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Full Length Research Paper

Prevalence of *Campylobacter foetus* and *Trichomonas foetus* among cattle from Southern Africa

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Trichomoniasis and campylobacteriosis are diseases caused by *Trichomonas foetus* and *Campylobacter foetus* respectively. These diseases pose economic losses due to infertility and abortion. The aim of this retrospective study was to estimate the prevalence of *C. foetus* and *T. foetus* among southern African cattle. Sheath washings and scrapings were subjected to polymerase chain reaction (PCR) for diagnosis of these microorganisms. Out of 3, 458 samples that were tested for *T. foetus*, 142 (4.1%) were positive. *Campylobacter foetus* was detected in 60 of the 3, 161 (1.9%) samples. The use of PCR was convenient for estimating the prevalence of *C. foetus* and *T. foetus.*

Key words: Species-specific PCR, diagnosis, venereal disease, *Campylobacter foetus*, *Trichomonas foetus.*

INTRODUCTION

Trichomoniasis and campylobacteriosis are venereally - transmitted diseases that are caused by parasitic protozoa *T. foetus* and *C. foetus* subspecies *venerealis* bacteria respectively. These sexually transmitted diseases continue to pose economic losses in Asia, America and southern Africa due to infertility and abortion (Kitching, 1999; Brookes et al., 2004). In southern Africa, campylobacteriosis is one of the most significant infectious causes of reproductive disorders such as poor calving (Schmidt et al., 2010). *C. foetus* subspecies *venerealis* is responsible for sporadic abortions, embryonic mortality and irregular oestrus cycles (Irons et al., 2004; OIE, 2008). Bulls that are infected with *C. foetus* subspecies *venerealis* are usually asymptomatic carriers and they pose a great challenge among herds as they infect cows during coitus (Garcia et al., 1983).

As numerous cattle are asymptomatic, accurate detection of infected animals is important for curbing the spread of *C. foetus* subspecies *venerealis* and *T. foetus* among herds (Eaglesome and Garcia, 1992). This scenario highlights the necessity for rapid and accurate diagnosis of *C. foetus* subspecies *venerealis* and *T. foetus*. The diagnosis of campylobacteriosis is usually based on analysis of samples taken from bulls, cows or aborted foetuses using classical microbiological methods, which involve cultivation, isolation and identification of *C. foetus* subspecies *venerealis* using biochemical tests (Brookes et al., 2004). The outcome of these classical methods relies on sampling techniques and transport media used to maintain these fastidious and fragile baceria (Monke et al., 2002). Diagnosis of *C. foetus* subspecies *venerealis* using conventional microbiological techniques is challenging due to low discriminatory power of biochemical tests and reduced viability of the microorganisms. Likewise, *T. foetus* may be mistaken for similar trichomonadid protozoa when light microscopy is used for identification (van Bergen et al., 2005). In South Africa, one of the challenges associated with maintaining the viability of *C. foetus* has been exacerbated by the extensive size of the breeding farms and the long duration required before sample analysis is conducted due to long travelling distances (Schmidt et al., 2010). These challenges regarding diagnosis may be circumvented by

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using polymerase chain reaction (PCR) (Schmidt et al., 2010). Some of the advantages of direct amplification of *C. foetus* DNA over classical microbiological methods are speed; specificity, sensitivity and the ability to detect non-viable bacteria (Willoughby, 2005). Even so, differentiation of the subspecies of *C. foetus* has proved to be challenging.

Little is known about the extent of *T. foetus* and *C. foetus* infection in southern African cattle. As trichomoniasis and campylobacteriosis are generally not associated with clinical illness and cattle may remain asymptomatic, it is paramount to know the extent to which *C. foetus* and *T. foetus* are circulating in herds. Therefore, the aim of this retrospective study was to estimate the prevalence of *C. foetus* and *T. foetus* among cattle in southern African countries based on validated species-specific PCR.

