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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

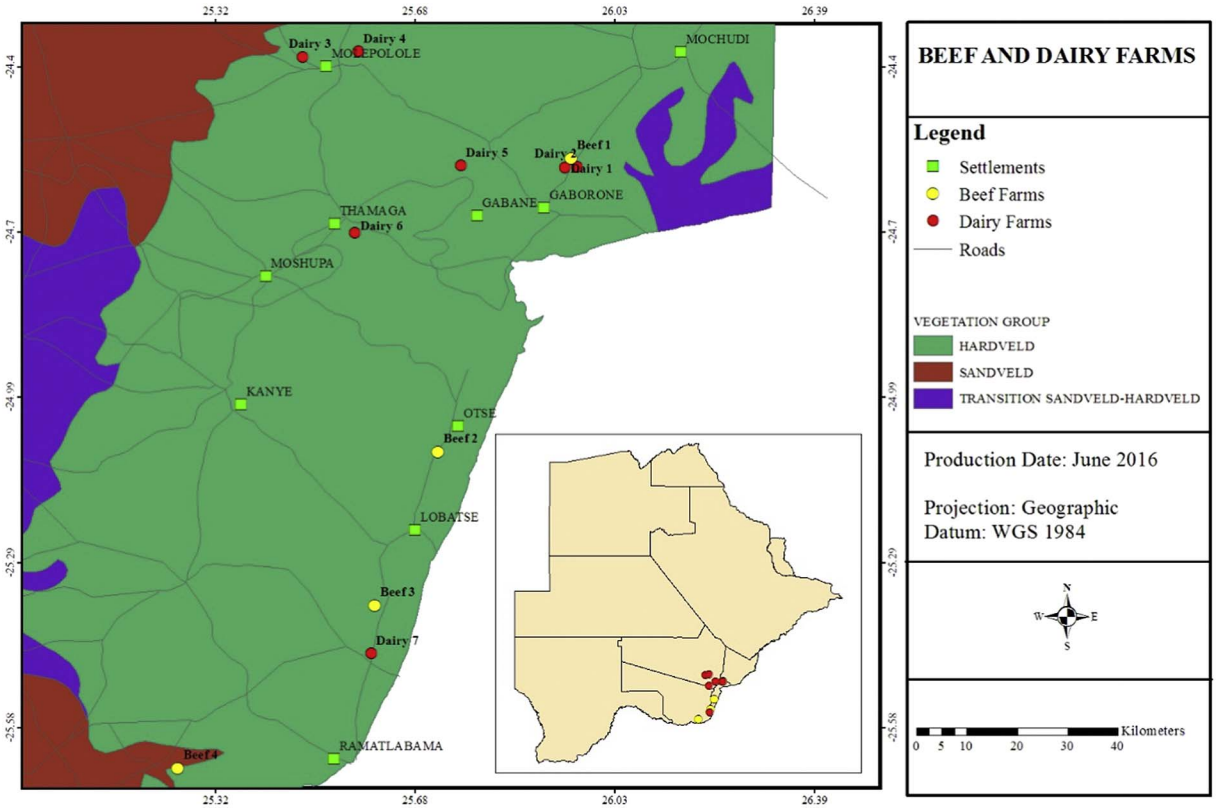

Fig. 1. Locations of the 11 herds of cattle tested for *Anaplasma* infection in the south east region of Botswana.