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Serological prevalence of *Babesia caballi* and *Theileria equi* in camels and donkeys from Karamoja sub-region, North-eastern Uganda

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Equine piroplasmosis is a severe disease of horses caused by the intra-erythrocyte protozoan, *Theileria equi* and *Babesia caballi*. *T. equi* and *B. caballi* infections were assessed in serum from camels and donkeys using competitive- enzyme-linked immunosorbent assay (cELISA) assay. A total 110 animals were studied including 25 donkeys and 85 camels from two districts viz. Moroto and Amudat in Karamoja sub-region, North-eastern Uganda. All the (100%) donkeys tested were positive for *Babesia/T. equi* while none of the camels had been exposed to the infection. All animals were negative to *B. caballi* cELISA. Our findings indicated that all donkeys sampled in Karamoja sub-region have been exposed to *T. equi* and this could be prevalent in equine population in Uganda. No exposure status to *B. caballi* was reported. This study represents the first report on the status of *T. equi* and *B. caballi* infection in Uganda.

Key words: Donkey, Camel, *Theileria equi*, *Babesia caballi*, Seroprevalence, cELISA, Uganda.

INTRODUCTION

Equine piroplasmosis (EP) is a tick-borne disease of horses caused by apicomplexan hemoprotozoan parasites *Theileria equi* and *Babesia caballi* of the order Piroplasmida (Wise *et al*., 2013; Scoles and Ueti, 2015; Sumbria *et al*., 2017). The nomenclature was changed from *B. equi* to *T. equi* based on evolutionary, morphologic, biochemical, and genetic evidence (Singla and Sumbria, 2017). The disease is also called biliary fever and affects all equid species including Horses, donkeys, mules and zebras (Friedhoff *et al*., 1990; Schein, 1988). Several genera of tick species including *Hyalomma*, *Rhipicephalus* and *Dermacentor* transmit both these parasites (De Waal, 1992; Sumbria *et al*., 2016a). Clinical signs include fever, anemia, icterus, hepatomegaly, edema, intra-vascular hemolysis, hemoglobinuria and even death (Schein, 1988; Uilenberg, 2006). The disease is distributed in tropical and sub-tropical areas including some temperate zones (Shkap *et al*., 1998; Steinman *et al*., 2012). The equine disease has a worldwide economic importance especially

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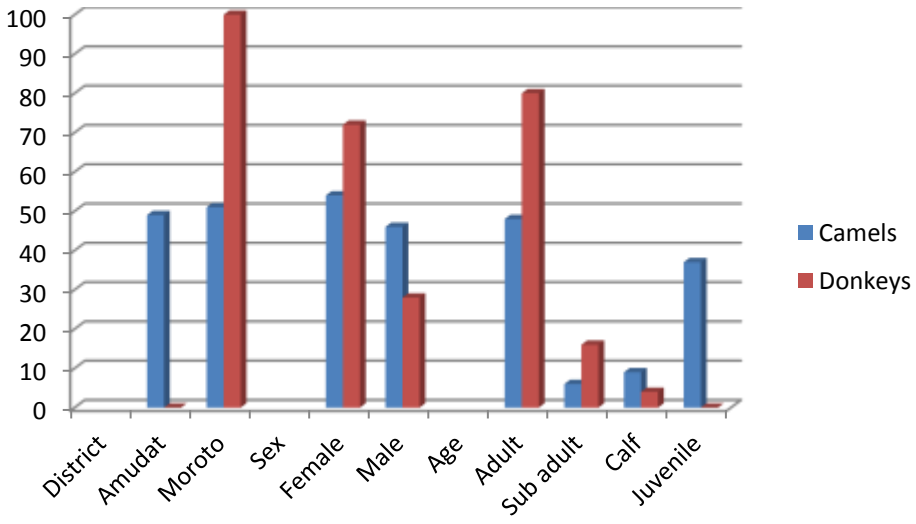

Figure 1. Demographic characteristics of sampled animals.

concerning international movement of horses because carrier horses and infected ticks can be introduced into disease-free countries (Friedhoff *et al*., 1990; Sumbria *et al.*, 2016b). The vectors of *T. equi* and *B. caballi* are the same (Abedi *et al*., 2014) although *T. equi* is more virulent than *B. caballi* (Friedhoff *et al*., 1990; Mehlhorn and Schein, 1998; Posnett *et al*., 1991). In endemic countries, mixed infections occur (Scoles and Ueti, 2015). Diagnosis depends on clinical observation especially in the acute phase of the disease and is confirmed by microscopic detection of intra-erythrocyte parasites in Giemsa-stained blood smears (Shkap *et al*., 1998). However, the latent phase of the infection is characterized by low parasiteamia (Kumar *et al*., 2008; Sumbria *et al*., 2015) hence the need for more sensitive diagnostics like enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (Friedhoff and Soule, 1996; Moretti *et al.,* 2010; Alsaad *et al*., 2012). Ponies, mules and donkeys act as natural reservoirs for disease transmission to the horses (Radostitis *et al*., 2008).

