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Short communication

Anaplasma infection prevalence in beef and dairy cattle in the south east region of Botswana

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ABSTRACT

Infection of cattle by the tick-borne intra-erythrocytic bacteria of the genus *Anaplasma* occurs worldwide. Nevertheless, prevalence rates in specific regions are still required to inform cattle farming management decisions. A study was carried out to determine *Anaplasma* infection prevalence in beef and dairy cattle in the south east region of Botswana. Two methods were used: competitive inhibition enzyme-linked immune-sorbent assay (cELISA) and conventional polymerase chain reaction (PCR). A total of 429 cattle consisting 207 beef and 222 dairy animals were sampled and tested. The prevalence was 91% and 31% by cELISA and PCR, respectively. A Z *test* revealed a statistical difference between the point prevalence as determined by cELISA compared to PCR ($p = 0$). There was no statistical difference between the point prevalence of *Anaplasma* infection as determined by cELISA ($p = 0.45$) between beef and dairy cattle. But there was a significant difference ($p = 0.001$) between the animals by PCR with the prevalence in beef cattle nearly double that in dairy cattle. Individual herd prevalence ranged from 79% to 100% by cELISA, and 0 to 100% by PCR. Though not statistically significant seroprevalence in both beef and dairy animals tended to be higher in urban/peri-urban areas compared to rural areas. The cELISA mean percentage inhibition (PI) for all cattle was found to be 58.6 (95% CI: 56.8–60.4). There was no statistically significant difference between the mean PI of sera from beef cattle (56.4 (95% CI: 54.1–58.7)) as compared to dairy cattle (60.7 (95%CI: 58.0–63.3)). However, there was a tendency towards statistical significance with beef animals having a lower PI value than dairy animals. *Anaplasma* infection was endemic in cattle in the south east region of Botswana with similar infection in beef and dairy animals. Further research should be done to identify the strains prevalent in the cattle herds.

1. Introduction

Tick transmitted bacterial pathogens cause important diseases of animals and humans worldwide. Several tick-borne diseases of ruminants are endemic in the tropics and sub-tropics. The most prevent tick-borne disease in cattle, is caused by *Anaplasma* species with *Anaplasma marginale* (the type species) manifesting the most severe disease (Palmer, 1989; Dumbler et al., 2001). Anaplasmosis rarely causes dramatic mortalities but significantly limit production with a negative impact on weight gain, milk yield, and fertility (Kocan et al., 2010; Suarez and Noh, 2011). Animals that survive the acute phase of the disease develop persistent infection (Kocan et al., 2003). Thus the economic impact of the disease at both farm and the national level, could be underestimated.

Knowing disease prevalence is essential for herd health management. Infection prevalence of *Anaplasma* organisms in cattle herds is variable. Most studies report a prevalence, as determined by ELISA, of around 30% in herds with endemic anaplasmosis (Palmer et al., 2004; Marufu et al., 2010; Fosgate et al., 2010). However the prevalence can approach 100% as was reported in South Africa (Mtshali et al., 2007; Khumalo et al., 2016), and Madagascar (Pothmann et al., 2016). Being tick transmitted, *Anaplasma* infections should reflect tick control practices in the herd. Thus increased exposure to ticks should result in increased infection prevalence. Variables such as dairy versus beef breed as is the difference in husbandry practices between such breeds could result in differences in infection prevalence (Simuunza et al., 2011). In the current study, we used two methods to determine *Anaplasma* infection prevalence in beef and dairy cattle in the south east region of Botswana. The first method was competitive inhibition enzyme-linked immune-sorbent assay (cELISA). This method has been

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shown to have a high sensitivity for detection of *Anaplasma* infection in cattle (Coetzee et al., 2007). The second method was conventional polymerase chain reaction (PCR). Albeit with comparative lower sensitivity than cELISA, PCR serves as a confirmatory test for *Anaplasma* infection. The study provides insight into the nature of tick-borne *Anaplasma* infection in cattle and forms a basis for further work to identify strains present in the cattle herds.

