Spatial and temporal epidemiology of bovine trichomoniasis and bovine genital campylobacteriosis in La Pampa province (Argentina)

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The venereal diseases bovine trichomoniasis (BT) and bovine genital campylobacteriosis (BGC) cause economic losses in endemic areas like La Pampa province in Argentina, where beef cattle are usually managed extensively. This study used data compiled under a Provincial Programme for the Control and Eradication of BT and BGC (PCE) to determine the spatio-temporal distribution of these diseases and identify spatial clusters. The study population comprised 29,178 non-virgin bulls drawn from 3766 herds, tested for BT and BGC in 2010. Preputial smegma samples were cultured for BT detection, while BGC was diagnosed by direct immunofluorescence testing of these samples. Campylobacter fetus infection was detected in 1.5% of bulls and 2.3% of herds, and Tritrichomonas foetus infection was found in 1.1% of bulls and 5.1% of herds. The proportion of positive tests was highest in February for BT, while in April it was highest for BGC, and was inversely related to the number of tests, which was greatest during the breeding season (spring). An elliptical spatial cluster of high risk for BGC and a circular cluster for BT were both identified in the south of La Pampa province, which could not be explained by cattle herd density. The spatial and temporal patterns identified in this study provide baseline data for monitoring the success of BT and BGC control activities in La Pampa.

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1. Introduction

Bovine trichomoniasis (BT) and bovine genital campylobacteriosis (BGC) are venereal diseases caused respectively by the flagellate protozoan Tritrichomonas foetus (Skirrow and BonDurant, 1988) and the Gram (-) Campylobacter fetus subspecies venerealis (Eaglesome and Garcia, 1992). Both agents are transmitted mainly during coitus, and colonize the reproductive tract of bulls and cows. In cows, infection can cause reproductive failures, including repeat of estrus, early embryonic death and abortions; in bulls, however, both infections are typically asymptomatic (Anderson, 2007; Mancebo et al., 1995). Infected bulls and cows can become long-term carriers (Corbeil et al., 2003).

In areas where beef cattle are mainly managed intensively, artificial insemination (AI) has largely displaced natural breeding; this tends to control the factors which typically favour these diseases (BonDurant, 2005). As a result, the incidence of both diseases in Europe, for example, is very low or negligible. However, in areas where production systems are typically extensive, based on communal pastures and natural breeding, incidence remains high (Gay et al., 1996; Mshelia et al., 2010). Moreover, it has been reported that the vaccines developed to date are unlikely to induce an effective immune response under field conditions, thus hindering the implementation of effective control plans in endemic areas (Cobo et al., 2004; Villarroel et al., 2004). Consequently, both diseases...
continue to be associated with economic losses due to breeding failure.

In Argentina BT and BGC are considered endemic and their adverse impact on the economy is considerable (Mardones et al., 2008; Jiménez et al., 2011). La Pampa province includes around 6% of the national herd (SENASA, 2012), and beef cattle production accounts for almost 16% of the province’s gross domestic product (DGEC, 2012). Awareness of the economic importance of this sector led in 2006 to the implementation of a Provincial Programme for the Control and Eradication (PCE) of BT and BGC. Participation in the programme is compulsory for all cattle farmers; however, due to the shortage of epidemiological and economic data on the regional incidence of the two diseases, only the control phase has been implemented so far, and the culling of positive bulls remains voluntary.

Recent research has recognized the importance of geographical location in identifying clusters of high-risk areas, which is essential to improve the intervention efforts (Kazembe et al., 2009). Geographical location is a proxy of factors that affect the prevalence of the disease. Spatial heterogeneity in these factors influences the disease transmission pattern (Allepuz et al., 2011). Consequently, efforts to reduce the prevalence of BT and BGC in La Pampa should include research on the influence of location on bovine diseases. These kinds of analyses are scarce for bovine venereal diseases and have been focused on spatial modelling of BT (Szonyi et al., 2012). Moreover, BT and BGC are influenced by some common factors such as herd size, animal density and pasture management. If similar patterns of geographical variation of both diseases could be identified, it might provide evidence of shared risk factors in La Pampa (Knorr–Held and Best, 2001).