MATERIALS AND METHODS

Data collection

The Bacterial PCR laboratory of the ARC-OVI holds records of species-specific-PCR for sheath washings and scrapings that were examined for *T. foetus* and *C. foetus*. This retrospective study examined the results of species-specific PCR that was used for diagnosis of *T. foetus* and *C. foetus*. The 3 458 samples for diagnosis of *T. foetus* were obtained from South Africa (n = 1 999), Namibia (n = 1 201), Botswana (n = 210), Zambia (n = 41) and Swaziland (n = 7) from January 2009 to May 2010. A total of 3, 161 samples were subjected to diagnosis of *C. fetus* (South Africa n = 1 912; Namibia n = 1 201; Zambia n = 41; Swaziland n = 7). The samples were examined for *T. foetus* and *C. foetus* within 72 h of collection in accordance with the standard operating procedures of the Bacterial PCR laboratory of the ARC-OVI. The data excludes sheath washes and scrapings that were submitted for culture to minimize the bias that may be introduced due to any of the following reasons:

1. limited sensitivity of culture-based methods for diagnosis of *T. foetus* and *C. foetus*;
2. Pseudoreplication for diagnosis of *T. foetus* and *C. foetus* for the same samples that were examined in the General bacteriology laboratory of the ARC-OVI.

Species-specific polymerase chain reaction

For diagnosis of *T. foetus*, 3, 458 tests were performed whilst 3, 161 analyses were carried out for *C. foetus* from sheath washes and sheath scrapings that were submitted to the PCR laboratory of the ARC - OVI from January 2009 to May 2010. Extraction of template DNA from sheath washes and scrapings was performed using the Silica-Guanidium thiocyanate method as described by Mukhufhi et al. (2003). Amplification of the C. *foetus* targeting 16S rRNA gene variable regions of the template DNA was conducted using a method that was developed by Oyarzabal et al. (1997) and the protocol was validated at ARC-OVI. Primers CF441F: 5'-gttagggaagaacaatgacgg-3' and CF995R: 5'-ttatctctaagagattagttgg-3' were used in the species-specific PCR for amplification of *C. foetus* (Oyarzabal et al., 1997). Amplification of *T. foetus* was done using MTFR3-F: 5'-cgggtcttcctatatgagacagaacc-3' and MTFR4:5'-cctgccgttggatcagtttcgttaa-3' (Felleisen et al., 1998). The amplification programme for *C. foetus* and *T. foetus* was as follows: Initial denaturation: 95°C for 15 s, followed by 35 cycles of denaturation at 94°C for 30 s, annealing: 60°C for 30 s, extension: 72°C for 30 s and final extension at 72 °C for 7 min. Reference strains were included alongside DNA from field samples. The PCR amplicons were analysed by electrophoresis through 1.5% ethidium bromide stained agarose (0.5 mg/ml) gels, followed by observation under ultraviolet light. The amplicon sizes were determined against a 100 bp-DNA ladder (Fermentas).

RESULTS AND DISCUSSION

The species-specific PCR for diagnosis of trichomoniasis and campylobacteriosis was rapid and specific. The PCR specificity was determined in the ARC-OVI PCR laboratory in previous studies (Mukhufhi et al., 2003). Expected band sizes of approximately 554 bp and 347 bp for *C. foetus* and *T. foetus* respectively were observed (Figure 1). Out of the 3, 458 samples that were tested for *T. foetus*, 142 (approximately 4.1%) were positive. *Trichomonas foetus* was not detected from the samples from Swaziland (7 samples) and Zambia (41 samples). Out of the 210 samples from Botswana, 7 (3.33%) tested positive for *T. foetus*. A total of 90 of the 1 999 samples (4.50%) from South Africa and 45 of the 1, 201 (3.75%) samples from Namibia were positive for *T. foetus*. The average prevalence of *C. foetus* was 1.9%, with 60 samples out of 3 161 testing positive. The *C. foetus* prevalence for Zambia was 2.44% (1 out of 41), followed by Namibia with 2.08% (25 out of 1, 201) and South Africa showed the least prevalence of 1.78% (34 out of 1 912). No *C. foetus* was detected from the 7 samples from Swaziland. There were no samples for diagnosis of *C. fetus* from Botswana.

