EPIDEMIOLOGICAL INVESTIGATION OF BOVINE TUBERCULOSIS OUTBREAKS IN URUGUAY (2011-2013)

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To my love, Zelmar, for your unwavering support, encouragement and sacrifice over

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Abstract

Bovine tuberculosis (BTB) is a chronic disease of cattle caused by infection with the

Mycobacterium bovis, and with an important zoonotic potential. Countries have widely

recognized BTB as a major animal and public health problem. Uruguay is a South

American country with a strong agriculture-based tradition and economy. Uruguay was

one of the pioneers in the fight against BTB in the world in the late 1800's, with

continuously update of BTB-control measures, especially in dairy herds, which resulted

in the design of the current national BTB control program. Under the current BTB control

program, surveillance is based on test-and-slaughter strategies for dairy farms, and

abattoir carcasses inspection looking for tubercles in all slaughtered cattle. When a farm

is reported as positive, control measures include movement restrictions, testing of

neighboring farms, and traceback of all animal movements performed up to two years

previous to the outbreak. As a consequence of this surveillance program, BTB prevalence

in Uruguay has been traditionally low (<11 outbreaks per year). However, between 2011

and 2013, the incidence of BTB increased, concerning farmers, industry, veterinarians,

and the government authorities. The goal of this study was to assess the spatial dynamics

of BTB in Uruguay in the 2011-2013 period, and the association between BTB and

demographic and movement factors in the Uruguayan dairy industry. Data provided by

the Uruguayan Ministry of Livestock, Agriculture, and Fisheries included information on

geolocation, BTB status (positive/negative), and onset date. Also, individual information

on movements from/to all farms during the 2008 to 2013 period in Uruguay was provided to obtain farm-level network characteristics. Fifteen, 26, and 16 BTB-outbreaks were reported in 42,759 (2011), 43,213 (2012), and 42,186 (2013) susceptible farms, respectively. The spatial distribution of incident outbreaks was assessed using the Cuzick-and-Edwards' test, and the Bernoulli model of the spatial scan statistic. Significant (P=0.05) clustering at the second order of neighborhood was detected in 2012, and significant high-risk clusters were identified in southwestern (2011, 2012, 2013), northwestern (2012), and southeastern (2012) Uruguay. The presence of spatial aggregation in southwestern Uruguay (consistently in 2011 and 2012, and corresponding to a dairy production land-use) suggests an association between land management and BTB risk. Global clustering detected in 2012 suggests dissemination by close contact of BTB. The increase of BTB incidence in disparate regions of the country suggests that live animal movements may have played a role in spreading the disease. A subset of the dairy Uruguayan farms was assessed with a conditional multivariate logistic regression model to investigate the risk factors most likely associated with BTB, with three controls (n=171) per positive farm (n=57), accounting for spatial dependency. Ten demographic, and 51 movement epidemiological factors were evaluated as possible risk factors. The final model included large herds, high number of incoming cattle to the farm (>44 cattle), and purchasing steers as factors increasing the odds of BTB. The first two factors were in agreement with previous studies, highlighting the importance of control in terms of disease spread this practice in the cattle industry. The association between BTB incidence

and purchasing steers may be associated with certain management practices for which this covariate may serve as proxy. Results from this study were presented to and discussed with Uruguayan Animal Health Bureau members to address applicability of results. These results and discussions contributed to the design and implementation of disease management strategies intended to enhance the effectiveness of BTB control programs in Uruguay, with the ultimate objective of preventing or mitigating the impact of the disease in the human and animal populations of the country.

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CHAPTER 1 - UNDERSTANDING BOVINE TUBERCULOSIS IN THE WORLD AND IN URUGUAY

1.1 Chapter summary

The goal of this chapter is to describe the fundaments of bovine tuberculosis (BTB) incidence, spread, and control, the importance of the disease worldwide, and its presentation in Uruguay. A description of disease epidemiology, transmission, pathogenesis, clinical presentation, and diagnosis are presented to understand important aspects of current control or eradication programs established to avoid the spread of disease worldwide and in the Uruguay cattle population.

Uruguay has an extensive history fighting BTB, in chapter one, I will address important aspects of the country which aim to understand the importance assigned by the country to this zoonotic disease for more than one century. However, disease farm and within-herd prevalence in Uruguay increased in the period between 2011 and 2013, raising concerns of farmers, veterinarians, and government officers.

Understanding the context and history of a disease in a country is prerequisite to develop an epidemiological investigation on any infectious disease. The analysis will help to interpret and discuss results obtained to develop accurate and applicable recommendations. Such recommendations will contribute to ultimately control or eradicate one of the most important zoonotic diseases for Uruguay and worldwide.

1.2 General aspects of bovine tuberculosis

The agent

Mycobacterium bovis (*M. bovis*), a member of the *Mycobacterium tuberculosis* complex, is the main etiological agent of bovine tuberculosis (BTB) (McMurray, 1941; Braun and Lebek, 1958; Francis, 1958; Schmiedel, 1966, 1968), and also a zoonotic agent responsible for one of the causes of human tuberculosis (Cosivi et al., 1998; Evans et al., 2007; Ojo et al., 2008; Rodriguez et al., 2009; Michel et al., 2010; Jenkins et al., 2011). In the last years, *Mycobacterium caprae* has also been described to a lesser extent as a cause of BTB; however that role has only been described in Central and Southern Europe (Aranaz et al., 2003; Rodriguez et al., 2009; Rodriguez et al., 2011; Muller et al., 2013; Zanardi et al., 2013).

M. bovis is an aerobic acid-fast bacillus; its structure involves a high lipid content wall and acid mycolic oil (Quinn et al., 2011). Even it is an obligate pathogen, and cannot survive heat treatments (such as pasteurization), this wall gives the bacilli some resistance to environmental factors, detergents, and disinfectants, in addition to macrophages intracellular killers. Those properties aim the bacteria to survive in soil, water and vegetation as described by observational studies with natural and artificial contamination of a variety of sites (Morris et al., 1994). *M. bovis* survivability in the environment depends on temperature, sunlight (particularly UV light), humidity, and organic matter (Wray, 1975), lasting between a few weeks in natural conditions(Menzies

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and Neill, 2000) to a few months under certain favorable circumstances (Quinn et al., 2011).

Although *M. bovis* infection mainly occurs in cattle, numerous domestic and non-domestic mammals had been reported as hosts for that microorganism worldwide. Livestock susceptible species include sheep (Malone et al., 2003; Houlihan et al., 2008; Munoz Mendoza et al., 2012), swine (Jenkins et al., 2011; Di Marco et al., 2012; Bailey et al., 2013; Barandiaran et al., 2015), goats (Gutierrez et al., 1998; Alvarez et al., 2008; Crawshaw et al., 2008; Daniel et al., 2009; Hiko and Agga, 2011; Broughan et al., 2013), and equines (Monreal et al., 2001; Keck et al., 2010; Sarradell et al., 2015). However, those reports may be considered incidental, and rarely associated with cattle infection. Cats and dogs were also reported to be infected with *M. bovis* (Ellis et al., 2006; Monies et al., 2006; Shrikrishna et al., 2009; Gunn-Moore et al., 2011), and because those domestic species are in close relation with human population, and there is a potential for public health importance. However, those reports were unrelated with BTB in cattle or potential transmission between them. Other bovid, such as buffalo, bison, and antelope (Wobeser, 2009; de Garine-Wichatitsky et al., 2010; Himsworth et al., 2010; Shury et al., 2015), and a wide range of wild hosts in different countries, are part of a vast list of reported hosts. Some of them include deer in the USA and Canada (Wobeser, 2009; Miller and Sweeney, 2013), badgers in the UK and Ireland (Gormley and Corner, 2013; Byrne et al., 2015; Buzdugan et al., 2016), wild boars in Spain (Aranaz et al., 2004;

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Gortazar et al., 2011), possums, and ferrets in New Zealand (de Lisle et al., 2005; Nugent et al., 2015), raccoons, coyotes, and a number of feline predators (lions, tigers, lynx, and leopards) (de Lisle et al., 2001). Such a wide range of animals that can be affected with BTB has been proposed as a relevant reason on BTB transmission and perpetuation of infection, especially for grazing cattle, increasing the challenge faced at the time of elaborating effective control and eradication programs for cattle populations.

Transmission

The most common route of transmission for *M. bovis* in adult cattle is by aerosol inhalation, and evidence is based on lesions found, most frequently at slaughter, in the nasopharyngeal, lungs (low respiratory track), and lymph nodes associated (Neill et al., 1994; Neill et al., 2001; Phillips et al., 2003). The second most commonly described route of infection is through ingestion of tubercle bacilli from contaminated feed, water, pastures or tuberculosis milk, particularly affecting suckling calves (Evangelista and De Anda, 1996; Menzies and Neill, 2000; Domingo et al., 2014). Additional routes of infection, such as venereal, trans-placental, intra-mammary, and cutaneous were reported (Vural and Tunca, 2001; Phillips et al., 2003; Menzies et al., 2012) but are believed to be infrequent due to active control and surveillance programs, and removal of animals with detected infections in most BTB-affected countries, which reduces the average age of infected cattle and the likelihood of transmission through atypical routes.

The dose required to effectively transmit the disease varies according to the route of entry. With aerosol droplets, only one bacillus may be sufficient to establish infection, when the proper location is reached at the pulmonary alveoli; however, for the oral route, millions of bacilli may be needed to cause infection (O'Reilly and Daborn, 1995), leaving the risk of infection from environmental contamination to be considered negligible (Humblet et al., 2009). Nevertheless, for respiratory infection, the size of the particles carrying the agent seems to be more important than the number of infectious particles reaching a susceptible animal. Studies suggest that particle sizes between 0.5 and 7 μm can contain *M. bovis* (Chambers et al., 2001), but, 1-5 μm particles are necessary to reach the targeted alveoli, and are more likely to establish and remain in the lung compared to large mass of particles (Wells et al., 1948).