The data compiled under the PCE provide reliable, essential information regarding the distribution and epidemiology of these diseases in La Pampa. They should therefore serve as the basis for decisions regarding the prevention, control and potential eradication of BT and BGC. Therefore, the objective of the present study, using official data from the PCE, was to determine the prevalence and spatio-temporal distribution of these diseases in the province of La Pampa.

2. Materials and methods

The study area was the province of La Pampa in Argentina, which included a total of 2,582,860 or 6% of the total cattle population of Argentina (INDEC, 2012). La Pampa is located in the geographical centre of Argentina and covers an area of 143,440 km² by approximately 5,2% of Argentina. Cattle production in La Pampa is typically extensive and involves two main production systems: herds that produce calves for fattening establishments (breeding herds), and herds where breeding, rearing and fattening is carried out on the same premises (full-cycle herds).

The study population is comprised by a total of 29,178 non-virgin bulls from 3766 herds, tested under the control and eradication programme (PCE) from January 1st to December 31st, 2010 (Fig. 1). La Pampa regulation requires the testing of all non-virgin bulls existing in a herd for BT and BGC prior to interprovincial or intraprovincial movement of any type of cattle (breeding bulls, non-breeding bulls, cows, calves) to another herd, feedlots or slaughterhouse (Res. 358/2008, SENASA). Therefore, the study population corresponds to all non-virgin bulls in breeding herds existing in La Pampa in 2010, except those few herds without animal movements during 2010.

All non-virgin bulls in La Pampa were tested twice a year as a part of the PCE. Samples were taken by 260 PCE-accredited veterinarians. Preputial smegma samples were collected by plastic brush scraper, Al pipette or preputial wash (Irons et al., 2002). Collected material was transferred to tubes containing 5 mL phosphate buffered saline (PBS, pH 7.0). The PBS suspension was thoroughly mixed to ensure a homogeneous mixture. Samples were analysed within 24 h of collection by one of the 16 accredited laboratories in La Pampa.

For detection of T. foetus, 10 mL of the PBS suspension were inoculated into modified Diamond medium containing thioglycolate. Samples were then incubated in darkness at 37 °C for 7 days (Parker et al., 2003) and microscopically examined (×200 for screening; ×400 for confirmation) on days 2, 4, 6 and 7 after collections. Parasites were identified by their characteristic morphological features and motility. C. fetus was identified using the direct immunofluorescence technique described by Duffy (1967), following Terzolo et al. (1992). Samples with at least one fluorescent bacterium displaying the morphological features characteristic of C. fetus were classified as positive. A bull was classified as negative if all results in two consecutive tests were negative, and positive if at least one test yielded positive results (Perez et al., 2006). Herds with at least one positive bull were classified as positive.

Herds were geolocated on a 254-cell grid system based on the grid system used by SENASA for the geolocation of all farming activity in Argentina. Each cell measured 0.223° latitude and 0.223° longitude, and comprised approximately 625 km² (with an error due to the geographical projection of 0.000016 km² per grid line), with the exception of the 34 boundary cells in the south–east of the province, which were irregular and smaller.

Data were aggregated at cell level. This approach was necessary to preserve confidentiality of animal owners. For each cell, prevalence of the two diseases was calculated as the ratio of positive bulls/herds to the total number of bulls/herds tested. In order to visualise prevalence, choropleth maps were created for each disease using the ArcGIS 9.3 software package (ESRI, Redlands, CA).

The scan statistics (SaTScan version 9.0) was used to identify spatial clusters of BT and BGC in La Pampa (Kulldorff, 1997). The number of T. foetus or C. fetus positive bulls in each cell was assumed to follow the Poisson distribution, while the total number of bulls tested per cell constituted the population at risk in each cell. The latitude and longitude of the centroids of each cell were used in the coordinate’s files. Both circular and elliptical spatial cluster shapes were investigated. This provides slightly higher power for true clusters that are long and narrow in shape (Kulldorff, 2010). The maximum spatial cluster size was set at 50% of the population at risk, according to Kulldorff’s (2010) recommendations. P values were
obtained based on Monte Carlo simulations after 999 random replications of the dataset, under the null hypothesis of spatial randomness. For the significant clusters ($p < 0.05$), the observed-expected ratio (OER) was calculated as the risk of a bull being positive within the cluster, compared to the population’s risk. From all the different clusters found with $P < 0.05$, we have chosen to show for each disease the clusters with higher observed-to-expected ratios.

