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Risk factors associated with *Tritrichomonas foetus* infection in beef herds in the Province of Buenos Aires, Argentina

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Abstract

Bovine trichomonosis (BT) is a contagious disease, characterized by reproductive failure, embryonic losses, infertility, and abortions, which directly impacts the reproductive performance of cattle. In this cross-sectional questionnaire-based study, 56 veterinarians provided information on the geographic location and on the demographic, husbandry, and sanitary characteristics of 173 beef herds located in 26 (21%) counties of the Province of Buenos Aires, Argentina. Herds rearing beef cattle (OR = 2.5, 95% CI = 1.0, 6.3), with low pregnancy rate in cows ($\leq$90%) (OR = 4.1, 95% CI = 1.5, 10.8), sharing livestock with other owners or farmers (OR = 5.4, 95% CI = 1.8, 16.5), and where trichomonosis was reported during the previous year (OR = 4.1, 95% CI = 1.7, 9.6) were at significantly greater risk of being BT positive. This study provided information for future risk assessment and/or management plans for the prevention and control of BT in a region where the disease is considered to be endemic.

Keywords: *Tritrichomonas foetus*; Bovine trichomonosis; Venereal disease; Beef cattle

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1. Introduction

Bovine trichomonosis (BT) is a contagious venereal disease, characterized by reproductive failure, early embryonic losses, infertility, and abortions, which directly impacts the reproductive performance of cattle (McDonald and McKay, 1960; Yule et al., 1989). The causative agent is *Tritrichomonas foetus*, a flagellated protozoan found only in the genitalia of cattle (BonDurant, 2007). In bulls, this anaerobic and non-invasive pathogen is confined to the epithelial surface of the penis, prepuce, and urethra, where it typically establishes an asymptomatic and chronic infection (Parker et al., 1999; Cobo et al., 2004). Coitus between susceptible cows and heifers and asymptomatically infected bulls is considered as the main route for *T. foetus* transmission (Yule et al., 1989; BonDurant, 2007). Thus, use of artificial insemination (AI) has resulted in a substantial reduction not only of trichomonosis incidence but also of other venereal infections (Yule et al., 1989; Foote, 1996; van Bergen et al., 2006). However, in regions where BT is endemic, mechanical transmission by contaminated instruments, semen, or gynecological examinations may also occur (Yule et al., 1989; Rae and Crews, 2006). Indirect losses

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related with *T. foetus* infection are mainly associated with culling and replacement of infected cattle (BonDurant et al., 1990; Rae et al., 1999, 2004).

Factors associated with high incidence of BT in a herd include large number of animals, high proportion of old bulls ($\geq$4 years of age), shared grazing, and high bull-to-cow ratio. Prevalence of *T. foetus* in Simmental, Charolais, and Angus breeds has been estimated to be higher compared with that in other pure breed and with cross-breed cattle (Rae et al., 1999; Villarroel et al., 2004; Rae and Crews, 2006).

Culture of a bull's preputial smegma has been considered the reference test for diagnosis of *T. foetus* infection (BonDurant et al., 2003; Parker et al., 2003). Median sensitivity and median specificity of culture from samples collected in the field ranges from 72 to 92% and from 95 to 100%, respectively (BonDurant et al., 2003; Parker et al., 2003; Villarroel et al., 2004; Perez et al., 2006a). Thus, it is generally accepted that one single positive result is sufficient to consider a herd *T. foetus*-infected; however, at least two or three consecutive negative results are necessary to demonstrate freedom from *T. foetus* infection (Perez et al., 2006a).

The median *T. foetus* herd-level prevalence in the Province of Buenos Aires, Argentina, was recently estimated at 28% (Perez et al., 2006b). Quantification of factors associated with BT will help to elucidate the role that epidemiological factors and forces play in the occurrence and spread of the disease, to design effective control and prevention programs, and to develop strategies to identify herds at high risk for the disease.

The purpose of this study was to quantify the association between husbandry and demographic factors and BT in range beef herds of Buenos Aires, Argentina.