2. Materials and methods

2.1. Study area and sampling design

The south east region of Botswana is sub-tropical, characterised by dry and semi-arid climate, erratic and variable rainfall averaging 600 mm per annum between September and March. According to Agricultural Census Report (2004), there were 647,697 cattle in the region of which 641,766 were beef and 5931 were dairy animals. Dairy breeds consist of Friesian, Jersey and Brown Swiss. Beef breeds are mainly Brahman, Semimetal, Tuli, Afrikaner, Charolaise, indigenous Tswana, and crossbreeds.

The study was designed to test beef and dairy herds for *Anaplasma* infection. Sampling was carried out during the warm season with sporadic rainfall (October 2014 to March 2015). Four beef herds were sampled in Gaborone, Otse, Lobatse and Ramatlabama (Fig. 1). Seven dairy farms participated in the study and were located in Gaborone, Moleplolole, Gabane, Thamaga, and Lobatse farms. Seventy five to 100% of cattle (≥ 6 months of age) in each herd or paddock were tested (Table 1). For dairy animals, the sample was taken from the whole herd in the farm whereas for beef animals the sample was taken from one paddock selected by the farm manager. Overall whole blood and serum samples were collected from 429 cattle consisting 207 beef and 222 dairy cattle. The blood was collected from either the jugular or tail vein.

Table 1
Cattle tested for *Anaplasma* infection in the south east region of Botswana.

FARM	Location	Breed(s) of animals	Herd or paddock size[a]	Sample size (% paddock or herd)
Beef 1	Gaborone (urban)	Tswana, Tuli, cross- breeds	60	50 (83)
Beef 2	Otse (rural)	Cross-breeds	65	50 (77)
Beef 3	Lobatse (rural)	Brahman	68	52 (76)
Beef 4	Ramatlabama (rural)	Brahman, simmental, cross-breeds	70	55 (79)
Dairy 1	Gaborone (urban)	Friesian	55	42 (76)
Dairy 2	Gaborone (urban)	Friesian	65	59 (91)
Dairy 3	Molepolole (urban)	Brown Swiss	26	23 (88)
Dairy 4	Molepolole (urban)	Friesian and Jersey	10	10 (100)
Dairy 5	Gabane (rural)	Jersey	36	29 (81)
Dairy 6	Thamaga (rural)	Friesian	32	24(75)
Dairy 7	Lobatse (peri-urban)	Friesian	47	37(79)

[a] Herd size applies to dairy animals. For beef animals the sample was taken from a paddock in the farm.

2.2. Competitive inhibition enzyme-linked immune-sorbent assay (cELISA)

Sera from all the cattle were tested using *Anaplasma* antibody test kit (VMRD inc. Pullman, WA, USA) according to the manufacturer's instructions. In this test, *Anaplasma* antigen (recombinant major surface protein (rMSP5)) is immobilised in wells. Sera from *Anaplasma* infected animals result in a high percentage inhibition (PI) of the enzyme reaction by binding to rMSP5 in the wells. A PI ≥ 30 was considered positive according to the manufacturer's instructions. The sero-prevalence (% positive) of beef cattle, dairy cattle, and all cattle was

