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

TRABAJO DE FIN DE MASTER

Leonardo Luís Molina

Córdoba, 2012
INFORMA

Que el trabajo de fin de master Spatio-temporal epidemiology of bovine trichomoniasis and bovine genital campylobacteriosis in La Pampa province (Argentina) que se recoge en la siguiente memoria y de la que es autor D. Leonardo Luís Molina ha sido realizada bajo mi dirección, cumpliendo las condiciones exigidas para que el mismo pueda optar al grado de Master en Zootecnia y Gestión sostenible: ganadería ecológica e integrada por la Universidad de Córdoba.

Lo que suscribo como director de dicho trabajo y a los efectos oportunos, en Córdoba a 15 de Julio de 2012.

Fdo. Dr. José Manuel Perea Muñoz
D. GUILLERMO MEGLIA, PROFESOR TITULAR DEL DEPARTAMENTO DE EPIDEMIOLOGÍA DE LA FACULTAD DE CIENCIAS VETERINARIAS DE LA UNIVERSIDAD NACIONAL DE LA PAMPA

INFORMA

Que el trabajo de fin de master Spatio-temporal epidemiology of bovine trichomoniasis and bovine genital campylobacteriosis in La Pampa province (Argentina), que se recoge en la siguiente memoria y de la que es autor D. Leonardo Luís Molina ha sido realizada bajo mi dirección, cumpliendo las condiciones exigidas para que el mismo pueda optar al grado de Master en Zootecnia y Gestión sostenible: ganadería ecológica e integrada por la Universidad de Córdoba.

Lo que suscribo como director de dicho trabajo y a los efectos oportunos, en Córdoba a 15 de Julio de 2012.

Fdo. Dr. Guillermo Meglia
Abstract

The venereal diseases bovine trichomoniasis (BT) and bovine genital campylobacteriosis (BCG) cause severe 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, identify spatial clusters, and estimate their impact on beef herd productivity in the province of La Pampa. The study population comprised 29,178 non-virgin bulls drawn from 3,766 herds, tested for BT and BGC in 2010. Preputial smegma samples were cultured for BT detection, while BGC was diagnosed by direct immunofluorescence test. *C. fetus* infection was detected in 1.50% of bulls and 2.28% of herds, while *T. foetus* infection was found in 1.06% of bulls and 5.15% of herds. For both diseases, the proportion of positive tests was highest in autumn, and was inversely related to the number of tests, which was greatest during the breeding season (spring). The scan statistics identified an elliptical spatial cluster of high risk for BGC, and a circular cluster for BT, both in the south of La Pampa province, which could not be explained by cattle herd density. It was estimated that BT and BGC reduced the number of weaned calves per cow by around 10% and 13%, respectively. 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.

Keywords: Cattle, Venereal diseases, Epidemiology, Argentina
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 García, 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 *et al*., 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 has largely displaced natural breeding; this tends to give rise to a more effective control of semen and in general of 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 in 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 severe economic losses due to breeding failure.

In Argentina, these two diseases 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, 2010), 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, so far only the control phase has been implemented, and the culling of positive bulls remains voluntary.

The data compiled under the PCE provide reliable, essential information regarding the distribution and epidemiology of these diseases in La Pampa, as well as an indication of their impact on cattle production. They should therefore serve as the basis for decisions regarding the prevention, control and potential eradication of BT and BGC not only in the province but in Argentina as a whole. Therefore, the objective of the present study, using official data from the PCE, was to determine the occurrence and spatio-temporal distribution of these diseases and to estimate their impact on beef herd productivity in the province of La Pampa.

**Material 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, 2010). 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 within the same premises (full-cycle herds). The study population comprised a total of 29,178 non-virgin bulls from 3,278 herds, tested under the control and eradication programme (PCE) from January 1st to December 31st, 2010.

All non-virgin bulls in La Pampa were tested twice a year. Samples were taken by 260 PCE-accredited veterinarians. Preputial smegma samples were collected by plastic brush scraper, AI 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 sent to one of 16 accredited laboratories within 24 hours of collection.