In Uganda, camels and donkeys are kept in Karamoja and Sebei sub-regions, North-eastern Uganda. They are kept by peasant farmers, pastoralists in this semi-arid region. They are kept for meat, milk, dowry, prestige, and carriage. These animals receive little or no veterinary care. Nakayima *et al.,* (2017) detected helminth parasitosis in these animals in the absence of veterinary intervention. No studies have been undertaken on equine piroplasmosis in Uganda. Therefore, the aims of this work were to determine the infection rate of EP in donkeys and possibly in camels given the fact that they occupy the same ecological setting and could therefore act as accidental hosts. Information from this study will help update current knowledge on the health of camels and donkeys in Uganda for their improved production and productivity.

MATERIALS AND METHODS

Serum samples were collected from Karamoja sub-region in two districts namely: Moroto: N 2° 31' 41.604", E 34° 39' 28.794" and Amudat: N 1° 47' 29.841", E 34° 54' 23.583" districts, Uganda. The study was conducted in March 2016. The camels and donkeys were classified as: Infant, juvenile, sub-adult and adult. Both sexes were sampled (Tables 1 and 2 and Figure 1). Blood was collected from the jugular vein of both camels and donkeys following restraint. 5 ml of blood was collected, 2.5 ml blood was put in anticoagulant ethylene diamine tetra acetic acid (EDTA) vaccutainers for parasitological and DNA analysis while 2.5 ml blood was put in serum tubes. The serum was collected into plain vaccutainer tubes without anticoagulant, serum was separated from blood cells by centrifugation at 2500 rpm for 15 min and stored at -20ºC until use in a competitive-ELISA (cELISA) (Kouam et al., 2010) for both *T. equi* and *B. caballi*. The total number of donkeys was 25, while camels were 85 giving a total of 110 animal samples collected. The sample size determination was based on purposive sampling based on the availability of the animals. The age and sex were recorded for each animal. The samples were transferred to the laboratory and stored at -20ºC until use. The serum from clinically healthy animals was examined for *T. equi* and *B. caballi* antibodies by two separate cELISA.

ELISA

Commercial cELISA kits were used to analyze sera from donkeys and camels for the presence of antibodies to *T. equi* and *B. caballi* as described by the manufacturer (VMRD Inc., Pullman, WA, USA). The cut-off values for positive infections was 40% of inhibition for both tests, as indicated by the manufacturer (VMRD Inc., Pullman, WA, USA) (Shkap *et al*., 1998; Kappmeyer *et al*.,1999). Thus samples with %I above 40 are considered as positive, and below 40, considered as negative. The results were expressed as a value of the percent inhibition (%I) according to the following formula:

(%I): %I = 100- {(sample O.D. × 100) / (mean negative control O.D.)}

Microscopic detection of hemoprotozoal parasites depends on morphological and biometrical parameters including the shape, site

Table 1. Infection of camels (Total = 85; all negative for both infections).

Category	Frequency	%
District		
Amudat	42	49
Moroto	43	51
Sex		
Female	46	54
Male	39	46
Age		
Adult	41	48
Sub adult	5	6
Calf	8	9
Juvenile	31	37

Table 2. Infection of donkeys (n=25) (All affected with *Theileria equi,* all negative for *Babesia caballi)*.

Category	Frequency	%
District		
Amudat	0	0
Moroto	25	100
Sex		
Female	18	72
Male	7	28
Age		
Adult	20	80
Sub adult	4	16
Calf	1	4
Juvenile	0	0

location and size of parasite in an infected erythrocyte in Giemsa-stained blood smears (Sadeghi Dehkordi *et al*., 2010). Morphological detection of the parasites could be described as single round, double round, single pyriform and double pyriform with obtuse or acute angle. Microscopically, *B. caballi* is a larger paired pyriform parasite, while *T. equi is a* smaller paired pyriform, rounded and tetrad or Maltese cross arrangement of merozoites (Kuttler, 1988; Levine, 1971).

Statistical analysis

Data were analyzed using SPSS (Version 16). A value of $p < 0.05$ was considered as statistically significant.

RESULTS

110 samples were analyzed with 25 donkeys and 85 camels. All donkeys were positive for *T. equi*. Donkey no. 22 was not done, the sample had dried out. All camels were negative for *Babesia/ T. equi* ELISA. Corrected ODs were calculated from sample ODs and blank ODs (Table 3). Sample Id represents animal species, age, sex and sample number.