Pathogenesis and clinical signs:

After the *M. bovis* enters into the host, its persistence depends on the ability to multiply and survive after been engulfed by hosts' macrophages and dendritic cells. Infection is disseminated through the lymphatic tissues by dendritic cells, and through other parts of the body by migration of infected macrophages (Quinn et al., 2011). The agent can survive, multiply, and be released from macrophages after interfering with lysosomal digestion, and then colonize new macrophages. Accumulation of macrophages on the primary site of infection is followed by secretion of cytokines which attracts lymphocytes aiming to the formation of a granuloma (typical lesion in response to *M.*

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bovis infection) containing the pathogen and essential to provide host's resistance to infection. Granulomas may be visible as early as the first three weeks after infection (Cassidy et al., 1998). Some animals in the herd may clear the infection or localized it in the granuloma, whereas in other animals, the disease may progress with the potential rupture of the tubercle and systemic spread of bacilli (Cassidy, 2008; Thoen et al., 2009). Some (9 to 20%) of the affected animals (Kameda and Kino, 1974; Menzies and Neill, 2000) may shed *M. bovis* through aerosol, by nasal or tracheal secretions; in addition, shedding may occur through feces, milk, and urine intermittently for up to 38 weeks (Neill et al., 1988; Neill et al., 1994), being a source of infection and transmission to other hosts.

BTB is a chronic debilitating disease and clinical signs are rarely evident in cattle until the advanced stages. Symptoms depend mainly on the localization and distribution of the lesions in the body (Thoen et al., 1981). Dyspnea, tachypnea, and cough are present in advanced stages of pulmonary tuberculosis, along with pyrexia, and sometimes lymph nodes are evidently enlarged (Une and Mori, 2007). When visceral infection is present, symptoms related to malfunction of spleen or liver have been reported, but are infrequent (Daniel et al., 2009), and when mammary gland is affected mastitis can be diagnosed. The most common clinical BTB presentation includes a chronic debilitation, emaciation, wasting and loss of body condition. However, in countries where a control

program is in place, such as Uruguay or the U.S., symptomatic presentation is uncommon and the disease typically course with no evident sign of infection.

For those reasons, clinical diagnosis of BTB is, at minimum, infrequent. However, different direct and indirect methods had been described and extensively used in control and eradication programs worldwide.

1.3 Worldwide bovine tuberculosis importance and animal health programs

Countries have widely recognized BTB as a disease of veterinary and public health concern. *M. bovis* zoonotic potential has been described since the early 20th century when evidence for animal-to-human transmission was irrefutable (Grange and Yates, 1994), and which resulted in the initial reason driving the implementation of animal health regulations in many countries (Pfeiffer, 2013; Bezos et al., 2014). Ingestion of raw, unpasteurized milk was linked to human tuberculosis infection even before *M. bovis* zoonotic potential was proved (Collins, 2000). Subsequently, pasteurization of milk and control programs applied in many countries reduced disease risk, and targeting the most frequent source of human infection to the airborne route among meat industry workers (Robinson et al., 1988). However, reports of human tuberculosis cases (caused by *M. tuberculosis* and *M. bovis*) are still frequent (Rodwell et al., 2010) and tuberculosis is still one of the leading infectious causes of death in humans worldwide (CDC, 2001-2004).

An additional incentive leading to implementing BTB control measures in different countries is the decrease in animal productivity (meat and milk) and welfare, motivating stakeholders to apply the recommended animal health measures in their cattle herds.

Nowadays, the control measure most commonly used by animal health authorities worldwide is the test-and-cull strategy (OIE, 2016), in which animals with positive result to the selected test are slaughtered, followed by carcasses inspection at slaughter, looking for BTB tubercles; sometimes movement restrictions, tracing back new animals introduced in the heard before outbreak was detected, is also applied (Pfeiffer, 2013).

In-vivo disease detection is mainly based on the delayed-hypersensitivity immunological response to a reagent (tuberculin) with intradermal administration (Monaghan et al., 1994; Schiller et al., 2010). Tuberculin skin test has been broadly used since first recommended by Koch in the late 19th century (Monaghan et al., 1994), although it was initially conceived as a treatment for the disease (Good and Duignan, 2011). There are two types of tuberculin skin test used today, namely, (1) the simple intradermal test (SIT), and (2) the comparative tuberculin test (CCT). The first cell-mediated response-based tests is conducted by the intradermal injection of a purified protein derivate (PPD) of *M. bovis* AN5 strain in the neck or the base of the tail (caudal fold) of the animal. A detectable increase (>2 or 4 mm) in skin-fold thickness at is maximal time reaction, at 72-hours post injection, is considered as a positive reaction

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(Monaghan et al., 1994). Although, bovine PPD is described as "purified", there is a complex mixture of components that are common to other mycobacterial species. CCT is used mostly to clarify the BTB-positive status after animals showed a reaction to the SIT and differentiate cross-reactivity resulting from exposure to other environmental mycobacteria. Towards that end, bovine tuberculin and avian tuberculin are injected intradermally in the neck of the animal in sites 12.5 cm apart. A positive result is computed when the skin thickness at the bovine injection site is 4 mm greater than the avian skin thickness (Monaghan et al., 1994).

Skin test diagnostic accuracy have been reviewed for the USA by Farnhan and others (Farnham et al., 2012). CFT sensitivity was estimated to range between 80.3 to 90.4%, and specificity between 89.2 and 95.2%. An increase in test specificity was reported for CFT-CCT combined (97.3-98.6%), but in detriment of the sensitivity values (74.4-88.4%). However, estimates from the European Union described lower values for CCT with mean values between 41 and 69% for the standard and severe interpretation respectively (European Food Safety Authority, 2012). Even tuberculin skin test remains as the most important diagnostic tool and PPD reagent is broadly used in livestock, its composition, and potency characteristics have been poorly described and have marked dissimilarities between manufacturers (Dobbelaer et al., 1983). In addition, different PPD inoculation sites can also affect the result of the test (Suther et al., 1974; Casal et al., 2015).

For BTB, the isolation of the agent from the tubercles is frequently considered as the "gold standard" technique to confirm infection; however, this technique is low sensitive, because the numerous stages of the disease lead to failure of detection (Gormley et al., 2014). Thus, finding at slaughter or post-mortem inspection of the typical tubercles has sensitivity limitations. It can be misdiagnosed with other granulomatous diseases (i.e. *Actynomices bovis*), and numerous studies report a detection of less than 50% of the presumptively infected animals (Álvarez et al., 2012a; Garcia-Saenz et al., 2015; Von Gehlen, 2015). Despite this limitation, inspection at abattoirs, to detect BTB-like lesions, followed by confirmation through PCR or culture has been an essential component of passive surveillance programs in every country attempting to control or even eradicate BTB.

Measures applied to reduce BTB prevalence in cattle has led to variable and inconsistent results in different countries. Reasons for such variation may include, for example, differences in the sources of infection such as wildlife (Fitzgerald and Kaneene, 2013; McCluskey et al., 2014) and domestic reservoirs (Guta et al., 2014; Pesciaroli et al., 2014); or social aspects, such as weak or nil awareness about the disease in stakeholders (Pfeiffer, 2013). Despite funds and efforts invested worldwide, disease eradication is still challenging in developed and developing countries, with Australia being the only documented example in which the disease has been eradicated (More et al., 2015).

1.4 Uruguay: the country and bovine tuberculosis

In numerous South American countries, BTB is considered a re-emerging disease, acknowledging this increase in incidence to variations in types of animal production, farms management, interactions between different species, and natural ecological changes (Proano-Perez et al., 2009). The prevalence and distribution of BTB Control programs implemented by countries in the South American Southern Cone include test and cull, disease free herd certifications, and slaughter surveillance (Max et al., 2011), targeting to reduce, control or eradicate the disease.

The Eastern Republic of Uruguay is a Latin American country, located in the Southern cone of South America, with an extension of 175.020 sq. km (Figure 1.1). The country is divided into 19 departments, each of which is divided into counties that are subsequently divided into the smallest administrative unit, referred to as "local units" (LUs). The vast majority of the area of Uruguay is utilized for agriculture production. According to last annual statistics, agro-industrial production accounts for 11.2 % of the gross domestic product (GDP) of the country, and 47.4% of agriculture GDP comes from livestock-related activities (DIEA, 2015).

Cattle production includes more than 45,000 farms and ~12 million head of bovine raised extensively, based mostly on grazing in a country with ~3.4 million inhabitants, which stress even more the importance of this industry. Farm size varies from 17,000 to <10 head of cattle for each farm with a Median of 76 animals per farm.

The type of production has been characterized by the Ministry of Livestock, Agriculture, and Fisheries in three basic types, namely, beef, dairy, and other (cattle production combine with agriculture). For the purpose of the study here I am considering only beef and dairy, according to different managements, production, and BTB-test and surveillance procedures.

History of bovine tuberculosis control programs in Uruguay

Uruguay was one of the pioneers in the fight against BTB worldwide, with activities starting as early as in the late 1800's, whereas in other countries, such as the USA (Bruning-Fann et al., 1998), New Zealand (Livingstone et al., 2015), or the UK and Ireland (Abernethy et al., 2013; Robinson, 2015), control and/or eradication programs started in the earlies 1900's,

Uruguay BTB control history and antecedents in cattle started in 1888, when Teodoro Visaires identified, rejected, and separated two sick animals (with lesions in lungs that later would be referred to as tubercles) from a dairy herd in Uruguay. Nine years later, in Montevideo (the capital city of the country), the first regulations addressing disease control of, and animal introductions into a dairy farm were dictated. The regulations demanded clinical inspection and skin testing of dairy animals in government facilities prior to entering into a dairy farm.

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In 1902, in addition to clinical inspection, veterinary authorization and skin test previous to the purchase of any dairy animal was made compulsory, as a result of the high percent (14,51%) of BTB-positive animals detected in Montevideo. Skin-test-positive animals with clinical signs were culled, and those without clinical presentation of disease, were identified in the neck and rejected to be included in a dairy herd, with compensation to the owners.