It was hypothesized that cattle herd density would be a strong predictor of the spatial BT and BGC risk distribution (Mardones et al., 2008; Jiménez et al., 2011). To test this hypothesis, Spearman correlation coefficients were calculated to quantify the relationship between herd densities indicators and BT and BGC risk at the cell level. Herd density indicators (number of herds, number of bulls, number of cows, cows per herd, cows per bull, bulls per herd) were constructed using data from the provincial census carried out by SENASA (2012). Risk was estimated as the proportion of BT or BGC positive bulls in each cell.

All statistical analyses were carried out at a significant level of alpha $\leq 0.05$ and using the software SPSS 15.0.

### 3. Results

The provincial grid comprised 254 cells, of which 241 were sampled; the remaining 13 cells were not sampled as they contained no beef breeding herds. The number of herds tested per cell ranged from 1 to 89 (median = 10), while the number of positive herds per cell ranged from 0 to 4 (median = 0) for *T. foetus* and from 1 to 9 for *C. fetus* (median = 1). The number of bulls tested per cell ranged from 1 to 654 (median = 93); between 0 and 11 tested positive (median = 0) for *T. foetus* and between 0 and 8 for *C. fetus* (median = 1).

*C. fetus* infection was detected in 437 bulls (1.50%, 95% confidence interval: 0.43–2.57%) and 86 herds (2.28%, 95% confidence interval: 0.91–3.65%). At the cell level, BGC prevalence (median = 0.27%) ranged between 0% and 100%. *T. foetus* infection was detected in 1.06% (95% confidence interval: 0.10–2.20%) of bulls and 5.15% (95% confidence interval: 3.10–7.20%) of herds. At cell level, prevalence (median = 0%) ranged between 0% and 100%. A total of 1.14% of herds contained bulls positive to both *T. foetus* and *C. fetus*, whilst 7.43% of herds contained bulls to at least one of the two diseases.

The number of tests carried out per month ranged from 152 to 11,988; sixty per cent of tests were performed between August and September. The highest BT positivity rate was observed in February (3.14%) and the lowest in December (0.35%). For BGC, the highest positivity rate was recorded in April (8.59%) and the lowest in June (0.35%) (Fig. 2). The spatial scan statistic detected a single significant elliptical cluster (proportion between the focal semi-distance and the major semi-axis in the ellipse, eccentricity = 2, penalty tuning parameter = 0.5) in the southeast.
of La Pampa province ($p = 0.008$) for *T. foetus* and a circular cluster in the south of the province for *C. fetus* ($p = 0.017$). No statistically significant secondary spatial clusters were detected. The prevalence of *T. foetus* and the elliptical cluster, which approximately matched the high-risk area (OER = 4.61), are shown in Fig. 3. The prevalence of *C. fetus* and the circular cluster, which approximately overlapped the high-risk area (OER = 2.80), are shown in Fig. 4.

Spearman correlation coefficients indicated no linear correlation between BT or BGC risk and any of the herd density indicators evaluated at the cell level (number of herds, number of bulls, number of cows, cows per herd, cows per bull, bulls per herd). However, high-risk cells for BGC were also high-risk cells for BT ($r = 0.721, p = 0.0014$).

### 4. Discussion

In this article data from the 2010 Official BT and BGC Control Program in La Pampa has been analyzed to determine the prevalence and temporal and spatial distribution for both diseases. Results show that BT and BGC distribution is non-homogeneous. Two spatial clusters in the south of the province were identified. This study provides baseline data for monitoring the success of BT and BGC control activities in La Pampa.

The population studied in this analysis was composed by non-virgin bulls in breeding herds undertaking annual movements during 2010, about 30,000 bulls and 3700 herds. This population closely matches the whole population of non-virgin bulls and breeding herds living in the Pampa, excluding only a few breeding productive units which did not move cattle during 2010, or those without non-virgin bulls. There might be breeding productive units not showing movements during 2010. However it is not frequent that a productive unit dedicated to the breeding of cattle does not move any type of animal along the year. It is also possible although improbable that some non-virgin bulls from tested productive units have not been diagnosed. Therefore, the results of the present study may thus be extrapolated to all breeding herds in La Pampa.