All the serum samples from donkeys and camels were negative to *Babesia caballi* Competitive-ELISA. This suggests no history of exposure to this parasite. 110 samples were analyzed with 25 donkeys and 85 camels. Donkey no. 22 was not done, the sample had dried out. Corrected ODs were calculated from sample ODs and blank ODs (Table 4). Sample Id represents animal species, age, sex and sample number.

DISCUSSION

Diagnosis of piroplasmosis can be achieved by clinical observation and confirmed by microscopy (Irwin, 2010). However, given the low sensitivity of parasitological diagnosis, there is need to combine parasitological diagnosis with molecular diagnostics (Abedi *et al*., 2014; Salib *et al*., 2013; Baptista *et al*., 2013; Rosales *et al*., 2013).

Camels were sampled alongside donkeys. Much as camels are not equines, they could serve as accidental hosts or reservoirs of the parasites since they are in the same ecological setting. However, all camels tested negative to *B. equi* cELISA (Table 3). The serological prevalence of *B. equi* cELISA was 100% in donkeys and 0% in camels (Table 3). Apparently, equine piroplasmids are enzootic in Uganda and their distribution pattern is likely affected by the presence and densities of suitable hosts rather than by ecological conditions. There is a significant correlation between host species and age with the distribution of EP. Horses are more susceptible to the infection than donkeys. Adult animals have a higher risk of infection. All the serum samples from camels and donkeys tested negative to *B. caballi* cELISA (Table 4). This suggests no history of exposure of these animals to this parasite or the fact that the infection is cleared in 1 to 4 years. All the 25 donkey serum samples (100%) were found positive for *T. equi* antibodies (Table 3), *B. caballi* was not detected in the study area (Table 4) while all the 85 camels tested negative to equine piroplasmosis (Tables 3 and 4). A study of equine piroplasmosis in horses in Sudan by Salim *et al*. (2008) recorded 100% prevalence of *T. equi* in Khartoum North (100%) and Atbara (100%); a low prevalence for *B. caballi* was reported and 0% prevalence was detected in Khartoum, Khartoum North, and Kosti areas. The infection rate of EP varies in different countries. Several factors account for this namely: disease management practices, tick vector abundance and climate. Climatic factors influence the habitat of tick vectors like rainfall, humidity and temperature (Oncel *et al*., 2007). In endemic countries, equines adopt to infection possibly because of the phenomenon of endemic stability. However, stress and immune-suppression could revert otherwise sub-clinical infection to overt disease. *T. equi* infection results into life-long carrier status (Brüning, 1996) while *B. caballi* could persist in subclinical form for at least 1 to 4 years

Table 3. *Babesia* / *Theileria equi* competitive-ELISA of camel and donkey serum.

S/N	Animal species	Sample ID	Corrected Ods	% Inihibition (%I)	Sample status
1	Donkey	D/A/M/01	0.262	75.40	Positive
2	Donkey	D/A/F/02	0.209	80.38	Positive
3	Donkey	D/SA/F/03	0.159	85.07	Positive
4	Donkey	D/A/F/04	0.218	79.53	Positive
5	Donkey	D/A/F/05	0.196	81.60	Positive
6	Donkey	D/A/F/06	0.395	62.91	Positive
7	Donkey	D/A/F/07	0.252	76.34	Positive
8	Donkey	D/A/F/08	0.572	46.29	Positive
9	Donkey	D/A/F/09	0.275	74.18	Positive
10	Donkey	D/A/M/10	0.173	83.76	Positive
11	Donkey	D/A/M/11	0.167	84.32	Positive
12	Donkey	D/A/F/12	0.291	72.68	Positive
13	Donkey	D/A/F/13	0.244	77.09	Positive
14	Donkey	D/A/F/14	0.232	78.22	Positive
15	Donkey	D/A/M/15	0.159	85.07	Positive
16	Donkey	D/A/M/16	0.147	86.20	Positive
17	Donkey	D/A/F/17	0.247	76.81	Positive
18	Donkey	D/A/F/18	0.241	77.37	Positive
19	Donkey	D/A/M/19	0.17	84.04	Positive
20	Donkey	D/SA/F/20	0.483	54.65	Positive
21	Donkey	D/SA/F/21	0.606	43.10	Positive
23	Donkey	D/A/F/54	0.48	54.93	Positive
24	Donkey	D/A/F/55	0.298	72.02	Positive
25	Donkey	D/SA/F/56	0.507	52.39	Positive
26	Donkey	D/C/M/57	0.328	69.20	Positive

