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Research Article

Evaluation of Haematological Parameters in Cattle, Detection, and Confirmation of Cattle *Anaplasma Marginale* Infection at BUAN Farm in the Southeast Region of Botswana

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Abstract

Anaplasmosis is a tick-borne disease caused by obligate intercellular gram-negative bacteria, *Anaplasma spp*. The present study evaluates dairy and beef cattle blood parameters at Botswana University of Agriculture and Natural Resources to determine *Anaplasma* infection in the herds. Thirty (30) dairy animals and 30 beef animals were randomly selected and tested for blood parameters using a haematology analyser and microscopic examination of Giemsa-stained blood smears was used to identify blood parasites. Subsequently, 48 dairy and 48 beef animals were randomly selected and tested for *Anaplasma* infection using competitive inhibition enzyme-linked immunosorbent assay (ci-ELSA). To confirm *Anaplasma* infection 43 dairy animals were tested using polymerase chain reaction (PCR). Nine PCR-positive animals were tested again with a PCR that is specific for *Anaplasma marginale*. Except for mean cell haemoglobin concentration (MCHC), blood parameters while within normal ranges, were higher in beef as compared to dairy cattle. There was a statistically significant difference in white blood cell count (WBC) ($P = 0.0162$) and granulocyte counts ($P = 0.0265$) with beef having higher counts compared to dairy cattle. The only blood parasite detected in both breeds was *Anaplasma marginale*. The study found a high level of *Anaplasma* infection with 98% (47/48) and 100% (48/48 of dairy and beef cattle testing positive by ci-ELISA receptively. PCR confirmed that 34.9% (15/43) of dairy cattle were positive for *Anaplasma* spp infection and *Anaplasma marginale* specific PCR confirmed the infection in 78% (7/9) of the animals tested. We conclude that cattle blood parameters at BUAN farm fall within normal ranges but that immune response cells were significantly lower in dairy compared to beef cattle. Also, a high-level endemic infection exists in the herd caused by *Anaplasma marginale*. The findings are discussed in the context of the utility of evaluating cattle health using diagnostic tests.

Keywords: *Anaplasma*; Infection; Cattle; ci-ELISA; Endemic

Introduction

Animal health is important for livestock productivity. Animal diseases both infectious and non-infectious are major determinants of animal health. Many animal diseases are subclinical and require laboratory-based diagnostic tests to be detected [1]. Cattle with subclinical disease go undetected if examined by the naked eye. It is known that certain diseases impact haematological parameters in cattle [2,3]. As an example, subclinical ketosis reportedly affects the number and activity of leukocytes in dairy cows [4]. Similarly, blood parasites can impact the health of animals and whether clinical or subclinical, require laboratory tests to be definitively diagnosed. Thus, evaluating the health status of a cattle herd using diagnostic tools including haematology, microscopy, serology, and polymerase chain reaction can provide insight into cattle conditions that would otherwise go undetected. Notably, analysis of blood parameters can reveal cattle breed differences in susceptibility to infection by blood parasites. A determinant of certain diseases resistance is whether the animal mounts a predominantly antibody-mediated immune response (AMIR) or cell-mediated immune response (CMIR) [5-7]. For certain intracellular pathogens including *Mycobacterium tuberculosis* CMIR is more effective than AMIR in suppressing the infection. Thus, haematological, and serological analysis can provide insight into the type of immune response cattle mount and reveal breed differences.

To investigate the health status of cattle at the Botswana University of Agriculture and Natural Resources (BUAN) farm we carried out four studies consecutively. First, we evaluated against normal values [8], the blood parameters of both beef and dairy cattle and used microscopic examination of Giemsa-stained blood smears to determine whether the cattle were infected with blood parasites. Second, we tested both cattle breeds to determine seroconversion for *Anaplasma* spp infection using competitive inhibition enzyme-linked immunosorbent assay (ci-ELISA). Third, we confirmed *Anaplasma* spp infection using polymerase chain reaction (PCR). Fourth, we used a specific PCR to confirm that the bacterium infecting the animals was *Anaplasma marginale.* The findings are discussed in the context of the utility of evaluating cattle health using diagnostic tests.