2. Materials and methods

2.1. Data source and sampling methods

The Province of Buenos Aires was selected as our study site. This province contains 18.6 million or 36.5% of the total cattle population of Argentina (INDEC, 2007). The production of beef cattle in Argentina involves two main schemes: herds that rear cattle until the weight of 150–250 kg, which here were referred to as 'rearing herds', and herds where breeding, rearing and fattening is carried out within the same premises, which here were referred to as 'full cycle herds'. Preputial fluid samples were taken from March to October 2005 by private veterinary practitioners from all bulls in the study herds by aspiration or scraping of the preputial cavity, inoculated directly into a modified Plastridge medium for *T. foetus* (Tricoazul®) (Perez et al., 2006a), and submitted to the diagnostic laboratory within 24 h. Cultured samples were incubated at 36–37 °C and examined daily for a week for motile *T. foetus* by placing a drop of each sample on a glass slide and observing at 100× magnification with light microscopy (Parker et al., 2003). Herds where all bulls were diagnosed negative to *T. foetus* infection in three sequential tests were considered free from the disease (controls). A case was defined as a herd in which at least one sample of preputial washings and/or scrapings from one or more bulls was positive to *T. foetus*.

2.2. Questionnaire

In this cross-sectional study, after a positive result for *T. foetus* infection was obtained from the laboratory, a questionnaire was sent to the veterinarian responsible for the sanitary status of the infected herd. Questionnaires were sent from October 2005 to May 2006 via mail and in some cases personally delivered to the veterinarian. Mailed questionnaires were followed by weekly telephone calls until the questionnaire was filled out and returned. Once the questionnaires for cases were received, the veterinarian was requested to fill out the same questionnaire for control beef herds, i.e., with three consecutive negative results, that were sampled in the same period of time, by the same veterinarian, and in the same region where the positive herd was located. Closed ended (checklist) questions for variables hypothesized to be biologically related to the disease at herd-level were included in the questionnaire. Variables assessed in the questionnaire referred to the last reproductive season. Demographic variables included identification of the beef herd type of production (rearing, full cycle), number of cows ($<$500, 500–1000, $>$1000), the unique national sanitary identification code for agricultural and livestock producers (referred to as *RENSPA* by the Argentine Animal Health Service, *SENASA*), and county where the herd was located. Variables related with reproductive management practices and sanitary history of the herd included use of AI (yes, no), use of natural mating (yes, no), pregnancy rates in cows and heifers ($\leq$90%, $>$90%), abortion (yes, no), purchase of pregnant cows or cows with young calves (yes, no), occurrence of unexpected pregnancies (yes, no), rectal palpation before mating or before AI in heifers (yes, no), performed capacity tests in bulls (yes, no), and confirmation of venereal diseases in the herd (yes, no). Variables related with general husbandry practices were rent or purchase of bulls (yes, no), share stock with other farmers (yes, no), rotation of bulls between different stocks of cows or heifers (yes, no), passage of bulls from contact herds (yes, no), and quality of fences (good, regular or bad). Information was coded and managed using spreadsheets in Excel® (Microsoft Corp., Redmond, WA) to maintain veterinarian and herd confidentiality.

2.3. Statistical analysis

Descriptive statistical analyses were performed for dichotomous variables to estimate the proportion of cases and controls for each of the variables assessed here. Strength of the bivariate association between each variable and *T. foetus* status was quantified using the Pearson's χ^2-test. The Fisher exact test was used when assumptions for the Pearson's χ^2-test were not met.

Variables for which the estimated value of the Pearson's χ^2 statistics was $P \leq 0.10$ were included as main effects in a multivariate regression analysis using a backward stepwise elimination process (P to enter ≤ 0.05, and P to exit >0.10). Because large number of interactions could identify false associations (Kleinbaum et al., 1998; Dohoo et al., 2003), only two-way interaction terms based on previous knowledge or biological reasoning were examined.

Models were compared using a likelihood ratio test and the goodness-of-fit of the final model was evaluated by calculating the Hosmer and Lemeshow statistic (Hosmer and Lemeshow, 1989).

Bivariate and multivariate analyses were performed, respectively, using the StatCalc application of Epi Info™ Version 3.4.3 (CDC, Atlanta, GA) and SPSS version 15.0 (SPSS Inc., Chicago, IL).