The prevalence recorded here refers to 2010, the first year of obligatory testing of all non-virgin bulls in La Pampa. Although it was initially intended that compulsory twice-yearly testing under the PCE should be implemented from
2008 onwards, the programme was not effectively implemented until 2010 due to economic difficulties prompted by the drought of 2008–2010. In Buenos Aires province, where similar livestock production systems are in use but with no obligatory BT or BGC testing programme since 1989, considerably higher prevalence rates are reported, in the range 0.5–2% for bulls and 4.2–15% for herds in the case of BGC, and 3.4–4.1% for bulls and 16.3–28% for herds in the case of BT (Campero, 2000; Perez et al., 2005; Rojas et al., 2011). The only previously-published data on BT and BGC prevalence in La Pampa province, reported by Fort et al. (2007), indicate for 2006 a T. foetus positivity rate of 2.53% in herds and 10.38% in bulls, and a C. fetus positivity rate of 1.72% in herds and 10.38% in bulls. These data refer only to the central area of the province, accounting for 6% of herds and 7% of non-virgin bulls (INDEC, 2012), and are based on a single test per bull, probably leading to an underestimation of prevalence. The results of the present study, therefore, indicate a greater decline in the prevalence of BT and BGC than suggested by a simple comparison of data. BT and BGC prevalence rates will need to be monitored in future years in order to determine whether the decline recorded here is part of a consistent trend.

However, the low levels of prevalence observed in this study may be attributable not only to the PCE programme but also to other factors including the severe drought of 2008–2010, the timing of sampling and the diagnostic procedures used. It should be stressed that under the PCE, the culling of positive cattle was recommended, but was not mandatory.

The drought that affected La Pampa between 2008 and 2010 led to a number of changes in breeding activities which may have favoured a decline in the incidence of BT and BGC. Because of the drought, the breeding season was delayed in every year, thus prolonging the rest period and possibly hindering the reinfecion of bulls and enabling positive cows to self-cure (Rae et al., 2004). Producers have adapted livestock density on farms to balance the availability of feed resources, either reducing or temporarily abandoning breeding activity. Generally, the first bulls to be eliminated from a herd are the oldest, which usually have the highest infection rates (Mendoza–Ibarra et al., 2011).

The timing of sampling may also have influenced the noticeable decline in prevalence. Similar trends were observed for both diseases: positivity rates peaked in autumn, thereafter progressively declining to the end of the year. This trend may be linked to the timing of sample collection, which was largely concentrated in the breeding season (i.e. September–November), when bacterial/parasite level in preputial fluid tend to be lower. Carrying out most sampling in the months prior to the breeding season would clearly help to reduce the false-negative rate prompted by low bacterial/parasite levels in preputial smegma; at the same time, it would enable bulls’ health status to be assessed prior to breeding, thus reducing the rate of reproductive failure. Since natural breeding tends to be non-seasonal, it would be advisable to rest bulls for 30–45 days prior to sampling, in order to increase pathogen levels in preputial smegma. However, the consistency of the temporal distribution noted in the present study would need to be confirmed in future years.

Effective control of BT and BGC requires highly-precise diagnostic techniques. In the present study, preputial smegma cultures were used to detect T. foetus, while the direct immunofluorescence test was used for C. fetus; both techniques are widely used to diagnose BT and BGC, respectively. Sensitivity and specificity of similar tests have been estimated by others. Perez et al. (2006) have reported a sensitivity of 72.04% and a specificity of 95.37% for the BT diagnosis test. Ferreira et al. (2002) have reported a sensitivity of 92.59% and a specificity of 88.89% for BGC direct immunofluorescence test. Under field conditions, technical factors influencing both the diagnosis itself (e.g. sampling interval, inappropriate sample storage) and the sample-collection process (e.g. collection technique, operator experience) tend to limit the sensitivity of both tests; for that reason, 2–4 consecutive tests are recommended in order to classify an animal as negative (Ferreira et al., 2002; Perez et al., 2006). The diagnoses in La Pampa were based on only two tests, which could lead to a high false-negative rate. Recent research suggests that real-time PCR techniques afford greater sensitivity and specificity under field conditions than the diagnostic methods used here, thus enabling infection to be ruled out by a single negative test (McMillen and Lew, 2006; Szonyi et al., 2012). Although, as Mendoza–Ibarra et al. (2011) have noted, the combined use of molecular and culturing techniques for routine diagnosis would be ideal, the feasibility of using real-time PCR methods in La Pampa would need to be examined. In any event, the timing of sample collection and the diagnostic methods used under the PCE may have led to a high false-negative rate, which would account in part for the low prevalences recorded in this study.