Materials and Methods

Haematological evaluation and microscopic examination of Giemsa-stained blood smears

Sixty female animals of either dairy (n = 30) or beef breed (n = 30) were randomly selected at Botswana University of Agriculture and Natural Resources (BUAN) farm with coordinates S -24.580874 E25.966465 located in the southeast region of Botswana. Whole blood was collected from the jugular vein. The dairy was Holstein Friesian, and the beef were native Tswana and Tuli breeds. Whole blood was centrifuged for 10 minutes in capillary tubes before reading with the QBC® VetAutoread hematology™ analyser (IDEXX Laboratories inc. Westbrook, Maine, USA) for haematocrit, differential cell count and hemoglobin concentration. To test for infection with blood parasites a small drop of fresh blood was put in the middle of one end of a glass slide and spread right across the slide using a second slide and then air dried. Blood films were fixed in absolute methyl alcohol for 5 minutes, stained in diluted Giemsa stain for 30 minutes and washed in distilled water and then dried. The slides were examined microscopically for blood parasites under oil immersion (1000X) magnification.

Testing for *Anaplasma ssp* infection using competitive inhibition enzyme-linked immunosorbent assay (ci-ELISA)

Dairy cows (n = 48) and beef cattle (n = 48) at BUAN farm were tested for Anaplasma spp infection using ci-ELISA as previously described [9]. Briefly, 70 µl of undiluted serum was added to a coated adsorption plate and incubated at room temperature for 30 minutes. Fifty µl of the adsorbed serum was transferred to a recombinant major surface protein 5 (rMSP5) coated plate and incubated at room temperature for 60 minutes. The serum was discarded, and the plate washed twice with diluted wash solution. Fifty µl per well of diluted monoclonal antibody/peroxidase conjugate was added to the rMSP5-coated plates and incubated at room temperature for 20 minutes followed by washing twice with wash solution. Fifty µl of substrate solution was added per well and the plate was covered with foil and incubated for 20 minutes at room temperature. After adding 50 µl per well of stop solution to the substrate solution already in the wells and gently tapping the sides of the plate to mix, the plate was read in a plate reader at 450 nm. Percentage inhibition (PI) was calculated according to the manufacturer's instructions. PI < 30 was considered negative and PI > 30 was considered positive.

Confirmation of *Anaplasma spp* infection using polymerase chain reaction (PCR)

To confirm *Anaplasma* spp infection we tested using polymerase chain reaction (PCR) 43 Holstein Friesian blood samples from the study above. Genomic DNA extraction was carried out according to the manufacturer's instruction (Qiagen, USA) and used as template in a major surface protein 5 (MSP5) based PCR as previously described (Ramabu., *et al*., 2018). Briefly, the primers used were forward 5′- ATG AGA ATT TCA AGA TTG TGT CT-3′, reverse 5′-CTA AGA ATT AAG CAT GTG ACC GCT G-3′. The reaction conditions were 5 minutes incubation at 95°C followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 50°C and 2 minutes at 72°C. The reaction was completed at 72°C for 10 minutes. The PCR products were analysed on 1% agarose gel in 1X TBE buffer and visualised using ethidium bromide and UV eluminator.

Confirmation of Anaplasma marginale infection using specific polymerase chain reaction (PCR).

To confirm whether the cattle infection consisted of *Anaplasma marginale* a PCR specific for the bacterium was carried out on nine samples from dairy animals that had tested positive by ci-ELISA and MSP5 based PCR. Genomic DNA from the samples was quantified using a Nano drop machine (Thermo Scientific™ NanoDrop™, USA). PCR was carried out to clone *Anaplasma marginale* major surface protein 1 beta (msp1b) with the following primers; forward 5' -CAGGCTTCAAGCGTACAGTG-3', reverse 5'-GATATCTGTGCCTGGCCTTC-3' previously used to detect the organism in ticks [10]. The PCR was run in an automated thermocycler, with the following conditions: 5 minutes incubation at 95°C, 35 cycles for 30 seconds at 94°C, 30 seconds at 50°C, and 2 minutes at 72°C. PCR was completed with the additional extension step at 72°C for 10 minutes. The PCR products were analyzed on 1% agarose gel in 1X TAE and visualized using ethidium bromide and UV-eluminator.

Results

Hematological evaluation and microscopic examination of Giemsa-stained blood smears

An evaluation of dairy and beef cattle blood reveals that, except for mean cell hemoglobin concentration (MCHC), parameters were higher in beef as compared to dairy cattle (Table 1). The beef parameters tended to be close to the upper limits of normal bovine ranges while the dairy parameters were closer to the lower limits of the normal ranges. Otherwise, both herds had in general parameters that were within normal bovine ranges as expected with exception of granulocyte counts in dairy which were below normal (Table 1). Of the seven parameters, measured there was no statistically significant difference ($P > 0.05$) between the two breeds in five of them. There was statistically significant difference in two being white blood cell count (WBC) ($P = 0.0162$) and granulocyte counts ($P = 0.0265$) both parameters being higher in beef as compared to dairy cattle. There was a tendency towards statistically significant difference ($P = 0.1531$) in the hematocrit of beef cattle as compared to dairy cattle with the latter being lower (Table 1).