2.4. Spatial analysis

Spatial distribution of cases and controls was explored to verify the absence of spatial clustering in the data. Formal assessment of the lack of spatial dependence in the data is important because if cases (or controls) were spatially clustered, then quantification of the association between risk factors and disease status needs to be adjusted by the geographical location of the herds. Conversely, if data are not spatially clustered, then accounting for the spatial location of the herds is not necessary. Spatial clustering was assessed using a first order of nearest-neighboring Cuzick and Edwards' test for inhomogeneous populations (Cuzick and Edwards, 1990). Herds *RENSPA*s were used to geolocate the herds into the grid system used by *SENASA* to refer the geographical location of any agricultural premises in the country. Briefly, each grid cell measures 0.25° longitude by 0.17° latitude and has been divided into six minor cells. For location-specific analysis, beef herds were assumed to be located in the centroid of the minor cell where the herd was located. Because some of the herds shared the same minor cell, and to evaluate the power of the spatial clustering in this study (Dohoo et al., 2003), we randomized the fourth decimal place of the estimated Cartesian coordinates and performed the Cuzick and Edwards' test 100 times using the SPSTAT v.4.7 (University of California, Davis, CA) for Excel®. Failure to identify spatial clustering ($P > 0.05$) in more than 80 of the simulations (test power >0.80) was considered sufficient evidence of absence of spatial bias in the dataset.

3. Results

3.1. Questionnaire response and demographics characteristics

A total of 465 questionnaires was sent to 118 veterinarians and 207 (45%) were answered and returned by 56 (48%) veterinarians. A total of 34 (16%) returned questionnaires was eliminated from the study because they referred to herds located in provinces other than Buenos Aires ($n = 7$), to production systems other than beef cattle ($n = 9$), or were incomplete ($n = 18$). Thus, 173 herds (cases = 42, 24%; controls = 131, 76%) from 26/123 counties of the Province of Buenos Aires were included in the bivariate analyses. Most ($n = 113$, 65%) of the herds were located in four counties, namely, Azul ($n = 64$), Tapalque ($n = 18$), General Lamadrid ($n = 16$), and Coronel Pringles ($n = 15$).

Because only one beef herd rented bulls and many (>30%) of the responses missed the information on pregnancy rates in heifers, these two variables were excluded from the analyses.

3.2. Statistical analyses

Seven of the 21 explored variables were associated ($P \leq 0.10$) with *T. foetus* status in the bivariate analysis and were tested as candidate variables for the multivariate logistic regression model (Table 1). The final multivariate logistic regression model included type of production (OR = 2.5), low pregnancy rate in cows (OR = 4.1), sharing livestock with other farmers (OR = 5.4), and BT status in the previous year (OR = 4.1) (Table 2). Interactions did not improve

Table 1
Point estimates and 95% confidence intervals (95% CI) of the odds ratios (ORs) for *T. foetus* infection in beef herds of the Province of Buenos Aires, Argentina, exposed to factors hypothesized to increase the risk for the disease, as measured in a bivariate analysis using data collected from October 2005 to May 2006