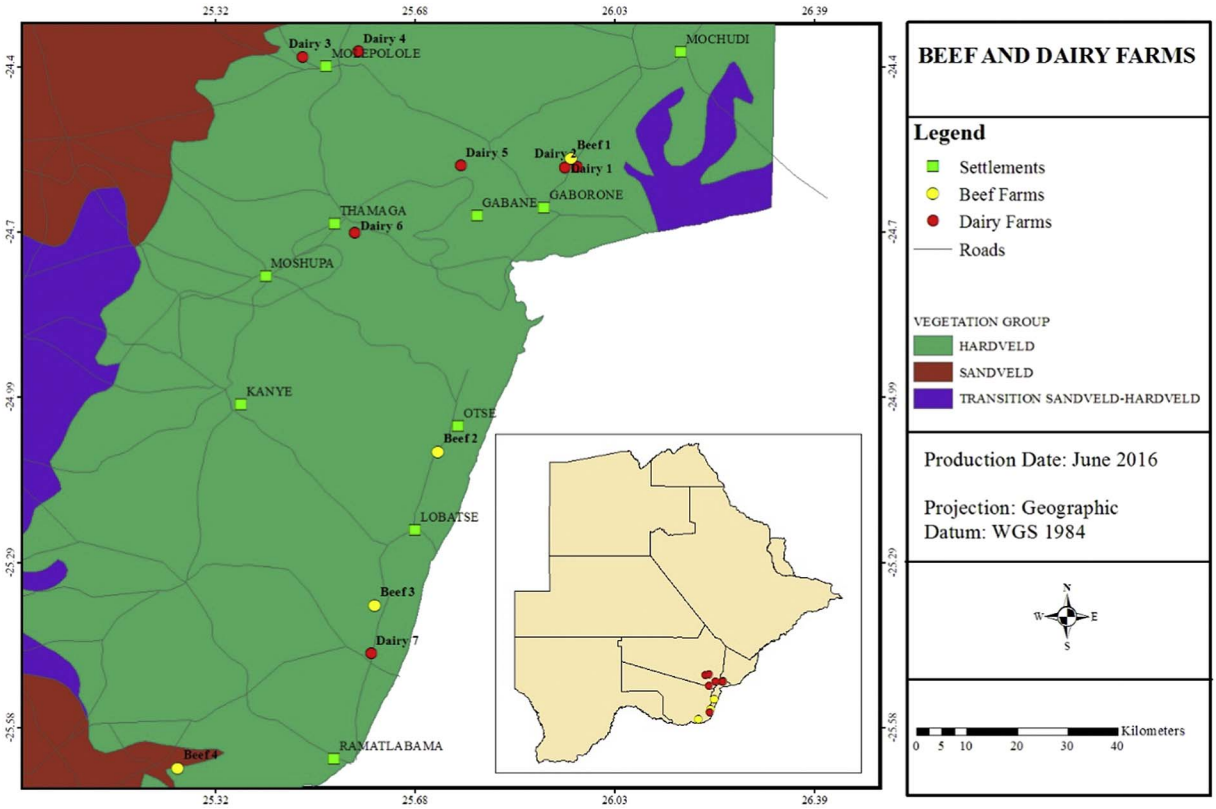

Fig. 1. Locations of the 11 herds of cattle tested for *Anaplasma* infection in the south east region of Botswana.

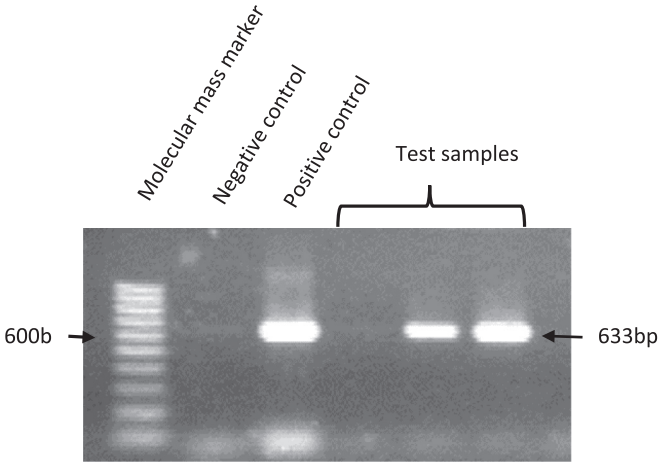

Fig. 2. Detection of *Anaplasma* infection in cattle using MSP5 based PCR.

calculated using simple arithmetic. Further, the mean PI values at 95% confidence interval for each beef farm, each dairy farm, all beef farms together, all dairy farms together, and all cattle were determined. The frequency distribution of PI values for all cattle was determined.

