Year05[2] + (1-0.00241)^18*d*o_f*mean(
      out$IF[((i-1)/by+1):(i/by)]); Year10[2] = Year05[2]
112  for(i in 61:120) Year10[2] = Year10[2] + (1-0.00241)^18*d*o_f*mean(
      out$IF[((i-1)/by+1):(i/by)]);
113  Year01[3] = milk*Year01[1]; Year02[3] = milk*Year02[1]; Year03[3] =
      milk*Year03[1]; Year05[3] = milk*Year05[1]; Year10[3] = milk*
      Year10[1]
114  Year01[4] = out$XX[12/by] - out$XX[1]; Year02[4] = out$XX[24/by] -

```

Listing 9.3 (Cont.): Code listing: BrucellaFunction.R

```

    out$XX[1]; Year03[4] = out$XX[36/by] - out$XX[1]
115 Year05[4] = out$XX[60/by] - out$XX[1]; Year10[4] = out$XX[120/by] -
    out$XX[1]
116 for(i in 1:12) Year01[5] = Year01[5] + mean(out$Rf[((i-1)/by+1):(
    i/by])) + mean(out$Rm[((i-1)/by+1):(i/by)]); Year02[5] = Year01
    [5]
117 for(i in 13:24) Year02[5] = Year02[5] + mean(out$Rf[((i-1)/by+1):(
    i/by])) + mean(out$Rm[((i-1)/by+1):(i/by)]); Year03[5] = Year02
    [5]
118 for(i in 25:36) Year03[5] = Year03[5] + mean(out$Rf[((i-1)/by+1):(
    i/by])) + mean(out$Rm[((i-1)/by+1):(i/by)]); Year05[5] = Year03
    [5]
119 for(i in 37:60) Year05[5] = Year05[5] + mean(out$Rf[((i-1)/by+1):(
    i/by])) + mean(out$Rm[((i-1)/by+1):(i/by)]); Year10[5] = Year05
    [5]
120 for(i in 61:120) Year10[5] = Year10[5] + mean(out$Rf[((i-1)/by+1):(
    i/by])) + mean(out$Rm[((i-1)/by+1):(i/by)])
121 managerTable = cbind.data.frame(Losses, Year01, Year02, Year03,
    Year05, Year10)
122 output$manager = renderTable(managerTable, align="rrrrrrr")
123 tte = min(which(out$IB/((out$S/12)+out$IF+out$IB+(out$Rf/12))
    >=0.20))
124 pop=out$S[length(times)]+out$M[length(times)]+out$Rf[length(times)
    ]+out$Rm[length(times)]+out$P1[length(times)]+out$IF[length(
    times)]+out$IB[length(times)]+out$PN[length(times)]+out$IM[
    length(times)]
125 prev=(out$P1[length(times)]+out$IF[length(times)]+out$IB[length(
    times)]+out$PN[length(times)]+out$IM[length(times)])*100/pop
126 sus= out$S[length(times)]
127 output$timeToEpi = renderText(paste("Time until first epidemic (
    months):", floor(times[tte])))
128 output$Finalpop = renderText(paste("Final Population:", floor(pop))
    )
129 output$Finalsus = renderText(paste("Final Susceptible Population:"
    , floor(sus)))
130 output$FinalPrev= renderText(paste("Final Prevalence %:", floor(
    prev)))
131 }
132 })

```