On April 13, 1910, the main Uruguayan animal health law was approved, given responsibilities and duties to the official veterinary service regarding animal diseases. That law (referred as order number 3.606) included bovine tuberculosis as a redhibitory disease, with compensation within the first 30-days post-purchase. Eight years later (1918), tuberculin test was declared mandatory in all dairy animals (>6 months) from farms located in urban areas or those who industrialized milk, and the skin test was made part of private veterinarians' duty.

Since 1941, a test-and-cull based program was approved for all dairy farms in Uruguay with incentives to those farmers with high health quality milk production; this legal frame led to the implementation of the current BTB control program in Uruguay.

Current Bovine tuberculosis control program in Uruguay

Current BTB control program in Uruguay is based on the Official Tuberculosis Free Herd declaration act. Test-and-slaughter strategies are based on the use of tuberculin skin test, and additional carcasses inspection at the slaughter plants.

Currently, all dairy >12 month animals are tested annually using the Caudal Fold Test (CFT) by accredited private veterinarians. Positive or inconclusive reactors in the CFT test are re-tested within 10 days, using the comparative intradermal tuberculin (CCT) test performed in the cervical area. In Uruguay, the CCT test is implemented by official veterinary services to differentiate cross-reactivity resulting from exposure to other environmental mycobacteria. Animals with a >4 mm increase in the skin fold thickness of the bovine PPD inoculation site, compared to the avian PPD inoculation site, are then considered reactors, leading to slaughtering of the reactor. The affected herd is consequently considered as an outbreak (or BTB-positive farm) and all animal movements in the herd are restricted until two negative CFT test results are obtained between minimum and maximum intervals of size and twelve months, respectively. Additionally, non-dairy cattle moved into markets or animal quarantine areas are also tested using the CFT test, prior to movement.

Once a herd is declared BTB-positive, an epidemiological investigation is conducted. Neighboring (sharing-fences) farms are tested and all movements within two

years are traced back to identify possible sources of infection or spread. Farms linked or associated with the outbreak are also tested using the CFT.

All CCT-positive animals are slaughtered and carcasses are inspected by the official veterinary services. All lesions are submitted to the National Veterinary Diagnostic Laboratory (DILAVE) for identification or isolation of the *M. bovis*. When infection is confirmed, the herd of origin is declared as an outbreak (or BTB-positive farm) and the procedure described above is followed.

Epidemiology of BTB in Uruguay

Historically, BTB has been linked to dairy production in Uruguay, mainly because this type of production was the only one with stocked animals giving the optimal conditions (close contact) for *M. bovis* to spread and maintain in the herd (Casas Olascoaga, 2013).

BTB prevalence in Uruguay has declined according to MGAP records after a relatively high prevalent start. Data obtained from 1937-1939 shows that 0.64% of the carcasses slaughtered and inspected at the time (~2.8 million) revealed BTB-like lesions. This number increased between 1943 and 1946 to 1.04% when replacement cows from dairy farms were included in the reports. Data on skin test results for a similar period (1942 to 1947) showed a total of 11.6% of reactive dairy animals at that time. However, the prevalence of BTB decreased markedly to less than 1% of positive cattle in the 1990s

(Gil, 2014 oral communication), and less than 11 positive-farms within ~4.500, in 2010 ((WAHIS_OIE, 2014) (Figure 1.2).

After this successful program, which suggested the potential for disease eradication, an unexpected change in BTB trend was observed. The mean number of annual outbreaks in the 2011-2013 periods duplicated the mean value observed in the previous decade as shown in figure 1.2. That increase was not only observed in the number of outbreaks, but also, the prevalence within farm was more than 20 times higher than the average reported between 2005 and 2010. The increment in BTB-positive farms, although does not represent a high growth in country prevalence, resulted in a rise of costs and resources focused on the control of BTB. For that reason, it represents an opportunity to review and ultimately improve the BTB-control program in Uruguay.

1.5 Figures

Figure 1.1: Location and administrative division of Uruguay.

Figure 1.2: Bovine tuberculosis positive farms reported per year in Uruguay (2003-2014).
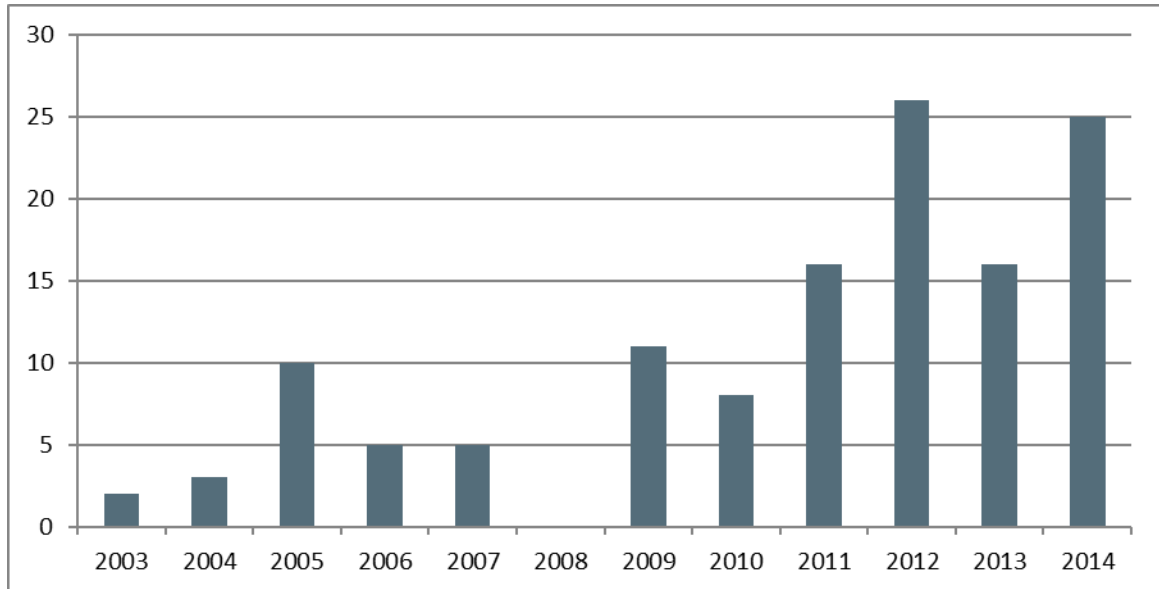
CHAPTER 2 – SPATIAL CLUSTERING OF BOVINE TUBERCULOSIS IN URUGUAY (2011-2013): IDENTIFICATION OF HIGHLY PREVALENT AREAS

2.1 Chapter summary

The goal of this chapter was to assess the spatial dynamics of BTB outbreaks reported in Uruguay between 2011 and 2013. Data provided by the Uruguayan Ministry of Livestock Agriculture and Fisheries included information on the location (latitude, longitude), status (positive, negative), and, if applicable, outbreak onset for cattle farms in the country. Fifteen, 26, and 16 outbreaks were reported in 42759, 43213, and 42186 susceptible dairy farms in 2011, 2012, and 2013, respectively. The spatial distribution of incident (new cases) outbreaks was assessed using the spatial scan statistic and the Cuzick-and-Edwards (CE) test, implemented in SatScan and Cluster Seer software, respectively. Significant (P<=0.05) high-risk clusters were detected in southwestern (2011, 2012, 2013), northwestern (2012), and southeastern (2012) Uruguay. In 2012, significant clustering at the second order of neighborhood was detected using the CE test. Presence of spatial aggregation in southwestern Uruguay consistently in 2011 and 2012 corresponding to a dairy production land-use, suggests an association between land management and BTB risk. Global clustering detected in 2012 might revealed local dissemination of BTB. In addition, the increase of BTB incidence in disparate regions of the country suggests that live animal movements (farm connectivity) may have played a role in spreading the disease in farther areas. The spatial characterization here contributed to understand the dynamics of BTB transmission in Uruguay, and, ultimately, to improve the effectiveness of the BTB control program in the country.

2.2 Introduction:

Spatial analysis tools may help to elucidate underlying epidemiological patterns for disease spread, identify high risk areas for disease, and, ultimately, implement mitigation and control strategies intended to prevent or reduce the impact of the disease. Since John Snow's work early in 1855, aimed at mapping cholera cases to study the patterns of the disease distribution, spatial analysis and Geographic Information Systems (GIS) have been keys at the time of visualizing epidemiological patterns, disease spread, and cluster detection in veterinary epidemiology.

In animal diseases, cases that tend to be less disperse in space (therefore more clustered), are more likely to have similar characteristics (environment, production, economy) than those far away located. At the same time, the site where the cases are grouped or aggregated might be a source of high risk for the disease under study. The study of cluster-site characteristics is essential to understand disease spread and patterns, and generate and test hypothesis for disease causation. Identification of clusters of disease could help to define areas for active surveillance and to identify factors associated with high disease incidence or prevalence.

Fundaments of cluster analyses techniques have been described, for example, by Ward and Capenter (2000). Basically, spatial analyses driven to detect spatial aggregation of cases (clusters) can be classified in three main groups: (a) global-cluster tests, (b) focused-cluster tests, and (c) cluster-detection tests. The global-cluster and cluster-

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detection test have been extensively used in veterinary medicine for more than thirty years; some examples include Hennebelle et al. (2013) assessment of spatial patterns of dogs Leptospirosis in California, Alvarez et al. (2012b) exploration of Coxiella burnetii in cattle in Spain, and Ekong et al. (2012) analysis of high pathogenic avian influenza outbreaks in poultry in Nigeria, and West Nile Virus in horses in Indiana, USA Ward et al. (2004). However, the focused-cluster tests have not been preferred for veterinary epidemiology, and I did not identify any report in the veterinary medicine peer reviewed literature.

Spatial epidemiological tools have helped to identify BTB distribution and epidemiological patterns worldwide. For example, spatial analysis helped to suggest an association between disease and areas related to dairy farms in Argentina (Perez et al., 2002), identify clusters in high prevalence counties as Madrid, Spain (de la Cruz et al., 2014), and categorize high and low risk areas based on historical spatial patterns in BTB-free status countries as France (Bekara et al., 2016).