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 (x 200 for screening; x 400 for confirmation) on days 2, 4, 6 and 7 after collection. Parasites were identified by their characteristic morphological features and motility. *C. fetus* was identified using the direct immunofluorescence technique described by Dufty (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 deemed negative if all results in two consecutive tests were negative, and positive if at least one test yielded positive results (Pérez 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 62,500 ha, with the exception of the 31 boundary cells in the south-east of the province, which were irregular and smaller. Data were aggregated at cell level. 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 method (SaTScan version 9.0) was used to identify spatial clusters of BT and BCG 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. Both circular and elliptical spatial cluster shapes were investigated. The maximum spatial cluster size was set at 50% of the population at risk. Critical 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 relative risk (RR) was calculated as the risk of a bull being positive within the cluster, compared to the population’s risk. The significant cluster with the highest relative risk was taken for each disease as the final cluster solution.
It was assumed 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, Pearson 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 (2010). Risk was estimated as the proportion of BT or BGC positive bulls in each cell.

It was also assumed that both diseases could cause severe economic losses due to reproductive failures. To estimate the losses prompted by BT and BGC infection in La Pampa, the number of weaned calves per cow as a function of herd health status (infection-free, BT-positive, BGC-positive, BT+BGC-positive) was compared by ANOVA. Data on cow and calf numbers were taken from the provincial census (SENASA, 2010).

**Results**

The provincial grid comprised 254 cells, of which 241 were sampled; the remaining 13 cells were not sampled since they contained very little or no cattle-rearing activity. 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%) and 86 herds (2.28%). At the cell level, BCG prevalence (median = 0.27%) ranged between 0% and 100%. *T. foetus* infection was detected in 1.06% of bulls and 5.15% 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 with at least one of the two diseases.
Figure 1. Monthly time series of the total number of *T. foetus* tests performed (solid) and the proportion of positive tests (dotted) during 2010.

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%) (Figure 1). For BGC, the highest positivity rate was recorded in April (8.59%) and the lowest in June (0.35%) (Figure 2).

Figure 2. Monthly time series of the total number of *C. fetus* tests performed (solid) and the proportion of positive tests (dotted) during 2010.
The spatial scan statistics detected a single significant elliptical cluster in the southeast of La Pampa province (p<0.05) for *T. foetus* and a circular cluster in the south of the province for *C. fetus* (p<0.05). No statistically significant secondary spatial clusters were detected for either disease using window shape setting. Prevalence rates for *T. foetus* and the elliptical cluster, which approximately matched the high-risk area (RR = 4.61), are shown in Figure 3. Prevalence rates for *C. fetus* and the circular cluster, which roughly overlapped the high-risk area (RR = 2.80), are shown in Figure 4.

**Table 1.** Pearson correlation coefficients between herd densities indicators and BT and BGC risk at cell level.

<table>
<thead>
<tr>
<th></th>
<th>BT risk</th>
<th>BGC risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cows</td>
<td>-0.145</td>
<td>-0.119</td>
</tr>
<tr>
<td>Number of herds</td>
<td>-0.128</td>
<td>-0.116</td>
</tr>
<tr>
<td>Cows per herd</td>
<td>-0.045</td>
<td>0.042</td>
</tr>
<tr>
<td>Bulls</td>
<td>-0.134</td>
<td>-0.122</td>
</tr>
<tr>
<td>Bulls per herd</td>
<td>-0.033</td>
<td>0.007</td>
</tr>
<tr>
<td>Cows per bull</td>
<td>-0.039</td>
<td>-0.014</td>
</tr>
<tr>
<td>BT risk</td>
<td>-</td>
<td>0.832</td>
</tr>
</tbody>
</table>
Figure 3. Choropleth map of bovine trichomoniasis in bulls in La Pampa, based on province–wide data from 2010. The most likely spatial cluster identified by the scan statistics are shown.
Figure 4. Choropleth map of bovine genital campylobacteriosis in bulls in La Pampa, based on province–wide data from 2010. The most likely spatial cluster identified by the scan statistics are shown.
Pearson correlation coefficients (Table 1) indicated no linear correlation between BT or BGC risk and herd density. However, high-risk areas for BGC were also high-risk areas for BT.