Our spatial analyses showed that those herds in the centre-south of La Pampa were more likely to contain bulls infected with T. foetus and with C. fetus than herds located in other areas of La Pampa. Moreover, the strong correlation between risk of BT and BGC infection and the large area in the south of the province which is shared by spatial clusters of both diseases is evidence of common variation of prevalence of BT and BGC. It is possible that both BT and BGC share common risk factors or because one disease can increase the risk of the other.

Studies conducted in Argentina in recent years have identified some common risk factors for both diseases (Mardones et al., 2008; Jiménez et al., 2011). Some of these factors co-exist in the co-infection area: the interchange of bulls and sharing pasture are frequent; so the co-infection area identified in this research could be considered evidence that BT and BGC share risk factors in La Pampa. However, other factors related to both diseases, for example, high herd numbers or high livestock density, are not located in the co-infection area or in the individual risk areas. An explanation for this fact could be that the PCE efforts in previous years have been focused in the northeast, an area with greater animal density, while in the south-west area 2010 was the first year for a complete evaluation.

The relationship between some of the above factors and BT and BGC risk could be assessed by including specific
covariates in a spatial model; however, the main objective of this study was to explore the spatial distribution of BT and BGC risk in La Pampa in order to summarize the spatial risk heterogeneity. In this sense, a joint formulation which simultaneously models spatial variations in the risk of BT and BGC allows an understanding of the epidemiology of both diseases at the population level. An appropriate model might be a shared component model, in which the underlying risk surface is decomposed into portions comprising shared and disease-specific variation (Knorr-Held and Best, 2001).

Apart from this, the high level of correlation between BT and BGC prevalence suggests that one disease can be a risk factor for the other. If we also consider that BT and BGC share the same clinical signs, this co-occurrence must be taken into account in a control program for either disease. These observations reinforce the idea that the effective intervention plans should be jointly focused on BT and BGC.

Better spatial resolution could have been obtained if the geo-referenced points had been taken into account. However they were not available. When exact farm locations are not available, data are often analyzed in an aggregate way by following a regular lattice (Abrial et al., 2005) or by making use of the lowest geographical unit for the available data (Allepuz et al., 2011). One disadvantage of using aggregated data is that the position of boundaries and the size of the areas do not usually show a spatial distribution of epidemiological factors. We have considered a regular aggregation routine where the boundaries keep approximately the same length (25 km) and the areas cover approximately the same surface (625 km²). This level of aggregation is similar to the one used by Szonyi et al. (2012) in BT in Texas. Nevertheless, different levels of aggregation can cause different statistical results, the modifiable area unit problem (Elliot and Wartenberg, 2004).

Since the introduction of the PCE programme in La Pampa, a negative-diagnosis certificate has been required when introducing non-virgin bulls from other provinces into local herds for breeding purposes. However, the movement of non-virgin bulls within the province depends entirely on the producer, since only a test certificate – whether negative or positive–is required. Whilst most producers are probably reluctant to accept infected bulls, it is not prohibited by law. Non-virgin bulls involved in commercial exchanges are the major cause of spread of both diseases. There are no BT-or BGC-related restrictions on trade in non-virgin cows; this may be significant, given the frequency of inter-provincial trade and the fact that none of the adjoining provinces are currently implementing venereal disease control programmes. The introduction of infected cows into breeding herds may contribute to the spread of BT and BGC. However, since cows tend to eliminate both pathogens from the reproductive tract within months of infection (Mancebo et al., 1995), non-virgin bulls remains the main source of spread. If the spread of these diseases is to be effectively controlled in La Pampa, it is essential to introduce measures to prevent both the use of infected bulls for breeding purposes and the movement of non-virgin bulls from one herd to another.

5. Conclusion

The spatial and temporal patterns identified in this study provide baseline data for monitoring the success of BT and BGC control activities in La Pampa. It is important to identify the factors contributing to the shared and disease-specific high risk areas of La Pampa, since this would provide information useful for optimising the control and eradication programme.

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References

Jiménez, D.F., Perez, A.M., Carpenter, T.E., Martinez, A., 2011. Factors associated with infection by Campylobacter fetus in beef herds in