2.3. Polymerase chain reaction (PCR)

Genomic DNA extracted from all the cattle using Quick-gDNA™ Miniprep (Zymo Research Corp. Irvine, California, USA) was used as template in a conventional PCR using the following primers; forward 5′-ATG AGA ATT TCA AGA TTG TGT CT-3′, reverse 5′-CTA AGA ATT AAG CAT GTG ACC GCT G-3′. The PCR clones a 633 bp single copy gene major surface protein 5 (MSP5) that is highly conserved in the genus *Anaplasma* (Visser et al., 1992). Amplification consisted of 5 min incubation at 95 °C, 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 2 min at 72 °C, and an additional 10 min at 72 °C. A cow previously tested positive by both cELISA and PCR was used as a positive control (Fig. 2). The PCR products were size-separated in 1% agarose gel, stained with ethidium bromide, and visualized with a UV-illuminator.

3. Results

3.1. Overall Anaplasma infection prevalence

The overall *Anaplasma* infection prevalence in cattle was found to be 91% and 31% by cELISA and PCR respectively (Table 2). Comparison using a Z test revealed statistical difference between the point prevalence of *Anaplasma* infection in the cattle as determined by cELISA compared to PCR ($p = 0$).

3.2. A comparison of Anaplasma infection between beef and dairy herds

Anaplasma infection prevalence in beef cattle was found to be 90%, and 43% when determined by cELISA and PCR respectively (Table 2). In dairy cattle, the prevalence was 92% by cELISA and 21% by PCR. There was no statistical difference between the point prevalence of *Anaplasma* spp. as determined by cELISA ($p = 0.45$) between beef and dairy cattle. Interestingly, there was statistical difference ($p = 0.001$) when comparing the infection prevalence between the breeds by PCR with the prevalence in beef cattle nearly double that in dairy cattle.

3.3. Herd by herd comparison of Anaplasma infection prevalence

The range of results was different when *Anaplasma* infection in the herds was tested by cELISA (79%–100%) or PCR (0–100%) (Table 2). Hundred percent sero-conversion was detected in one beef and two dairy herds. It was also found that two dairy herds were negative for infection by PCR whereas all the four beef herds tested were positive by PCR. Although not statistically significant, sero-conversion in herds that were in urban/peri-urban areas tended to be higher than those that were in rural areas. Conversely, PCR detection of *Anaplasma* infection tended to be lower in herds that were in urban/peri-urban areas compared to those in rural areas (Table 2).

Table 2
Anaplasma infection prevalence (% positive), determined by cELISA and PCR in beef and dairy cattle in the south east region of Botswana.

Factors	Number of cELISA + ve animals	Proportion cELISA + ve (%)	Number of PCR + ve animals	Proportion PCR + ve (%)
All cattle	383	91	135	31
Breed				
Beef	180	90	89	43
Dairy	203	92	46	21
Source				
Urban/peri-urban Beef 1, Dairy 1–4 & 7	208	92	62	26
Rural Beef 2–4, Dairy 5 & 6	175	85	73	35
Farms				
Beef 1	48	100	23	46
Beef 2	46	90	24	44
Beef 3	44	88	32	64
Beef 4	42	81	10	19
Dairy 1	40	95	23	55
Dairy 2	56	95	9	15
Dairy 3	23	100	5	22
Dairy 4	8	100	0	0
Dairy 5	24	83	7	24
Dairy 6	19	79	0	0
Dairy 7	33	92	2	5

3.4. Nature of humoral response of the herds to Anaplasma infection

The cELISA mean percentage inhibition for all cattle was found to be 58.6 (95% CI: 56.8–60.4). There was no statistically significant difference between the mean percentage inhibition of sera from beef cattle (56.4 (95% CI: 54.1–58.7)) as compared to dairy cattle (60.7 (95%CI: 58.0–63.3)) (Table 3). However, there was a tendency towards statistical significance with beef animals having a lower mean PI value than dairy animals. Two groups of farms were identifiable. One group had PI values that were higher at 95% level and it consisted of dairy farms in Gaborone and Molepolole and a beef farm in Gaborone (Table 3). The other group had lower PI values. This group consisted of dairy farms in Gabane, Thamaga and Lobatse and the rest of the beef farms. A graphical representation of PI values of all cattle had a pattern similar to those previously reported (Knowles et al., 1996; Molloy et al., 1999) (Fig. 3).