In the chapter here, a global-cluster test and a cluster-detection test were selected to assess the spatial dynamics of BTB outbreaks reported in Uruguay between 2011 and 2013, and identify spatial clusters and geographical association between positive farms, to ultimately assess, in next chapters, if spatial clustering was associated with the presence of specific epidemiological factors that promoted or prevented disease occurrence in the country.

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2.3 Methods

Data source and case definition

Location, demographic characteristics, and BTB status (positive/negative), based on the animal health program described before, of all farms in Uruguay during the study period were provided by the Animal Health Department at the Ministry of Livestock Agriculture & Fisheries of Uruguay (MGAP) (figure 2.1). Due to confidentiality concerns, farms were de-identified in the database by eliminating their unique ID number.

Geolocations (Universe Transverse Mercator coordinates) were provided at the local unit (LU) level, the smallest administrative unit of the country. LUs aggregate into counties (n=261) and departments (n=19). Typically, a farm will take up one LU, but may also extend over more than one.

Data analyses

Farms were assumed to be located at the centroid of the LUL; an average centroid was computed for farms geographically extended over more than one LUL.

Two tests for detection of spatial clusters were used, namely, the Cuzick-and-Edwards' test for inhomogeneous populations (Cuzick and Edwards, 1990), and the spatial scan statistic test (Kulldorff and Nagarwalla, 1995), which were implemented

using ClusterSeer software (version 2.5) and SaTScan software v9.3

(http://www.satscan.org/) respectively.

Briefly, the Cuzick-and-Edwards' test is a global-cluster test useful when an exact location of the unit of analysis is known, and makes no assumption on the distribution of the underlying population. Cuzick-and-Edwards' test detects case space aggregation and at which level of neighborhood the cluster exist. The test compares the sum of the observed cases that are nearest neighbors to another case at different levels of neighborhood (h), with the expected cases assuming a random distribution of cases and controls. For this test, we assume that cases and controls are included in the same population. For each location z_i, exists k nearest neighbors, were d^k_i has a value of one if the neighbor corresponds to an infected farm and zero if is a free of disease farm. The formula $T_k = \Sigma^k_{i=1} \delta_i d^k_i$ gives the T statistic value, were δ_i is one if the z_i location matches with an outbreak and zero if not. When high numbers of farms closer to a case are also cases, T_k value tends to be large and we might presume that large clusters with few farms included will be more powerful than small clusters with numerous farms. Cuzick-and-Edwards' leaves to researcher's criteria definition of neighborhood order (k) to be analyze, for instance if $k=3$ we want to study the third closest farm to a case z_1, d^3_1 will be equal to 1 if is a positive farm and zero if is a control, so $\delta_1 d^3_1 = 1$. However, if the farm of origin is a control then $\delta_1 d^3_1 = 0$. Monte Carlo simulations (999) are adjusted

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for every k, generating a p-value of the comparison between observed T_k and expected by chance after simulation.

The Bernoulli model of the spatial scan statistics is a cluster-detection test to identify significant geographical aggregations of cases. The test delimits a circular window of variable sizes on a map where all the farms under study are situated, and allocates its centroid in all possible locations. Afterwards, the test compares the observed number of cases and controls within each window with the expected assuming a random distribution of cases and controls. Centroid and size of the window changes randomly, from zero to 50% of the population at risk. A Bernoulli distribution is adjusted considering the incidence of the disease per year, with a mean value equal to the observed incidence. After thousands of randomly distributed simulations over the windows, an estimation of expected cases under the Bernoulli distribution is compared to the observed cases. Higher reported cases than expected define a spatial cluster. Expected distributions for both the Cuzick-Edward's test and the spatial scan statistics were generated via 999 Monte Carlo simulations in which case and control labels were assigned randomly to each location.

ArcGIS software 10.2.2 (Redlands, CA, US) was used for visualization of the farm distribution and the results of the spatial analyses.

2.4 Results

Descriptive results:

The total number of farms with complete information in all datasets included for the analyses was 42,759 (2011), 43,213 (2012), and 42,186 (2013), whereas 2,884 (6.3 %), 2,392 (5.2 %), and 1,971 (4.5%) farms were removed from the analysis due to missing data on each year, respectively. The number of BTB outbreaks detected each year was 16 (2011), 26 (2012), and 16 (2013), for an annual cumulative incidence of 4, 6 and 4 herds per 10,000 each year, respectively. BTB-positive farms were located in eleven of the nineteen departments of the country (Figure 2.2). A high proportion of positive cases were detected in dairies (57/58) and all of them by active (CCT testing) surveillance.

Cluster detection results:

Significant ($P<0.02$) clustering of positive herds up to the second level of neighborhood was detected with the Cuzick-and-Edward's test in 2012, whereas no spatial pattern was observed on any of the other two years. In contrast, the Bernoulli model of the spatial scan statistic test identified significant clusters in the three years of study (Figure 2.3). In 2011, one single large cluster (102 km radius) was detected, located in the southwestern area of the country and including seven departments and the 16 BTB-positive farms detected that year. In 2012, three small (radio <42 km) clusters were detected ($P<0.05$), located at the southwestern, northwestern, and southeastern regions of

the country, and including 2, 4, and 7 outbreak farms respectively. Finally, a large (radius=235 km) cluster in southwestern Uruguay, including 15 of the 16 cases detected in the year, was observed in 2013 (table 2.1).

2.5 Discussion

BTB eradication is still challenging in most of the countries with a significant cattle population. Despite the increase in annual BTB-positive farms reported in Uruguay between 2011-2013 compared with previous years, prevalence in the country was lower than what has been described in other countries endemically infected in which similar test-and-cull strategies are in place (Abernethy et al., 2013; de la Cruz et al., 2014; Livingstone et al., 2015).

The use of spatial tools to understand BTB distribution in different animal species has been described previously in several regions of the world (Perez et al., 2002; Boadella et al., 2011; de la Cruz et al., 2014; Bekara et al., 2016), but consider effect of local characteristics (cattle production systems, farm connectivity, presence of wildlife, control strategies, etc.) is essential to understand differences in the dynamics of disease spread. Our study is the first BTB spatial analysis in the South American south cone using farm-level information collected at the farm level through multiple years.

To avoid possible biases as a result of different attitudes toward risk from farmers (e.g. depopulation), data from each of the three years were analyzed independently;

therefore, only new positive farms reported each year were considered BTB-positive farms. Misclassification bias due to limited sensitivity of tests used for BTB detection (CFT, CCT, slaughter detection) (Monaghan et al., 1994; Farnham et al., 2012; Álvarez et al., 2012a; Garcia-Saenz et al., 2015; Von Gehlen, 2015) was assumed to be non-differential across years.

Combination of the CE test and Bernoulli spatial scan statistics provides information aiming to prove significant spatial aggregation and complementary, which geographic locations are responsible for those clusters identified.

The Cuzick-and-Edwards' method has been described and used for more than twenty years now in different veterinary medicine fields, (Ekong et al., 2012; Alvarez et al., 2012b; Hennebelle et al., 2013) , and was particularly suited for our data due to the heterogeneous distribution of cattle production types in Uruguay. Uruguayan cattle production as mentioned before is mostly based on grazing, so the majority of Uruguay lands are occupied by cattle with a heterogeneous spatial distribution.

The only year in which significant clustering was detected in the Cuzick-and-Edwards' test was 2012 (2nd level of neighborhood), suggesting BTB-positive farms tended to be closer to other infected farms. This pattern was not identified in the previous and following years, which could be due to variability in factors affecting between-farm animal contacts.

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Bernoulli spatial scan statistics was assessed using circular windows considered appropriate to Uruguay shape and to reduce as much as possible edge effects bias. Maximum window sizes were set varying from zero to 50% of population at risk because evaluating high risk areas inside a window that encompasses up to 50% of the population at risk is equal to assessing for low risk areas outside the window and vice versa (Kulldorff, 1998). Significant (P<0.04) BTB clusters with high incidence identified had different locations for each year (Figure 2.3). This pattern may be counterintuitive if considering possible localized persistence and transmission of the disease, but may be a result of the high network connectivity of a relatively low number of farms. Such "super-spreading" farms thus could spread the disease to different regions if infected, as described for Uruguay by VanderWaal et al. (2016) & VanderWaal et al. (submitted).

The cluster identified in 2011 was located in an area with high dairy production (DIEA, 2015) whereas the large clusters in 2012 included half of the 19 Uruguayan departments, suggesting multiple introductions through long-distance transmission factors, such as animal movements (VanderWaal et al., 2016). Cluster detected in 2013 should be interpreted carefully since could be the product of the parameters set for the spatial scan statistics test (upper limit for maximum cluster size including up to 50% of population at risk).

Presence of local clusters in 2011 and 2013 when global aggregation is not significant, may be effect of "BTB wells" that could not have adequate population size or connectivity to represent higher risk to its neighbors.

Cluster sensitivity analysis accounting for window size, case (incident vs prevalent) and control population (dairies vs beef) was evaluated obtaining similar cluster locations as results presented above, for that reason they were not presented in this manuscript.

In conclusion, this chapter shows the spatial evolution of BTB incidence in Uruguay between 2011 and 2013. This results show inconsistent clusters over the three years, but a two-year steady high risk region at south-west correspondent to agriculture and dairy production suggests association between land management production and BTB risk. Local transmission may be revealed by global significant statistics in 2012 period in addition to the role that farm connectivity could play (live animal movements) to spread the disease to farther areas of the country the same year. While BTB has been proven difficult to eradicate since more than a century, spatial characterization of recent outbreaks may contribute to understand disease transmission and also being an input to characterize risk factors involved in these outbreaks to improve effectiveness of the BTB control program in Uruguay and reduce incidence in the region.