Variable	Category	*T. foetus* status			OR 95% CI		
		N	Cases (%)	Controls (%)	OR	Lower	Upper
Type of production	Rearing	**100**	**31 (31)**	**69 (69)**	**2.45**	**1.14**	**5.29**
	Full cycle	**71**	**11 (15)**	**60 (85)**	**1**		
Herd size	>1000	44	11 (25)	33 (75)	0.77	0.338	1.770
	501–1000	45	7 (16)	38 (84)	0.43	0.168	1.086
	<500	82	24 (29)	58 (71)	1		
Artificial insemination of cows	Yes	36	10 (28)	26 (72)	1.26	0.55	2.89
	No	137	32 (23)	105 (77)	1		
Artificial insemination of heifers	Yes	37	9 (25)	28 (75)	1.00	0.43	2.34
	No	136	33 (24)	103 (76)	1		
Natural mating of cows	Yes	167	41 (25)	126 (75)	1.63	0.18	14.33
	No	6	1 (17)	5 (83)	1		
Natural mating of heifers	Yes	122	27 (22)	95 (78)	0.68	0.33	1.43
	No	51	15 (29)	36 (69)	1		
Pregnancy rate in cows	**≤90%**	**104**	**36 (35)**	**68 (65)**	**5.56**	**2.19**	**14.08**
	>90%	**69**	**6 (9)**	**63 (91)**	**1**		
Perform capacity test in bulls	Yes	13	4 (31)	9 (69)	1.43	0.42	4.90
	No	160	38 (24)	122 (76)	1		
Purchase pregnant cows	Yes	60	17 (28)	43 (72)	1.39	0.68	2.85
	No	113	25 (22)	88 (78)	1		
Purchase cows with young calves	Yes	19	8 (42)	11 (58)	2.38	0.89	6.38
	No	154	36 (23)	118 (77)	1		
Pre-calving palpation to heifers	Yes	56	15 (27)	41 (73)	1.22	0.59	2.53
	No	117	27 (23)	90 (77)	1		
Observed unexpected pregnancies	Yes	70	17 (24)	53 (72)	1.00	0.49	2.03
	No	103	25 (24)	78 (76)	1		
Quality of the fences	Regular or bad	60	18 (30)	42 (70)	1.59	0.78	3.24
	Good	113	24 (21)	89 (79)	1		
Shared livestock with others	Yes	**23**	**11 (48)**	**12 (52)**	**3.49**	**1.41**	**8.66**
	No	**149**	**31 (21)**	**118 (79)**	**1**		
Purchase of bulls	Yes	104	29 (28)	75 (72)	1.64	0.78	3.43
	No	68	13 (19)	55 (81)	1		
Rotation of bulls	Yes	**34**	**13 (38)**	**21 (62)**	**2.35**	**1.05**	**5.25**
	No	**139**	**29 (21)**	**110 (79)**	**1**		
Passage of bulls from in-contact herds	Yes	**58**	**19 (33)**	**39 (67)**	**1.93**	**0.94**	**3.94**
	No	**114**	**23 (20)**	**91 (80)**	**1**		
Abortion	Yes	**71**	**24 (34)**	**47 (66)**	**2.36**	**1.16**	**4.78**
	No	**101**	**18 (18)**	**83 (82)**	**1**		
T. foetus reported in previous year	Yes	**37**	**18 (49)**	**19 (51)**	**4.30**	**1.97**	**9.40**
	No	**133**	**24 (18)**	**109 (82)**	**1**		

Variables significantly associated ($P < 0.1$) with *T. foetus* infection are bolded.

Table 2
Point estimates and standard error (S.E. β) of the regression coefficients (β), point estimates and 95% confidence intervals (95% CI) of the odds ratios (ORs), and *P*-value of the association between *T. foetus* infection and epidemiological factors estimated using a multivariate logistic regression model and data collected in beef herds in the Province of Buenos Aires, Argentina, from October 2005 to May 2006 ($n = 170$; d.f. 6, *P*-value of the Hosmer–Lemeshow statistic = 0.995)

Variable	β	S.E. β	OR	95% CI	*P*-value
Intercept	−3.36	0.58			
Type of production (rearing)	0.91	0.47	2.49	1.00, 6.26	0.052
Pregnancy rate in cows (≤90%)	1.40	0.50	4.07	1.53, 10.82	0.005
Shared livestock with others (yes)	1.68	0.57	5.37	1.75, 16.49	0.003
T. foetus reported in previous year (yes)	1.40	0.44	4.06	1.71, 9.63	0.001

the fit of the model. The model provided a good fit to the data (Hosmer and Lemeshow test, $P > 0.05$). The categorical variable type of production was borderline significant ($P = 0.052$).

3.3. Spatial analysis

Statistical evidence ($P < 0.05$) of spatial clustering was not found in 91 of the 100 runs of the first order nearest-neighboring Cuzick and Edwards' test. Therefore, the distribution of cases in our dataset was considered not to be spatially clustered (test power >0.80).