Of the 3,766 herds initially included in the study, reliable data on number of cows and number of weaned calves was available for only 1,517 herds (40.3%). Of these, 1,345 (88.7%) herds were negative for both diseases, 46 (3.0%) contained *C. fetus*-positive bulls, 105 (6.9%) contained *T. foetus*-positive bulls, and 21 (1.4%) had bulls positive for both diseases. The mean number of weaned calves per cow for all tested herds was 0.77 (Table 2); in disease-free herds, the mean was 0.78. Mean productivity in BT-positive herds was 9.8% lower (p<0.05), whilst in BGC-positive herds, the mean was 13.0% lower (p<0.05). In herds positive for both BGC and BT, productivity displayed a significant mean decline of 23.8%.

Table 2. Weaned calves per cow according to the herd health status (mean ± S.D.).

<table>
<thead>
<tr>
<th>Herd health status</th>
<th>n</th>
<th>Weaned calver per cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection–free</td>
<td>1,345</td>
<td>0.78 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BGC–positive</td>
<td>46</td>
<td>0.69 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BT–positive</td>
<td>105</td>
<td>0.71 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BT+BGC–positive</td>
<td>21</td>
<td>0.63 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall</td>
<td>1,517</td>
<td>0.77 ± 0.14</td>
</tr>
</tbody>
</table>

*Mean with different letters are different (p<0.05)*

Discussion

The PCE programme requires the twice-yearly obligatory testing of all non-virgin bulls, whether or not involved in commercial exchanges. If a herd is not tested, movement of all animals, even for slaughter purposes, is banned (Res. 358/2008, SENASA). The results of the present study may thus be expanded to all breeding herds in La Pampa.

The prevalence rates recorded here refer 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 there has been 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; Pérez 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 well have favoured a decline in the incidence of BT and BGC. Because of the drought, the breeding season was delayed every year, thus prolonging the rest period and possibly hindering the reinfection of bulls and enabling positive cows to be cured (Rae et al., 2004). Livestock producers were also obliged to adapt herd density to the availability of food resources, either reducing or temporarily abandoning breeding activity. Generally, the first bulls to be eliminated from a herd are the oldest, which usually presenting 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 to 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 established prior to breeding, thus reducing the rate of reproductive
failure. Since natural breeding tends not to be season-limited, 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. In 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; Pérez 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 in 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.

Analysis of spatial distribution revealed that herds in the centre-south of La Pampa are
more likely to contain bulls infected with *T. foetus* and/or with *C. fetus* than herds located in
other areas of La Pampa. 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 highlights epidemiological similarities between BT and BGC in terms of risk of infection. Recent research into risk factors for BT and BGC in the province of Buenos Aires, whilst not evaluating joint risk, have certain common factors, including the exchange of bulls (Mardones et al., 2008; Jiménez et al., 2011). In the south of La Pampa province, the breeding season tends to be uncontrolled, mainly due to adverse agroecological conditions. Herds are extensively managed, in low inputs systems where few veterinarians and advisers are available. Herds tend to share rangelands and involuntary exchange of bulls is common. All these factors may help to account for the presence of high-risk areas for BT and BGC. However, identification of the factors responsible for high local prevalence of BT and BGC would provide essential information for identifying the areas concerned and controlling the spread of both diseases.

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 wholly 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 is 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 remain 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.
The impact of BT and BGC on productivity in La Pampa was found to be similar to that reported by Jiménez et al. (2011) for BGC in Buenos Aires and by Cima (2009) for BT in Texas. The data obtained may be of value in evaluating the economic benefits of eradicating both diseases in La Pampa.

Conclusions

In 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. The results obtained highlight two key issues that need to be addressed in order to improve the success of the PCE programme for eradicating BT and BGC: the possible high false-negative rate and the need to restrict the movement of non-virgin bulls not provided with a negative-test certificate, particularly from the high-risk areas identified here. To reduce the rate of false negatives, the following measures might usefully be considered: concentrate sampling in the months prior to the breeding season and/or encourage compulsory resting of bulls over the 30-45 days prior to sampling, and improve the sensitivity of the diagnostic techniques used, either by increasing the number of consecutive tests required to classify an animal as disease-free from 2 to 3, or by combining molecular and culture-based techniques, or by using real–time PCR.

Finally, it is important to identify the factors contributing to the high risk of BT and BGC in certain areas of La Pampa, since this would provide information useful for optimising the control and eradication programme.
References


de tres departamentos de la provincia de La Pampa. Año 2006 – Programa de Control. 17
Reunión Científica Técnica de la Asociación Argentina de Veterinarios de Laboratorios