110 samples were analyzed with 25 donkeys and 85 camels. All donkeys were positive for *T. equi*. Donkey no. 22 was not done, the sample had dried out. All camels were negative for *Babesia*/ *T. equi* ELISA. Corrected ODs were calculated from sample ODs and blank ODs. Sample Id represents animal species, age, sex and sample number.

before being eliminated. This could be attributed to the fact that *T. equi* parasites are not completely eliminated from the blood of equines after treatment or natural recovery (de Waal and van Heerden 1994) as compared to *B. caballi*. Therefore, failure to detect *B. caballi* by ELISA is most probably due to the parasites clearance from the circulating blood by the host or reduction to a level beyond the detection of the host immune response or the diagnostic test. There was a significant difference between donkeys and camels with respect to the seroprevalence of *T. equi* and *B. caballi* ($P<0.05$).

Treatment strategy for piroplasmosis in equines depends on the endemic status of the country. So it is either parasite elimination in disease free countries or resolution of clinical disease in endemic countries. It is not recommended to eliminate the parasite from equids in endemic countries because the animals need endemic stability due to constant exposure to the parasite at low levels (Donnellan and Marais, 2009). Several drugs can be used to treat EP. Generally, *B. equi* has been reported to be more refractory to babesiacidal drugs than *B. caballi*. More common drugs include imidocarb and diminazene. However, donkeys are more susceptible to imidocarb toxicity than horses (Donnellan and Marais, 2009). Imidocarb causes a dose-dependent hepatotoxicity and nephrotoxicity (Donnellan and Marais, 2009). Other drugs could include artesunate, arteether, buparvaquone. Equine piroplasmosis is endemic in Karamoja sub-region and possibly Uganda at large due to the distribution of equine species and the tick vectors.

Conclusion

All donkeys tested positive to *Babesia* / *T. equi* cELISA while all camels were negative. This exposure status indicates that this piroplasm could be endemic in Karamoja sub-region and in the equine population in Uganda in the absence of veterinary intervention. No exposure status to *B. caballi* was reported. Camels are not accidental hosts or reservoirs of equine piroplasmosis. The results of this study will help inform policy on the improvement of the health and welfare of these animals through veterinary intervention. To our knowledge, this is the first report on epidemiology of

Table 4. *B. caballi* competitive-ELISA of camel and donkey serum.

No.	Animal species	Sample ID	Corrected Ods	% Inihibition (%I)	Sample status
1	Donkey	D/A/M/01	1.115	-2.6	Negative
2	Donkey	D/A/F/02	1.257	-15.7	Negative
3	Donkey	D/SA/F/03	1.292	-18.9	Negative
4	Donkey	D/A/F/04	1.055	2.9	Negative
5	Donkey	D/A/F/05	1.168	-7.5	Negative
6	Donkey	D/A/F/06	1.175	-8.1	Negative
7	Donkey	D/A/F/07	1.138	-4.7	Negative
8	Donkey	D/A/F/08	1.08	0.6	Negative
9	Donkey	D/A/F/09	1.114	-2.5	Negative
10	Donkey	D/A/M/10	1.263	-16.2	Negative
11	Donkey	D/A/M/11	1.248	-14.8	Negative
12	Donkey	D/A/F/12	1.15	-5.8	Negative
13	Donkey	D/A/F/13	1.055	2.9	Negative
14	Donkey	D/A/F/14	1.127	-3.7	Negative
15	Donkey	D/A/M/15	1.181	-8.7	Negative
16	Donkey	D/A/M/16	1.151	-5.9	Negative
17	Donkey	D/A/F/17	1.044	3.9	Negative
18	Donkey	D/A/F/18	1.185	-9.0	Negative
19	Donkey	D/A/M/19	1.27	-16.9	Negative
20	Donkey	D/SA/F/20	1.174	-8.0	Negative
21	Donkey	D/SA/F/21	1.076	1.0	Negative
23	Donkey	D/A/F/54	1.098	-1.0	Negative
24	Donkey	D/A/F/55	1.141	-5.0	Negative
25	Donkey	D/SA/F/56	1.151	-5.9	Negative
26	Donkey	D/C/M/57	1.136	-4.5	Negative

All the serum samples from donkeys and camels were negative to *Babesia caballi* Competitive-ELISA. This suggests no history of exposure to this parasite. 110 samples were analyzed with 25 donkeys and 85 camels. Donkey no. 22 was not done, the sample had dried out. Corrected ODs were calculated from sample ODs and blank ODs. Sample Id represents animal species, age, sex and sample number.

equine piroplasms in Uganda. Molecular studies for accurate diagnosis of equine piroplasmosis are essential for providing baseline information about its epidemiology, distribution, and prevalence in the affected equine population and for effective control measures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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