4. Discussion

In this study it was estimated that sharing livestock with other farmers, and production of beef cattle on a farm, which involved only the rearing stage (150–250 kg.) were significant risk factors for *T. foetus* infection in beef herds of the Province of Buenos Aires, Argentina. Beef herds with low pregnancy rates in cows (≤90%) and in which BT was reported in the previous year were also at significantly greater risk of being *T. foetus*-infected. Lack of evidence of spatial clustering in the dataset suggests that the design used here was successful in controlling the dependence in the spatial distribution of cases.

Sharing livestock, which may be considered a likely practice when more than two producers share land through common employees, structures and pastures at the same time, increased the odds that a herd would have at least one *T. foetus*-positive bull by 5.4 times. There is a general agreement that the likelihood of spreading a sexually transmitted disease increases when the number of sexual contacts increases. Previous studies also noticed this common association with *T. foetus*, when commingled grazing was identified as a risk factor for trichomonosis in beef herds (Gay et al., 1996) and also in a simulation model (Villarroel et al., 2004), where shared grazing was found to be the most significant risk factor for a decreased calving incidence due to *T. foetus*. Indeed, implementation of population segregation has been proposed as an effective 'herd-level treatment' for trichomonosis (BonDurant, 2005, 2007; Rae and Crews, 2006).

The odds of identifying *T. foetus* infection in rearing beef herds were 2.5 times higher than the odds of diagnosing BT in full cycle beef herds. Because of the venereal nature of BT transmission, one would expect that the individual risk of establishing an effective contact is higher in rearing beef herds compared with full cycle herds. In other words, there is a higher proportion of reproductively active animals in rearing beef herds compared with full cycle herds. Thus, for the same number of animals in rearing and in full cycle herds and fixing at the same values all other variables and conditions, there will be more effective contacts in the former than in the latter, increasing the risk for disease spread.

The link between beef herds with pregnancy rate ≤90% and *T. foetus* supports the belief that infertility is the primary manifestation of *T. foetus* infection, which was clinically evidenced as a high percentage of non-pregnant cows. Considering that the mating season in Argentine range beef herds usually lasts 3 months, i.e., on average, three estrus cycles per cow, and that the expected pregnancy rate per cycle is approximately 0.6, one would expect that under normal conditions, approximately 95% of the cows in a herd will be pregnant throughout the mating season. Therefore, pregnancy rates ≤90% might be considered evidence for reproductive disorders in a herd. In a previous study (Stewart et al., 1998), *T. foetus* was found to be statistically significantly associated with reduced pregnancy rates in *T. foetus*-infected (84%) compared with non-infected (94%) cows. Lower pregnancy rates resulted in increased calving intervals in cows, resulting in reduced herd performance and profitability (Rae, 1989).

BT reported in the previous year was associated with 4.1 times more risk for the presence of at least one *T. foetus*-positive bull. This finding is consistent with the contagious nature of the disease and the biological characteristics of the agent. *T. foetus* is an insidious pathogen that can persistently infect males in the absence of clinical signs, which facilitates the persistence of the disease in a herd. This finding also suggests that intra-herd control or eradication programs for BT have had limited success in the region (Mancebo et al., 1995).

Potential pitfalls of this study could be related to the absence of a formal randomization procedure to select the assessed herds. Information was voluntarily provided by practitioners and for that reason the selection process might be biased. Because confidentiality issues in the availability and use of the information would have substantially decreased the response rate of the questionnaires, targeting practitioners and producers willing to participate in the study was preferred. Because the ratio between cases and controls provided by each veterinarian was heterogeneous, results could potentially be biased by the individual criteria for responding to the questionnaire and by the ability of the practitioner to collect and deliver the samples to the laboratory. Assessment and comparison of the ability and professional competence of the 56 practitioners that submitted information used on this study were infeasible, and fall beyond the scope of this work. Age of bulls is a factor that could potentially be associated with BT status but was not assessed here.

In summary, the results reported here will be useful for future risk assessment and/or management plans for the prevention and control of *T. foetus* in an economically important region where trichomonosis is considered to be endemic. For example, the multivariate model fitted here may be used to estimate the probability that beef herds in the Province of Buenos Aires are *T. foetus*-infected, based on husbandry practices and herd demographics. Herds at a predicted high risk of infection may be selectively targeted for testing and eventual implementation of disease control strategies and as part of a surveillance and eradication program in the region.

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