2.6 Figures

Figure 2.1: Total cattle farms location in Uruguay.
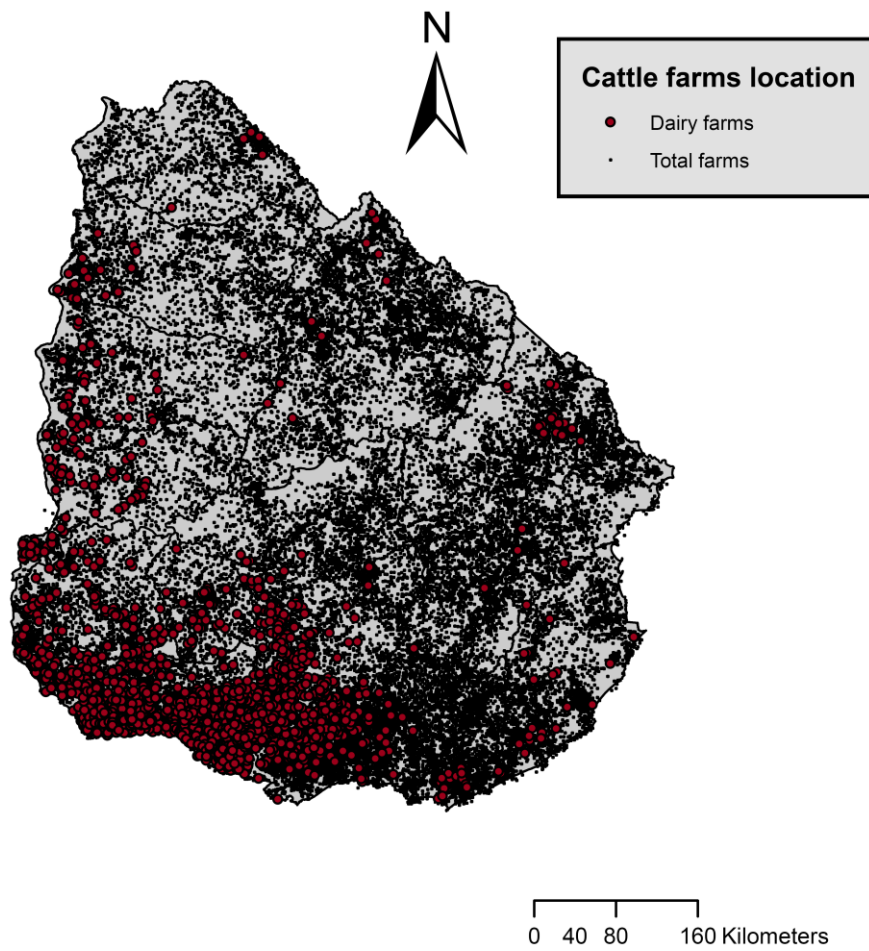
Figure 2.2: BTB-positive farms reported and recorded from the Animal Health Bureau, (MGAP) in 2011 (+), 2012 (▲) and 2013 (*).
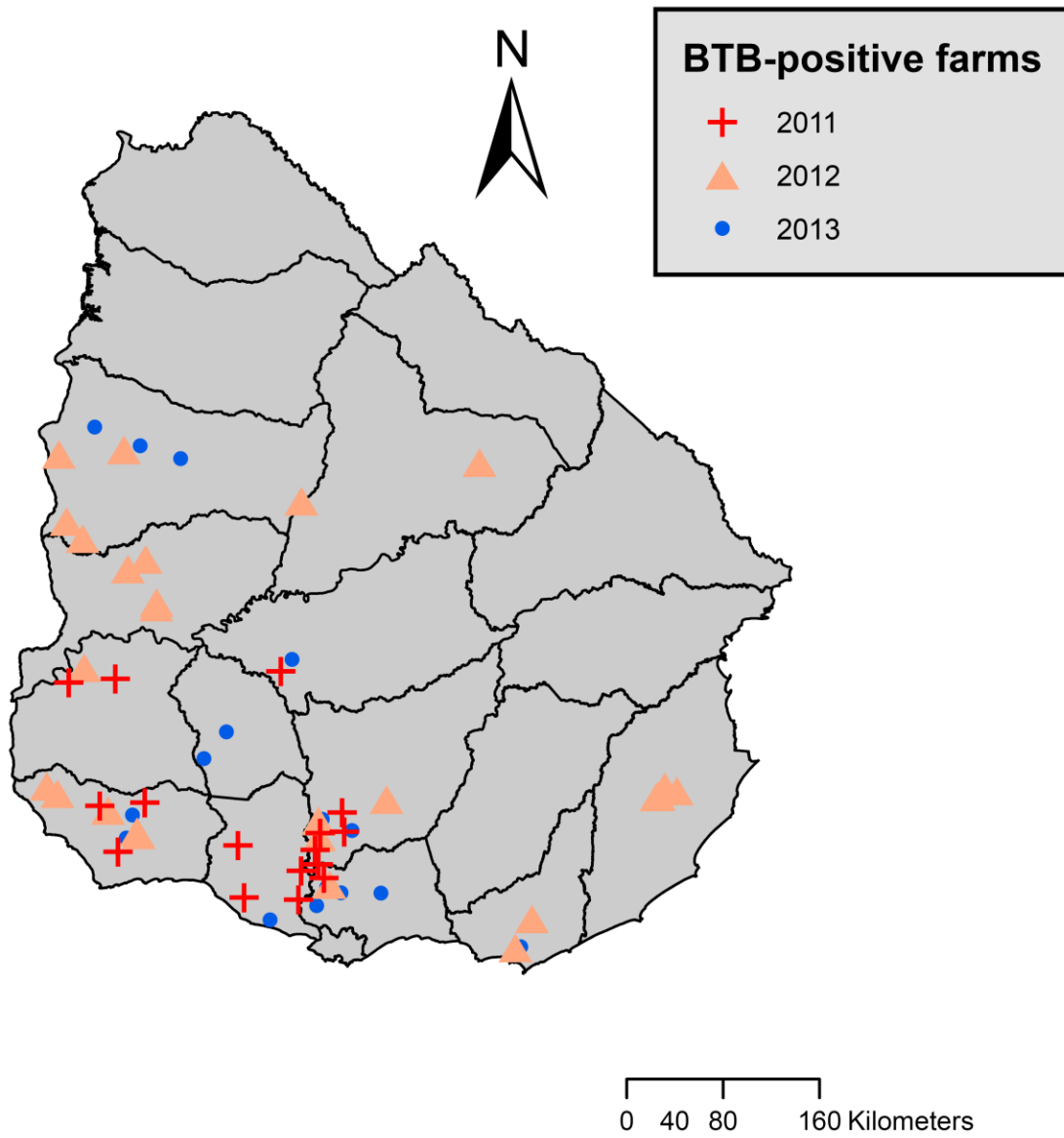
Figure 2.3: BTB spatial clusters detected in Uruguay per year of study, 2011(yellow), 2012(lilac) and 2013(blue).
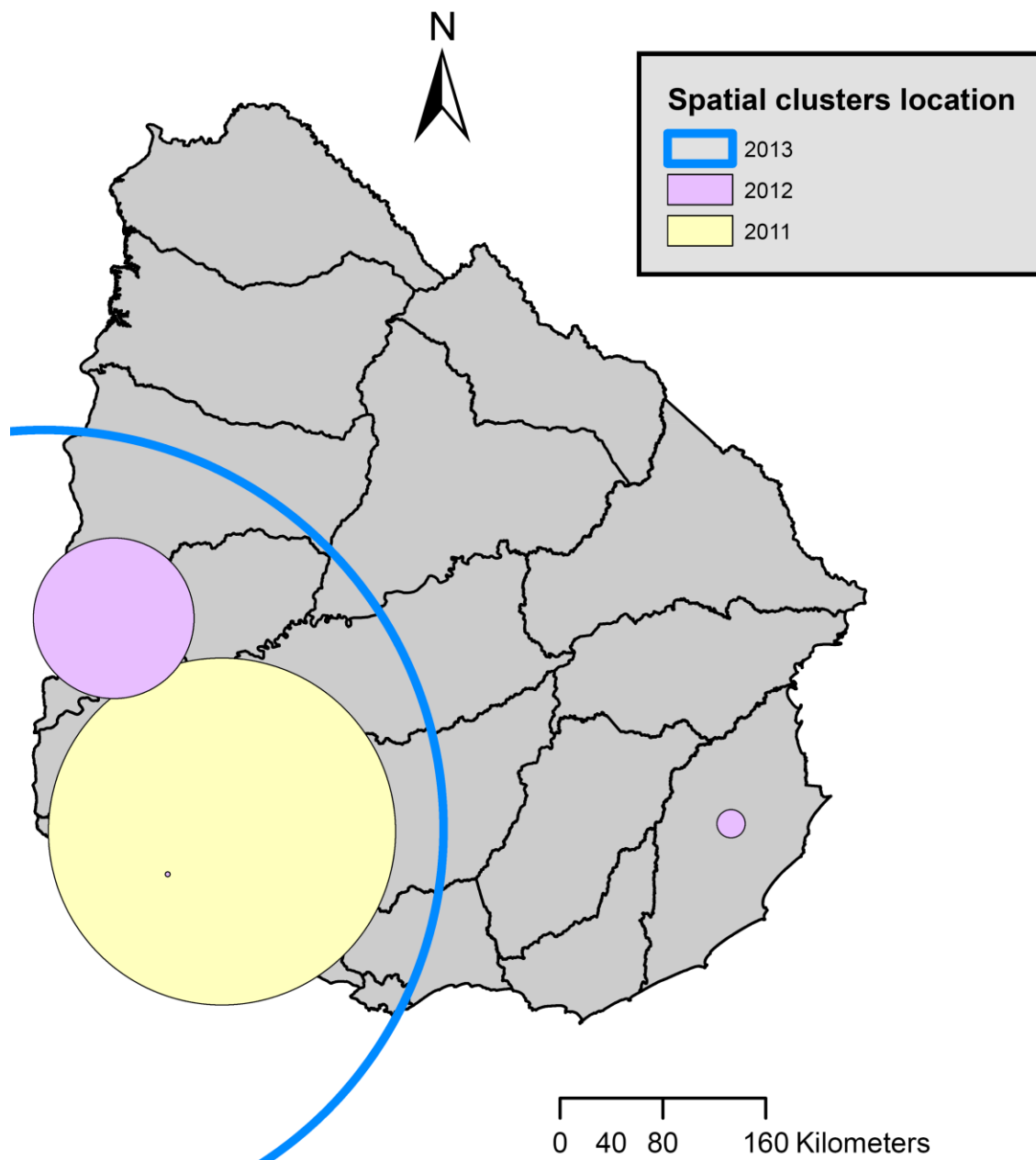
2.7 Tables

Table 2.1:Cluster results from the Bernoulli model of the spatial scan statistic for BTB-positive farms (2011, 2012 and 2013).