4. Discussion

Anaplasma infection in cattle is common and world-wide. However, determination of its prevalence is important to inform herd health management in both beef and dairy farms. In the current study, two test methods established an endemic status in beef and dairy herds in the south east region of Botswana consistent with previous findings in the United States and South Africa (Palmer et al., 2004; Mtshali et al., 2007; Coetzee et al., 2010). The difference in prevalence between cELISA (91%) and PCR (31%) was attributable to the variation of both the

Table 3
cELISA percentage inhibition (Mean and 95% confidence interval) of sera from beef and dairy cattle in different locations in the south east region of Botswana.

FARMS	Location	Sample size	cELISA percentage inhibition	
			Mean	95% CI
BEEF				
Beef 1	Gaborone (urban)	50	65.7[a]	62.8–68.7
Beef 2	Otse (rural)	50	56.1[b]	51.7–60.5
Beef 3	Lobatse (rural)	52	53.1[b]	48.4–57.9
Beef 4	Ramatlabama (rural)	55	51.1[b]	46.1–56.1
All beef		207	56.4	54.1–58.7
DAIRY				
Dairy 1	Gaborone (urban)	42	72.6[a]	66.5–78.7
Dairy 2	Gaborone (urban)	59	72.6[a]	66.5–78.7
Dairy 3	Molepolole (urban)	23	69.4[a]	65.4–73.4
Dairy 4	Molepolole (urban)	8	64.5[ab]	52.7–76.2
Dairy 5	Gabane (rural)	29	51.7[b]	43.9–59.6
Dairy 6	Thamaga (rural)	24	49.2[b]	40.7–57.7
Dairy 7	Lobatse (peri-urban)	37	54.2[b]	48.2–60.2
All dairy		222	60.7	58.0–63.3

[ab] Means within the same column with different letters differ at 95% confidence interval.

sensitivities and specificities of the tests (Reinbold et al., 2010; Pascale and Paré, 2012). The cElISA results place the region in the category of high level prevalence comparable to findings in Costa Rica (Shebish et al., 2012) and part of Texas in the USA (Hairgrove et al., 2015). High prevalence of *Anaplasma* infection is indicative of endemic stability (Alfredo et al., 2005; Tembue et al., 2011) unless in the phase of disease outbreak. The PCR result is consistent with endemic status reported in the USA, South Africa, and Puerto Rico (Palmer et al., 2004; Marufu et al., 2010; Fosgate et al., 2010). Conventional PCR is prone to false negatives, in particular during the period when the organism is suppressed by the immune system. Nevertheless PCR has the value of confirming *Anaplasma* infection in a herd.

There was no statistical difference between *Anaplasma* infection prevalence between beef and dairy cattle as determined by cELISA. This suggests that beef and dairy animals are equally susceptible to *Anaplasma* infection. Interestingly, there was statistical difference ($p = 0.03$) in the infection prevalence between the animals as determined by PCR with the prevalence in beef cattle nearly double that in dairy cattle. Dairy cattle had a slightly higher PI value (60.7 (95%CI: 58.0–63.3)) than beef cattle (56.4 (95% CI: 54.1–58.7)). The difference was not statistically significant at 95% confidence level yet it shows that dairy cattle mounted a stronger humoral response. The immune response could have suppressed *Anaplasma* organisms in the blood to a level below detection by PCR, in some of the animals as was suggested by Hairgrove et al. (2015). Alternatively, some cELISA positive animals were infected with unknown rickettsial organisms. Antibodies to such organisms would cross-react with cELISA but would be PCR negative. Notably, clinical anaplasmosis had not been reported in all the beef and dairy herds tested. In beef animals, persistent *Anaplasma* infection is not associated with decreased production (Van Donkersgoed et al., 2004). On the other hand anecdotal evidence suggests that the infection is production limiting particularly in dairy herds.

