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SHORT COMMUNICATION

Prevalence of *Tritrichomonas foetus* in beef bulls slaughtered at two abattoirs in northern Australia

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Bovine trichomoniasis, caused by the protozoal parasite *Tritrichomonas foetus*, is a highly contagious venereal disease characterised by early pregnancy loss, abortion and pyometra. Persistently infected bulls and cows are the primary reservoirs of infection in infected herds. This research investigated the prevalence of *T. foetus* infection in bulls from properties located across northern Australia and New South Wales. Preputial samples were collected from 606 bulls at slaughter and tested for *T. foetus* using the VetMAX-Gold Trich Detection Kit (Thermo Fisher Scientific). The apparent prevalence of *T. foetus* infection varied between regions, with northern regions in the Northern Territory, Queensland and Western Australia showing a prevalence of 15.4%, 13.8% and 11.4%, respectively. There was some evidence of an association between infection and postcode ($P = 0.06$) and increasing bull age ($P = 0.054$). This study confirms that *T. foetus* infection is likely to be present in many beef breeding herds and contributing to lower than expected reproductive performance, particularly across northern Australia.

Keywords bovine trichomoniasis; bovine venereal disease; economic loss; prevalence; productivity; *Tritrichomonas foetus*

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Bovine trichomoniasis is a venereal disease of cattle caused by *Tritrichomonas foetus*, an extracellular protozoan that colonises the preputial cavity and penis in males and the vagina and uterus in females.[1] Infected bulls are generally asymptomatic, whereas infection in females can result in returns to service and abortion,[1–3] with economic losses of up to 35% in return per cow in an infected herd over a breeding season based on modelling.[4] Visible impacts in some herds are limited to alterations in calving patterns, with the result that the disease is often overlooked.

The prevalence of *T. foetus* in bulls varies by age, breed, herd and herd management practices.[5] Young bulls are generally resistant to infection, while bulls older than 3–4 years are commonly persistently infected and become permanent sources of infection.[1] Relatively few control measures are available, especially in Australia where there are no commercially available vaccines. Diagnostic testing remains challenging with molecular tests offering minor improvements in speed and ability to detect infected cattle.[1,6] Effective control therefore relies on management practices including the exclusive use of younger bulls.[7]

There has been little awareness of the disease in Australia in recent years. Historically, bovine trichomoniasis varied from being uncommon in more intensive beef regions to common in northern Australia's extensively managed herds, with prevalences of 25% for bulls[3] and 66% for herds in some areas during the 1970s and 1980s.[8] Since then, the disease has received little attention, with reports limited to one study which found no evidence of infection in dairy herds in Victoria[9] and one report from a beef herd in the Northern Territory in recent years.[10]

This study aimed to estimate the prevalence of *T. foetus* infection in slaughtered beef bulls from Queensland, New South Wales, Northern Territory and Western Australia.

Genital tracts from bulls were sampled post mortem between October 2018 and August 2019 at an abattoir in Queensland sourcing bulls from New South Wales, the Northern Territory, South Australia and Queensland and an abattoir in Western Australia slaughtering bulls from northern Western Australia and Northern Territory. The required sample number (574) was computed using EPITOOL's prevalence calculator (https://epitools.ausvet.com.au/) with inputs of 0.05 and 0.1 for estimated proportion affected, 0.02 and 0.05 for precision and a confidence level of 0.95. The desired number of samples from the two abattoirs was set in proportion to 2018 bull population estimates for the respective feeder areas derived as described by Fordyce[11] (Appendix).

Samples were collected on days when abattoirs slaughtered larger numbers of bulls. The first and every second or third bull of a batch were sampled at the Queensland abattoir to increase the property spread. Consecutive bulls were sampled at the Western Australia abattoir.

For each bull sampled, the sheath containing the penis and prepuce was removed, and preputial smegma samples were collected within 2 h of slaughter. The preputial orifice was blotted free of