Year	Cluster	BTB-farms in cluster/total*	Cluster location (UTM)	Radius (km)	Obs-to-Exp ratio	RR	P-value
2011	1	16 /16	6245740, 476808	102.9	4.7	∞	<0.001
2012	1	7/26	6250320, 776662	8.3	443.5	52	<0.001
	2	4/26		47.3	15.1	3.9	0.016
	3	2/26	6371400, 413092	1.5	1108.7	4	0.031
			6220520, 444885				
2013	1	15/16	6247350, 372388	234.9	2.6	27.1	0.036

*Number of BTB-positive farms included in the cluster out of the total BTB-positive farms reported in each specific year.

CHAPTER 3 – RISK FACTORS ASSOCIATED WITH BTB-POSITIVE DAIRY FARMS IN URUGUAY (2011-2013)

3.1 Chapter summary

Between 2011 and 2013, BTB-positive farms increased in Uruguay, a country with an important agricultural-based economy. Results from spatial clustering assessment showed before, highlighted a high steady area of BTB risk at the southwestern region of Uruguay. This land area in majority relates to dairy cattle production. In addition, aggregation of cases was detected in disparate regions of Uruguay during 2012 suggesting that farm connectivity (animal movements) could be playing a role spreading disease in numerous areas of the country. The goal of this study was to assess the association between BTB and factors related to farm-demography and connectivity (movement factors) in the Uruguayan dairy industry between 2011 and 2013. A subset of data-set provided by the Ministry of Livestock, Agriculture, and Fisheries of Uruguay including all dairy farms was assessed with a conditional multivariate logistic regression model to investigate the risk factors most likely associated with BTB, with three controls (171) per positive farm (57). BTB-negative dairy farms during the three years of study, and nearest neighbors of a case were selected as controls accounting for spatial dependency. Ten demographic factors were assessed; including herd size (total number of cattle), age and gender demographics of animals in the herd, and presence of sheep in the farm for each year. In addition, fifty-one movement epidemiological factors were evaluated as possible risk factors, including incoming movements (categorized by origin and animal category) and network centrality index. The final model included large herds,

high numbers of animals purchased (>44 cattle), and incoming steers as factors increasing the odds of breaking with BTB. The first two factors were in agreement with previous studies, and the association with incoming steers may suggests management factors for which this variable may serve as proxy. Results from this study will contribute to the design and implementation of disease management strategies intended to enhance the effectiveness of BTB control programs in dairy farms in Uruguay.

3.2 Introduction

Epidemiological factors associated with bovine tuberculosis in cattle

Understanding of BTB transmission and relation between infection, disease, and infectious animals is important to address epidemiological factors associated with disease prevalence. Transmission of BTB have been described to occur most likely by aerosol route, via "direct" contact between animals in close proximity (Bourne, 2007; Skuce et al., 2012); although, less important route can include oral ingestion.

Given the plausibility of transmission routes, different studies aiming to assess and quantify epidemiological factors associated with higher risk of BTB have been done worldwide. BTB has been found to be associated with individual factors such as age, gender, breed, body condition, and immune status (Cleaveland et al., 2007; Inangolet et al., 2008; Belchior et al., 2015; O'Hagan et al., 2015). Also herd-level factors including herd size (Johnston et al., 2011; Winkler and Mathews, 2015), and type of production and

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management (Griffin et al., 1993; Johnston et al., 2005; Álvarez et al., 2014; Bekara et al., 2016) were related with BTB. Other factors have been extensively explore such as presence of wildlife (Fitzgerald and Kaneene, 2013; McCluskey et al., 2014) or domestic reservoirs (Kaneene et al., 2010; Boadella et al., 2011; Zanardi et al., 2013; Guta et al., 2014; Pesciaroli et al., 2014). Previous analyses highlighted animal movements as an important factor in the maintenance and spread of BTB in a population (Gilbert et al., 2005; Clegg et al., 2013; Ribeiro-Lima et al., 2015; Vial et al., 2015), and the existence of higher risk movement-categories has been hypothesized (Gilbert et al., 2005).

The aim of this chapter was to assess the association between BTB and demographic and movement factors in the dairy cattle industry of Uruguay. Results will contribute to the design and implementation of disease management strategies intended to a better allocation of resources for enhance the effectiveness of BTB control programs in Uruguay.

3.3 Methods

Data source and case definition

A subset of the Uruguayan data-base provided by the MGAP and described in chapter section 2.2 was used for this analysis. BTB-positive farms were defined based on the Uruguayan control program described before. Onset date (defined as the date when the outbreak was confirmed) was also recorded for BTB-positive farms.

In addition, individual information on movements from/to all farms (between-farm) during the 2008 to 2013 period was also provided by the MGAP (Directory of Animal Identification System, SIRA).

Demographics epidemiological factors

Regarding demographic characteristics, farms included in the study were sub-classified in six production types as described elsewhere (VanderWaal et al., 2016) (dairy farms and five sub-categories of beef production types): farms with <5 animals in the premises were considered small farms; farms with >5 animals were categorized based on the steers/breeding cow ratio (Molina, 2012) in breeding (ratio <0.3), fattening (ratio >1.0), and complete cycle (ratio 0.3-1.0) farms. The remaining type of production including solely heifers and/or calves was classified as growing production. This classification was used to assess network characteristics at farm-level.

Herd size (total number of cattle), age and gender demographics of cattle in the herd [calves, heifers1-2 years, heifers 2-3 years, steers 1-2 years, steers 2-3 years, and bulls, fattening and breeding cows (all above 3 years)], and presence of sheep in the farm (yes/no) were additional attributes included in the demographic dataset for each year.

Farm-level network characteristics

Network characteristics were determined based on the movements carried during the three previous years of the BTB onset-date. Number of contacts, defined as total

number of unique farms from which the focal farm received animals in a given year (i.e. in-degree), and total incoming number of animals into the focal farm differentiated by production type and animal category in a given year were assessed.

Network centrality metrics were also included in the analyses. The number of directed paths passing through the focal node when the shortest path is traced between all other pairs of nodes (i.e. betweenness), and the estimation on how closely connected by incoming movements a focal farm is to all other farms in the network (i.e. in-closeness) (Martinez-Lopez et al., 2009) were quantified. The temporal sequence of edges was ignored (Martínez-López et al., 2009; VanderWaal et al., 2016). Origin of the incoming contacts, and animals sent to the dairy farms under study are shown in figures 3.1 and 3.2.

Data analyses

All BTB-positive dairy farms (cases) and three controls (BTB-negative farms throughout the study period) per case matched by production (i.e., dairy farms) and location (closest dairies to the case farm) were included to investigate the factors that were most likely associated with an increased risk of BTB outbreak.

After selection, a formal assessment of spatial independence of BTB in the subpopulation included in the study was verified by running a third-order nearest-neighbor Cuzick-and-Edward's test and the Bernoulli model spatial scan statistics.

Variables from the demographic and between-farm movement datasets for the year in which the outbreak was detected were initially explored to evaluate the distribution and proportions of BTB-positive and negative farms for each variable in the study. Continuous variables were categorized in thirds or dichotomized (presence vs absence) based on their distribution. Due to the high amount of right skew and extreme assortment of farm-betweenness (a network centrality metric), it was ranked for better understanding and interpretation by giving the largest value to the highest betweenness index.

Strength of bivariate associations between BTB-status and epidemiological factors was estimated using a univariate conditional logistic regression. Covariates with P-value ≤0.2 were selected for potential inclusion in multivariate models, and a Spearman's correlation coefficient was used to assess collinearity between variables. If two variables were found to be correlated (Spearman's test>0.7), the one with the lowest AIC value in the univariate model was selected and included as main effect in the multivariate conditional regressions. The final model was selected using a backward stepwise elimination procedure (P>0.2 to be excluded from the model) and the lowest AIC value. Two-way interactions between variables retained in the final model were also assessed. Statistics analyses were conducted using the free software R (v3.1.0, R Foundation for Statistical Computing).

3.4 Results

A total of 57 BTB-positive dairy farms and three-matched controls per farm were included in the multivariate conditional logistic regression. No association between location and BTB status in the selected herds was detected using the CE and spatial scan statistics tests, suggesting that the matching strategy was successful in accounting for spatial dependence (figure 3.3). Ten demographic (based the classification mentioned in section 3.2) and 51 movement-related factors were initially assessed as factors potentially associated with the occurrence of BTB outbreaks in dairy farms. Twenty-nine between-farm factors with at least one movement within the category were kept for the analyses (table 3.1).

Fifteen variables were selected in the bivariate analysis due to their potential association (P<0.2) with BTB status, of which five were excluded due to correlation with other predictors with a lower AIC in a univariate model [animals incoming from fattening herds, incoming dairy calves, contacts introduced (total/dairy/non-dairy)], leaving 10 covariates for the multivariate stage (table 3.2).

The best-fit multivariate conditional logistic regression model included three epidemiological factors, namely, herd size, total incoming animals to the farm, and incoming steers (table 3.3). Two-way interactions between them were not significant.