Breeds of cattle	Hematocrit (%)	WBC (10^9/L)	Hemoglobin (g/dL)	PLT (10^9/L)	GRANS (10^9/L)	Lymph/mono (10^9/L)	MCHC (g/dL)
	A	A	A	A	A	A	A
Beef	42.4 ± 9.8	15.5 ± 5.8	13.1 ± 3.7	615.6 ± 201	2.0 ± 0.7	11.9 ± 4.7	31.8 ± 11.7
	(25 - 42)	(4 -12)	(8– 14)	(175 -500)	(2 - 6)	(3.0- 7.5)	(27 –34.9)
	A	B	A	A	B	A	A
Dairy	38.7 ± 7.5	11.4 ± 3.9	11.5 ± 2.9	576.3 ± 205.8	1.6 ± 0.6	9.3 ± 3.5	33 ± 14.3
	(25 - 42)	(4 - 12)	(8 – 14)	(175 -500)	(2 – 6)	(3.0 - 7.5)	(27 – 34.9)

Table 1: Comparison of hematological parameters (mean) of beef and dairy cattle at BUAN farm. The numbers in brackets are normal ranges for bovine (Blood and studdert, 1988). A, B – t-test Grouping (means with the same letter are not significantly different).

WBC: White Blood Cell Count; PLT: Platelet; GRANS: Granulocytes; Lymph/mono: Lymphocytes/Monocytes. MCHC[a]: Mean Cell Hemoglobin Concentration

The only blood parasite detected in both breeds was *Anaplasma marginale* (Table 2). Other parasites considered endemic in sub-saharan Africa including *Babesia spp*, *Theileria spp*, and *Trypanosoma spp* were not detected by microscopic examination of Giemsa-stained blood smears. The prevalence of *A. marginale* was almost twice as high in dairy as compared to beef cattle (Table 2).

Breed	No. of cattle examined	Anaplasmosis[a]		Anaplasmosis[a]	
		No. Positive	%	No. Negative	%
Beef	30	5	16.7	25	83.3
Dairy	30	9	30	21	70

Table 2: Prevalence (%) of Anaplasmosis among beef and dairy cattle at BUAN farm.

[a] – Data obtained by microscopic examination of Giemsa-stained blood smears

Testing for *Anaplasma spp* infection using competitive inhibition enzyme-linked immunosorbent assay (ci-ELISA)

Of the 48 dairy animals tested, 98% (47/48) were positive for *Anaplasma* infection and 100% (48/48) beef animals tested positive. Thus, there was high level prevalence of infection in the cattle. Comparing the dairy and beef cattle there was a discernible difference in the distribution of percentage inhibition (PI). There were more dairy animals, 81.3% (39/48) with a PI over 80% compared to 54.2% (26/48) beef cattle.

Confirmation of *Anaplasma spp* infection using polymerase chain reaction

Of the dairy animals tested 34.9% (15/43) were positive for *Anaplasma* spp infection. A PCR product of the expected 633 bp was present in the lanes loaded with test samples and the designated positive control and not in the negative control (Figure 1).

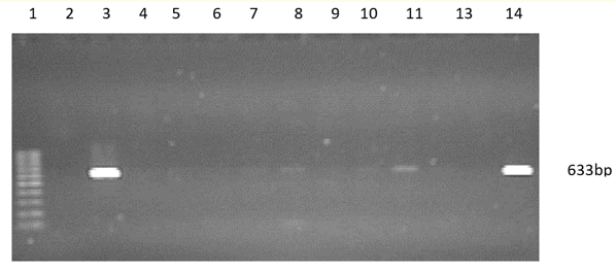

Figure 1: Detection of *Anaplasma* infection using polymerase chain reaction. Lane 1: Molecular mass marker. Lane 2: Negative control. Lane 3: Positive control. Lane 4- 14: test samples.

Confirmation of Anaplasma marginale infection using specific polymerase chain reaction (PCR)

Of the nine animals tested 78% (7/9) tested positive for *Anaplasma marginale* based on the MSP1β based PCR (Figure 2). A PCR product of the expected 85bp was present in the test samples and the positive control and absent in the negative control.