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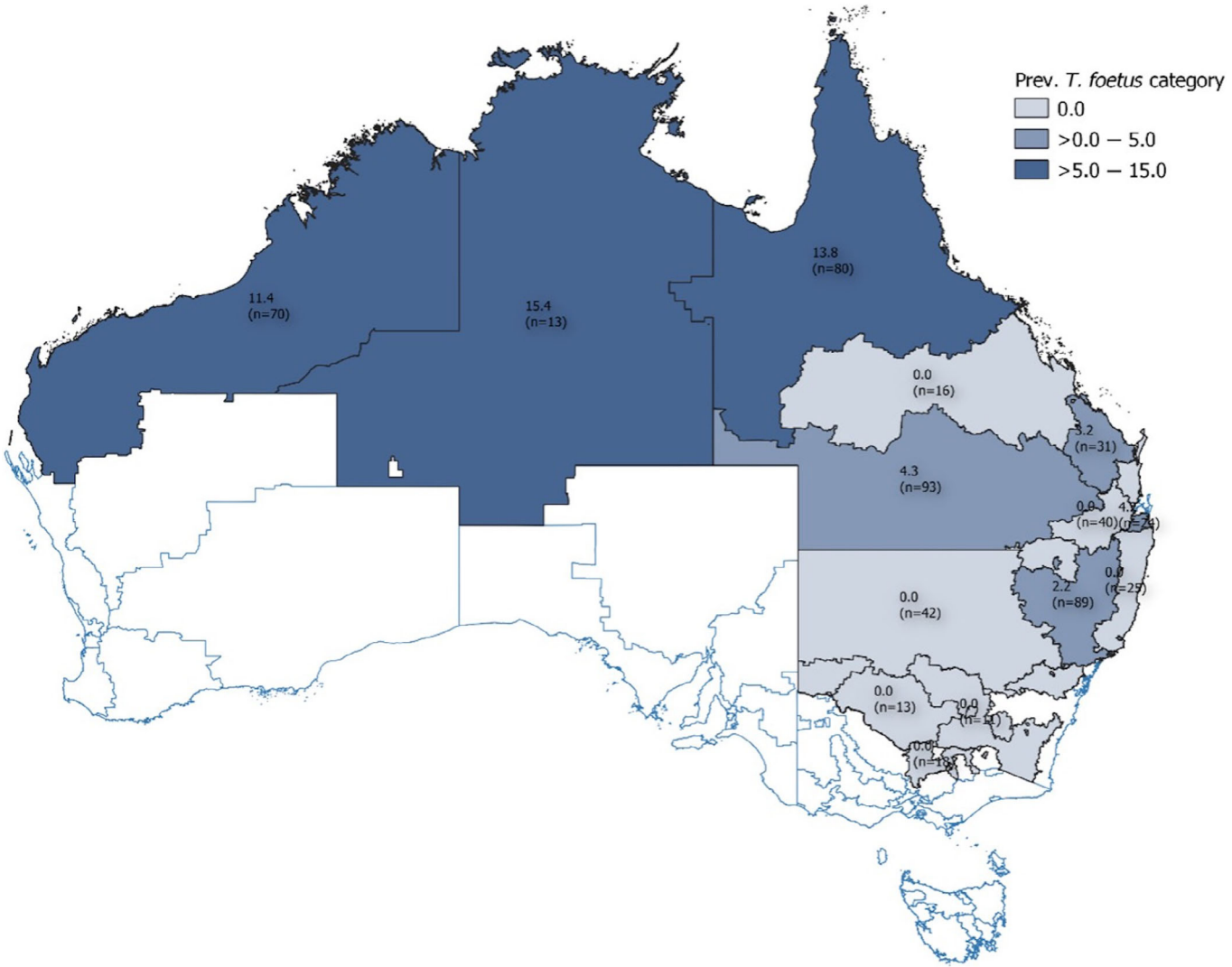

Figure 1. **Map of apparent prevalence (%) of *Tritrichomonas foetus* infection and number of bulls sampled for each postcode region of Australia sampled (scale 1:13000000).**

blood using a paper towel, and then one person held the preputial orifice while another inserted a Tricamper™ sampling device and scraped it backward and forwards approximately 8 times across the preputial mucosa and surface of the penis. The device head was agitated inside a 10 mL tube containing 4 mL Diamond's Modified media (*T. foetus* enrichment-culture media), cut off and the tube capped. Samples were incubated at 37°C for 24 hrs and then frozen at −20°C until shipment on dry ice to the Elizabeth MacArthur Agricultural Institute laboratory, Menangle, New South Wales, for analysis.