3.5 Discussion

Herd size was associated with increased BTB risk at the farm level as described elsewhere (Griffin et al., 1996; Brooks-Pollock and Keeling, 2009; Bessell et al., 2012). Having more animals in the herd increased the risk for BTB, which may be explained by higher density at the paddock, longer periods of time waiting in the pre-milking area, or even if the density is not higher (because larger herds pasture in larger areas), there is more opportunities of contact fence-to-fence with other herds, increasing the likelihood of cattle-to-cattle transmission. Alternatively, considering that BTB test specificity is imperfect, the higher the herd size, the higher the chances of a false positive result, artificially increasing the herd-level prevalence and the likelihood for being designated as BTB-positive.

Number of animals moved into the farm was also associated with high risk of BTB-positive results in agreement with previous reports in the UK (Gilbert et al., 2005), northern Italy (Marangon et al., 1998), and Ireland (Clegg et al., 2013). This finding may be related to the introduction of false-negative infected animals into the herd due to the limited sensitivity of diagnostic tests.

Introduction of steers was also associated with a 2.88 (95% CI 1.12-7.37) increase in the odds of BTB. Interestingly, purchase of steers in dairy premises was not frequent (13% of farms under study), but the attributable fraction was 0.21. This association may be pointing at other management practices increasing the BTB risk, such as having at least some beef production in the farm, a practice that may be present in larger and non-

traditional dairy farm systems. Because beef animals are not routinely tested, mixing beef and dairy animals may result in an increase for disease maintenance and spread.

One potential limitation of the study is the time structure used in the analyses: each outbreak was assigned to one calendar year according to the onset date, and demographic information was also assessed per calendar year. However, cases reported at the beginning (January/February) of the year could be less related to the controls selected in the spatial/risk factor analysis for that specific year. Still, this situation only occurred in 5/57 outbreaks, and thus it is unlikely that had a major impact in our results.

The assessment of epidemiological factors associated with BTB was targeted to dairy farms in Uruguay. The exclusion of the only beef-positive farm in the data-set from the analysis was done to avoid possible confounding with known risk factors associated with BTB detection based on different beef/dairy production systems such as management (Menzies and Neill, 2000; Amanfu, 2006; Munyeme et al., 2008), animal movements (VanderWaal et al., 2016), culling rate (Humblet et al., 2009), and diagnostic test frequency in Uruguay. This could represent a possible limitation given dairy farms in Uruguay represent ~10% of the total farms in the country, leaving a high proportion of the cattle population unassessed that could be involved in BTB dynamics. Further studies are needed to address risk factors in the entire cattle population in Uruguay.

3.6 Figures

Figure 3.1:Boxplot of total incoming movements, and categorized by origin in dairy and non-dairy.
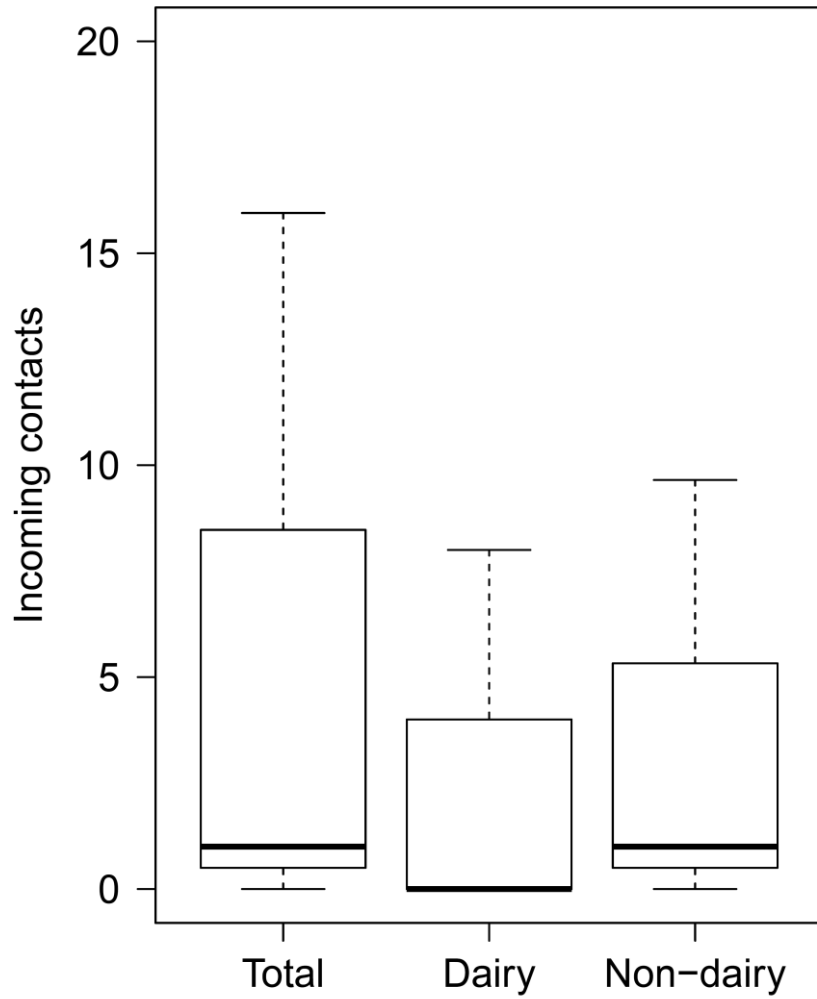
Figure 3.2: Number of incoming animals categorized by farm of origin according to

production type.
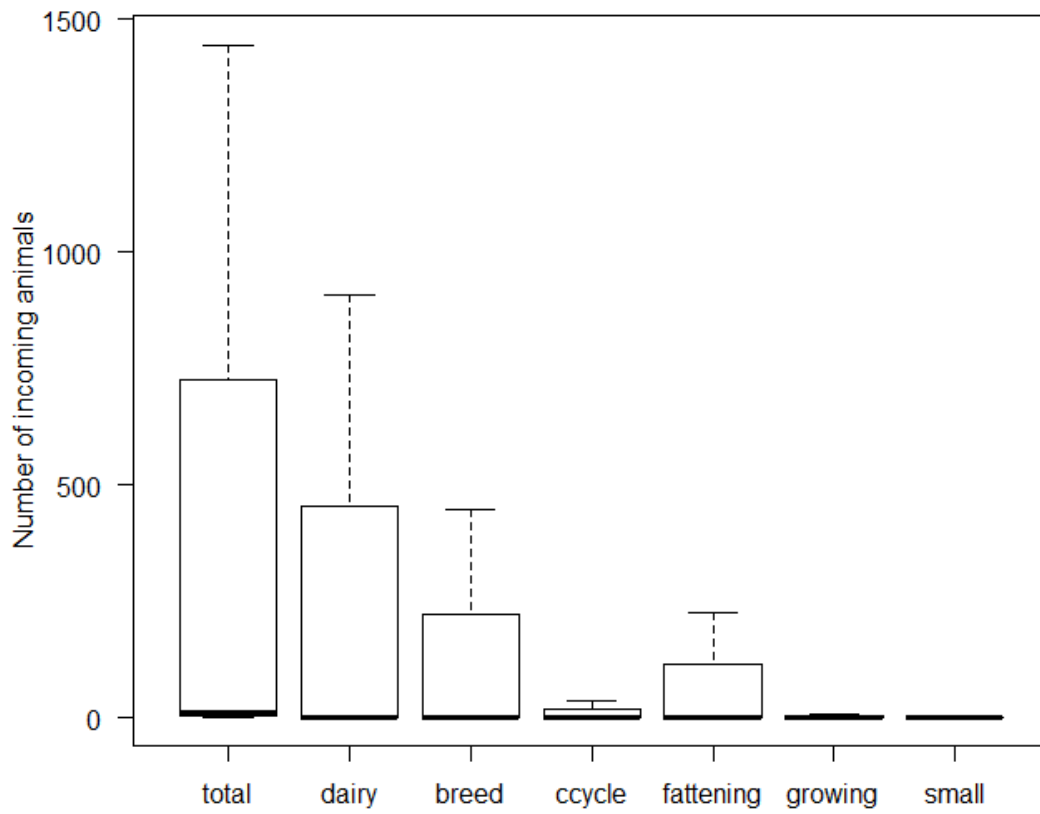
Figure 3.3: Location of the 57 dairy BTB-positive farms and 171 controls (i.e. BTB-negative farms) selected to assess high risk epidemiological factors with a conditional multivariate logistic regression model assessment.
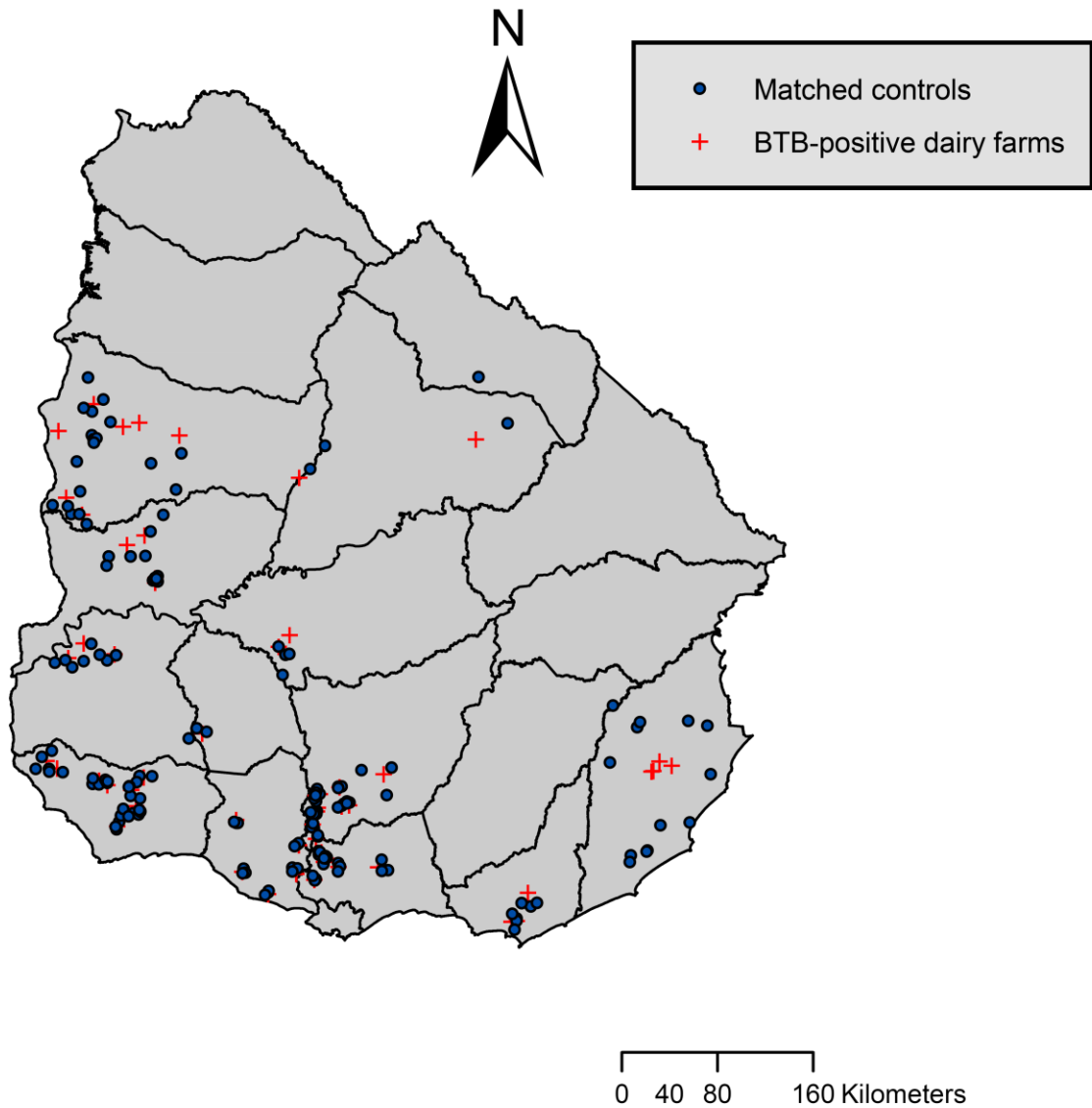
3.7 Tables