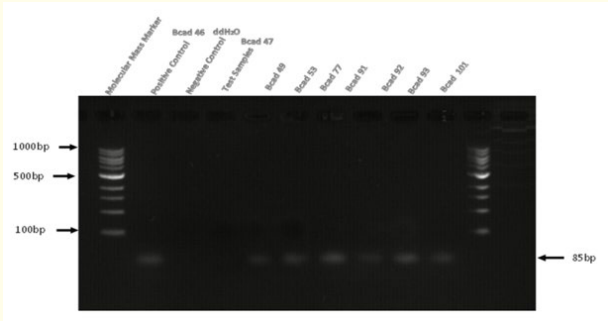

Figure 2: Detection of *Anaplasma marginale* using MSP 1β based PCR.

Discussion

Hematological parameters can reveal subclinical conditions including anemia and leukopenia [11]. We evaluated hematologocial parameters of both dairy and beef cattle at BUAN farm and found that they fell within normal ranges for cattle. Interestingly, except for mean cell hemoglobin concentration (MCHC) all blood parameters were numerically higher in beef compared to dairy cattle. Two, white blood cell count (WBC) and granulocytes were significantly higher in beef compared to dairy cattle. Dairy cattle are exposed

to more demanding and stressful production conditions compared to beef cattle. The findings suggest that stressful production conditions have a suppressive effect on blood parameters and in particular the immune system by decreasing WBC count and specifically granulocyte count. Similar findings were reported by Meglia., *et al*., (2005) who found significantly lower white blood cell count in dairy cattle as compared to beef cattle. Suppression of leukocyte count in dairy cows was found to be attributable to negative energy balance around calving and in early lactation [12]. Also, it has been asserted that dairy cows have decreased disease resistance, in particular immune responsiveness, during the peripartum period [13]. Thus, dairy animals are likely to be more susceptible to disease than beef cattle. With a lower packed cell volume (PCV), dairy cattle appear to compensate by having a higher MCHC to meet their oxygen needs.

Interestingly, the only blood parasite detected by microscopic examination of Giemsa-stained blood smears was *Anaplasma marginale*. The organism has been determined to be endemic in cattle in the area [14]. In agreement with previous findings high level *Anaplasma* seroprevalence was found in the current study [14,15]. The prevalence of bacteremia was almost twice as high in dairy as compared to beef cattle consistent with dairy animals being less suppressive to the infection. The 30% prevalence in dairy animals suggests that at any time only a third of animals persistently infected with *Anaplasma* are detectable by microscopic examination of Giemsa-stained blood smears (Dumler., *et al*., 2001). Thus, microscopy is capable of high specificity but has a low sensitivity compared to ci-ELISA. Comparing percentage inhibition (PI) which is a proxy for circulating antibody, between dairy and beef cattle, it appears that dairy animals had a higher humoral response than beef cattle. Perhaps beef cattle mounted a stronger cell-mediated immune response hence the comparatively lower bacteriemia. It is documented that for specific intracellular pathogens including *Mycobacterium tuberculosis* cell- mediated immune response tends to be more effective compared to antibody-mediated immune response in clearing the infection [16-18]. *Anaplama marginale* is also intracellular and for the bacteremia to be higher in dairy cattle evidently mounted a higher humoral response than beef cattle indicate agreement with findings pertaining to intracellular pathogens. Also, our findings suggest that the genetics of the animal determines susceptibility to infection as was previously reported [19,20].

PCR confirmed *Anaplasma* infection in dairy cattle. Major surface protein 5 is a single copy gene conserved in *Anaplasma* strains and MSP5 based PCR and ci-ELISA have previously been used to test ruminants for *Anaplasma* infection [14,15,21,22]. Like microscopic detection reported in this study PCR detected *Anaplasma* infection in a third of the cattle population that based on ci-ELISA had about 100% infection. An msp1β-based PCR was previously used to identify animals infected specifically with *Anaplasma marginale* [10]. The same msp1β-based PCR was used in the current study to confirm *Anaplasma marginale* infection in dairy cows constituting to our knowledge the first-time confirmation of such infection in Botswana. An interesting feature of cattle infection with *Anaplasma* spp in the southeast region of Botswana is the lack of widespread clinical disease and mortality. Could the strain infecting the animals be nonpathogenic? This calls for genotyping of the *Anaplasma* organism and comparison with the known pathogenic strains.

Conclusion

We conclude that dairy cattle blood parameters tend to be lower than those of beef cattle and for immune response cells specifically white blood cells and specially granulocytes, the difference was significant with dairy animals having fewer cells than beef animals. The only blood parasite detectable by microscopy in the cattle was *Anaplasma marginale.* There is a high level *Anaplasma* infection in the cattle herds. We confirm the infection to be caused by *Anaplasma marginale*.

Conflicts of Interest Statement

All of the authors declared that no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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