DNA samples for qPCR were extracted using a MagMAX™ CORE Nucleic Acid Extraction Kit, simple workflow (Thermo Fisher Scientific, Seventeen Mile Rocks, Queensland, Australia) and the KingFisher Flex semi-automated purification system (Thermo Fisher Scientific, Seventeen Mile Rocks, Queensland, Australia), following the manufacturer's instructions. Lysate aliquots (300 µL) were assayed using VetMAX-Gold Trich Detection Kit (Thermo Fisher Scientific, Seventeen Mile Rocks, Queensland, Australia) following the manufacturer's instructions, which included an internal DNA-positive control in each test sample. Real-time PCR was performed on an ABI7500Fast Real-Time PCR System. A no-DNA template control (nuclease-free water) was included in each run to control for extraction performance, inhibition and contamination. Assay results were interpreted following kit directions.

The body numbers of sampled bulls were matched with electronic slaughter data, providing details on property of origin and dentition for each bull. Each was classified to a region using the first two digits of the postcode for the property of origin (derived primarily from vendor identification details or the Property Identification Code on the National Livestock Identification Scheme database).

The apparent prevalence of *T. foetus* was calculated by dividing the number of positive bulls by the total number of bulls sampled

Table 1. Prevalence of *Tritrichomonas foetus* infection in different age groups based on dentition at slaughter

Dentition	Age in months	Bulls (n)	Prevalence	95% confidence intervals	
				Lower	Upper
0	0–18	37	0.0%	–	–
2	18–30	69	0.0%	–	–
4	24–36	48	2.1%	0.3%	13.4%
6	30–42	55	1.8%	0.3%	11.8%
8	36+	397	6.8%	4.7%	9.8%

expressed as a percentage. Logistic regression was used to assess associations between age (based on dentition) and property location and *T. foetus* test results. All models incorporated a random effect coding for property identity to adjust for correlations between bulls within the same property. Estimated prevalences for the levels of each variable of interest were generated by utilising the margins function. The transform_margins function was subsequently specified to generate log-transformed confidence intervals to avoid sub-zero estimates. All analyses were conducted in Stata, version 16.1 (www.stata.com).

A total of 606 bulls were sampled: 536 and 70 from the Queensland and Western Australia abattoirs, respectively. A postcode could be defined for 96% (583/606) of bulls sampled. Bulls sampled represented 134 unique properties across 17 postcode regions, with between one (50% of properties) and 48 bulls sampled per property. Two regions with one bull sampled from each were excluded from regional prevalence determination ('25' in New South Wales and '41' in Queensland).

Overall, 29/606 (4.8%) bulls tested positive for *T. foetus*. After adjusting for clustering at the property level, the estimated apparent prevalence was 1.9% (95% confidence interval 0.6% to 5.4%), reflecting marked clustering of infection at the property level.

The apparent prevalence of *T. foetus* infection in bulls for 15 postcode regions is presented in Figure 1. In three regions of northern Australia, the apparent prevalence of infection was greater than 10%.

There was some evidence of an association between post code region and infection ($P = 0.06$) after adjusting for clustering at the property level, with higher prevalences in northern regions.

There was some evidence of a positive association between bull age (estimated by dentition) and infection ($P = 0.054$) as shown in Table 1. There was no evidence of infection in bulls with dentition 2 or less (below 30 months of age) and a prevalence of 6.8% in bulls with dentition 8 (over 3 years of age). This concurs with previous findings of increased prevalences in older bulls.[12, 13]

This study confirms that *T. foetus* infection of bulls is present in the major beef breeding regions of Australia and that prevalences in northern areas have not declined over three decades despite the lack of reports in the interim. Bovine trichomoniasis is likely to have a major negative effect on reproductive fecundity in these areas and justifies further research and consideration of currently available control measures such as annual diagnostic testing of bulls over 3 years of age and culling of infected bulls. The absence of an effective commercially available vaccine is a major constraint to effective control of this venereal infection in Australia. The importance of the infection should be further investigated and preventative strategies employed.

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Conflicts of interest

The authors declare no conflict of interest for the work presented here.

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APPENDIX

Table A1. Estimated number of bulls mated in Australia from Fordyce et al[11]

	Number of bulls
State/territory distribution	
Queensland	185,310
New South Wales	65,621
Victoria	26,773
Northern Territory	35,841
South Australia	12,686
Western Australia	35,660
Tasmania	4984
Total (Australia wide)	366,875
Regional distribution	
South and East Australia (New South Wales, Victoria, South Australia and Tasmania)	110,064
North and Western Australia – nutritionally endowed zones	124,147
North and Western Australia – nutritionally nonendowed zones	132,663
Total (Australia wide)	366,874