Table 3.1: Summary of movement risk variables assessed in this study. Sub-classification per origin is shown if total number of category-animals incoming to the focal farm was greater than zero.

Movement factor	Origin farm	Animal category	Levels
In-Degree			
Tot contacts introduced	Any	Any	Thirds
Contacts from dairy introduced	Dairy	Any	Thirds
Contacts from non-dairy farms introduced	Beef/Small	Any	Thirds
Total number of animals			
Tot animals entered	Any	Any	Thirds
Tot bulls entered	Any	Bulls	None
Tot b. cows entered	Any	Breed cows	None
Enter steers	Any	Steers	Binary
Tot heifers entered	Any	Heifers	None
Enter calves	Any	Calves	Binary
Tot animals d. entered	Dairy	Any	Thirds
Enter steers from dairy	Dairy	Steers	Binary
Enter calves from dairy	Dairy	Calves	Binary
Tot animals breed entered	Breeding	Any	Thirds

Enter steers from breed	Breeding	Steers	Binary
Enter calves from breed	Breeding	Calves	Binary
Enter animals from ccycle	Complete cycle	Any	Binary
Enter steers from ccycle	Complete cycle	Steers	Binary
Enter calves from ccycle	Complete cycle	Calves	Binary
Enter animals from fatten	Fattening	Any	Binary
Enter steers from fatten	Fattening	Steers	Binary
Enter calves from fatten	Fattening	Calves	Binary
Enter animals from grow	Growing	Any	Binary
Tot steers grow entered	Growing	Steers	None
Enter calves from grow	Growing	Calves	Binary
Enter animals from small	Small farm	Any	Binary
Enter steers from small	Small farm	Steers	Binary
Enter calves from small	Small farm	Calves	Binary

Network centrality metrics

Betweenness	na	na	Ranked
In-closeness	na	na	Cont.

Table 3.2: Epidemiological factors selected in the bivariate analysis (P<0.2) after exclusion of correlated factors.

Epidemiological Factor	Type of factor	Category	N	Cases (%)	Controls (%)	P-value
Incoming animals from Compete cycle farms	Movement	Yes	30	13 (23)	17 (10)	0.016
Incoming steers	Movement	Yes	30	18 (31.6)	12 (21)	<0.001
Incoming calves	Movement	Yes	78	28 (49.1)	50 (29.2)	0.007
Incoming calves from breeding farms	Movement	Yes	37	17 (28.9)	20 (11.7)	0.002
Incoming calves from complete cycle farms	Movement	Yes	14	6 (10.5)	8 (4.7)	0.120
Fattening cows in herd	Demographic	Yes	39	17 (29.8)	22 (12.9)	0.004
Steers in herd	Demographic	Yes	93	30 (52.6)	63 (36.8)	0.037
Total incoming animals	Movement	≤1	78	34 (59.6)	44 (25.7)	<0.001
		>1-≤44	71	10 (17.5)	61 (35.7)	
		>44	79	13 (22.9)	66 (38.6)	
Herd size (cattle)	Demographic	<116	76	3 (5.3)	73 (42.7)	<0.001
		116-360	73	16 (28)	57 (33.3)	
		>360	79	38 (66.7)	41 (24)	
Total number of bulls in herd	Demographic	None	75	14 (24.6)	61 (35.7)	0.005
		≤2	102	21 (36.8)	81 (47.3)	
		>2	51	22 (38.6)	29 (17)	

Betweenness (ranked)	Net. centrality	Ranked	N/A	0.005
In-closeness	Net. centrality	Continuous	N/A	0.055

Percentages (%) are based on the total number of BTB-farms (57), and 171 matched controls.

Table 3.3:Odds Ratio (OR) with their 95% confidence interval (95% CI) and P-value of the variables selected by the multivariate conditional logistic regression model.

Variable	Type of factor	Category(N)	OR	95% CI	P-value
Herd size (cattle)	Demographic	<116 (76)	1.00		
		116-360 (73)	5.79	1.58-21.21	0.008
		>360(79)	14.38	4.06-50.90	<0.001
Incoming steers	Movement	No (198)	1.00		
		Yes (30)	2.88	1.12-7.37	0.027
Total incoming animals	Movement	≤1 (79)	1.00		
		>1-≤44 (71)	0.77	0.29-2.06	0.608
		>44 (78)	1.90	0.81-4.44	0.138

CONCLUSIONS

Bovine tuberculosis is a chronic, zoonotic infectious disease of cattle that has been a priority for governmental animal health programs worldwide since more than a century ago. Although eradication has not been broadly successful, a marked decrease in prevalence was observed in countries with an active surveillance and control strategies.

Uruguay as a South-American developing country with a substantial and important agriculture economy has overturned efforts to control BTB in cattle population with, limited resources since 1897. The impact of control measures was perceived in the reduction of BTB-positive animals reported until 2010, suggesting that the country may have been close to eradication of the disease. Such accomplishment may have been remarkable, given that only one country in the world (Australia) was successful in eradicating the disease thus far. However, during the 2011-2013 period, the average number of BTB-positive reports for that period duplicated the trend observed the decade before, suggesting that Uruguay may not be as close to eradicate the disease as believed one decade ago. To understand possible factors that could be affecting or influencing BTB dynamics in the Uruguay setting, a spatial assessment of BTB-positive farms jointly with an evaluation of most-likely epidemiological risk factors that could play a role in disease re-emergency were studied in chapters 2 and 3.

From chapter 2, I concluded that BTB-positive farms were not randomly distributed in the cattle population in Uruguay. The global aggregation detected suggests that affected farms could be infected as a result of close contact with infected animals from neighboring positive farms until the second order of neighborhood. In addition, spatial clusters detected indicate a steady area of outbreak aggregation associated to the south-west of the country, in a land area occupied mainly with dairy farms. Far away located (>100km) clusters suggested that animal movements may have played an important role in disease spread. I also concluded that BTB outbreaks were more likely to occur in dairy land areas, compared to the rest of the country, and that close proximity between infected and susceptible farms, along with animal movements, may explain the observed aggregation of positive cases.

In the third chapter, an assessment of most likely epidemiological factors associated with BTB-positive farms confirmed the association with animal movements, after controlling by spatial location. Purchasing a large number of animals (>44) in a period of three years was associated with higher odds of reported the farms as BTB-positive; and the purchase of steers was also associated with disease. These findings, along with large herd size, were factors associated with high risk of reporting a BTB-positive farm. Results here showed the importance of testing animals for BTB previous to move and commingle them at the destination herd. Results also suggest a possible reservoir of disease in an

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untested population as steers that could be linked to management practices related to untraditional large (>116 animals) dairy farms.

The ultimate goal of this study was to contribute to the design and implementation of disease management strategies intended to enhance the effectiveness of BTB control programs in Uruguay. To address this goal, different meetings and discussions with members from the headquarters and Epidemiology Division of Uruguayan Animal Health Bureau at the Ministry of Livestock, Agriculture and Fisheries were conducted. These meetings aimed to show, present, and transmit the results obtained, and discuss the most accurate interpretations based on field characteristics and expert opinions.

The study here helped to identify the nature and extent of the association between BTB and epidemiological factors in Uruguay. Future directions of this study include the assessment of strategies that may help to improve surveillance and control of BTB in Uruguay. For example, formulation of a mathematical model for BTB would help to identify cost-effective surveillance and control measures. Those measures may include, for example, giving incentives to slaughter workers at inspection, or targeting surveillance in the field, such as increasing frequency of testing protocols on high geographical risk areas, on those dairy farms which purchase large number of animals to their premises, or maybe testing those farms whom besides the dairy production concentrates beef animals as a secondary income. Ultimately, this work will help to

create the foundations for a revision of the BTB control and eradication program in

Uruguay.

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