This study highlighted the presence of *T. foetus* and *C. foetus* in southern African cattle, which is important for management of breeding programmes. These measures include biosecurity, elimination of the infected animals after identification and vaccination to elicit resistance. This can significantly reduce delayed conception, embryonic or foetal deaths.

The circulation of *C. foetus* in South African animals has been demonstrated in previous studies. For instance, Schmidt et al. (2010) observed a prevalence of 4.2% in 212 diagnostic samples that were analysed for *C. foetus* over a ten month period from South Africa and were tested using PCR. Our results are further corroborated by the observations of Irons et al. (2004) who indicated that the prevalence of *C. foetus* in South Africa is low. Nevertheless, the estimated prevalence is adversely affected by the challenges associated with diagnosis of *T. foetus* and *C. foetus*, (Schmidt et al., 2010); hence, it is tempting to interpret these results with caution. For instance, it would be interesting to determine the prevalence of the *T. foetus* and *C. foetus* for samples that are collected with improved sampling procedures that make

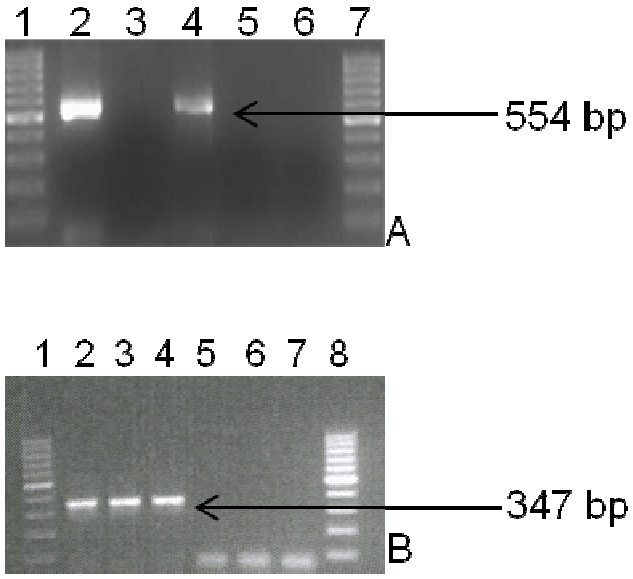

Figure 1. Typical gel picture showing PCR amplicons of C. foetus (A: Lanes: 1 and 7; 100 bp DNA ladder, lane 2; positive field sample; lanes 3, 5 and 6; negative field samples and lane 4; positive control) and T. foetus (B: Lanes 1 and 8; 100 bp DNA ladder, lane 2; positive control, lanes 3 and 4; positive field samples , lane 5; negative control, lanes 6 and 7; negative field isolates).

use of the tricamper (Lew et al., 2006).

The impact of bacterial contaminants on the species-specific PCR for diagnosis of *T. foetus* and *C. foetus* was not determined in this retrospective study. Other studies indicated that DNases produced by some bacteria could negatively affect the PCR results (Gilardi, 1985). However, the studies by Schmidt et al. (2010) suggested that such an occurrence is unusual. As the samples from this retrospective study include other countries in southern Africa, it is challenging to directly compare the prevalences with those obtained by Schmidt et al. (2010).

Taken together, the results of this retrospective study prompt comprehensive research regarding the prevalence of *T. foetus* and *C. foetus*, particularly among rural communities and emerging farmers in southern Africa. This includes active regular surveillance of cattle that are slaughtered at abattoirs and investigating a broader spectrum of pathogens that cause abortions. These include *Brucella* spp, *Listeria monocytogenes*, *Leptospira* spp., *Chlamydia* spp. and some viruses. The surveys should take into consideration the factors that may influence prevalence of microorganisms.

These factors include herd size, management factors, age, geographical area, improved sampling and sensitive diagnostic tests. In addition, well-designed cross-sectional studies will be important for providing accurate prevalence estimates in future. The information generated from such comprehensive studies could be translated to management decisions.

Conclusion

The use of PCR was convenient for estimating the prevalence of *C. foetus* and *T. foetus* in southern African countries. The prevalence of *C. foetus* and *T. foetus* among cattle in southern Africa warrant further investigations in the form of well-designed cross-sectional studies and regular surveys in order to improve intervention strategies.

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