Notably, herds in urban areas (Gaborone and Molepolole) tended to have higher PI values irrespective of whether they were beef or dairy than those that were in rural areas. Presumably, these herds received a higher tick challenge and consequently more frequent pathogen challenge resulting in higher antibody titres. There was also a tendency for dairy herds to have higher PI values than beef herds which correlated with dairy animals having a lower *Anaplasma* prevalence by PCR as compared to beef animals. Simuunza et al. (2011) noted that strong immunity conferred by repeat challenge may keep parasitaemia below PCR detection limit. Previously, Tebele et al. (1991) reported a negative correlation of high antibody titres with indicators of parasitaemia including packed cell volume and haemoglobin. In addition, the profile of PI values from cattle in the south east region of Botswana was similar to that reported previously by other workers (Knowles et al., 1996; Torioni de Echaide, et al., 1998; Molloy et al., 1999) and is consistent with *Anaplasma* infection that is endemic.

5. Conclusion

We conclude that there is a high level prevalence of *Anaplasma* infection, as determined by cELISA in both beef and dairy cattle in the south east region of Botswana. In the absence of disease the high prevalence represents endemic stability. Dairy animals tend to have higher antibody titres represented by higher mean PI values in cELISA compared to beef animals. The PCR detection level of *Anaplasma* infection in dairy cattle was lower than in beef cattle attributable to suppression of the organism in the blood by high circulating antibody levels. Interestingly cattle in urban areas whether beef or dairy, tended to have higher mean PI values compared to those in rural areas. Further work is needed to identify strains of *Anaplasma* organisms endemic in the cattle herds and to determine the presence and distribution of competent tick vectors.

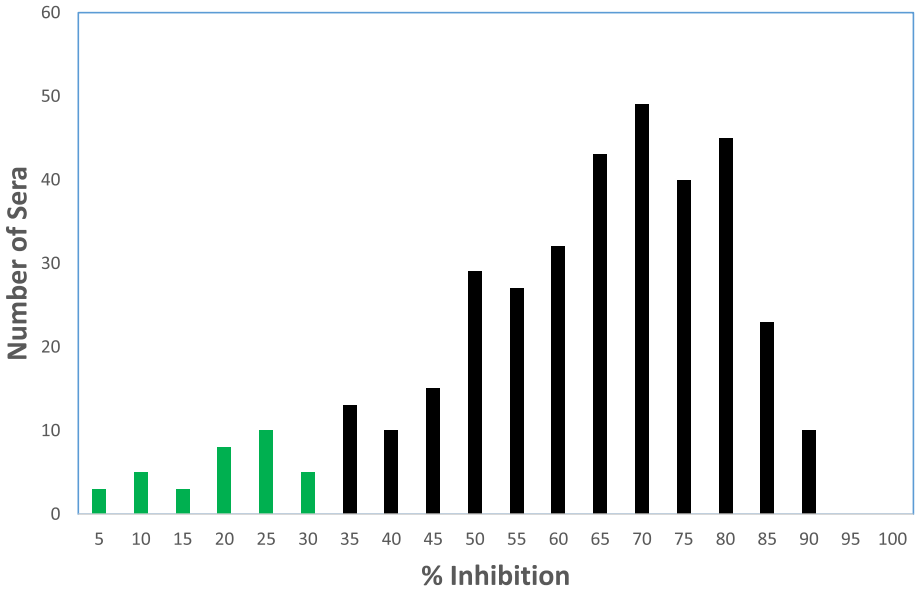

Fig. 3. Frequency distribution of cELISA percentage inhibition values of sera from cattle (207 beef and 222 dairy) in the south east region of Botswana. Green bars represent negative result. Black bars represent positive result. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Conflict of interest

We declare that we do not have conflict of interest and there is no private company or special interest group associated with the study except the institution where the study was carried out.

Ethical statement

As the principal investigator I would like to state that the handling of animals during the performance of this study, met the terms of the International guiding principles for biomedical research involving animals. The study was under the auspices of the Animal Care and Use Committee (ACUC) of the University of Botswana.

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