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A Field Survey for the Seroprevalence of *Theileria equi* and *Babesia caballi* in Donkeys from Nuu Division, Kenya

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Abstract

Equine piroplasmosis is one of the most significant tick-borne disease of equids. The prevalence of this disease in donkeys of semi-arid Kenya remains largely unexplored. The primary objective of this study was to demonstrate the extent to which donkeys in Nuu division, Kenya have been exposed to the haemoprotozoans *Babesia caballi* and *Theileria equi,* the causative agents of equine piroplasmosis. The study also assessed the effect of age and sex on seroprevalence. A stratified sampling approach was used and three hundred and fourteen donkeys were sampled across nine sub-locations in Nuu division, Mwingi district. Serodiagnosis was via competitive inhibition enzyme linked immunosorbent assays (cELISA). The seroprevalence of *T. equi* was 81.2% (95% CI: 76.4-85.4). There was no significant difference in sub-location seropositivity, gender seropositivity or age related seropositivity. Antibodies against *B. caballi* were not detected (95% CI: 0-1.2). Findings from this study suggest that *T. equi* infection is endemic in Nuu division, Mwingi where it exists in a state of endemic stability. Existence of the infection should be communicated to animal health practitioners and donkey owning communities in the area.

Keywords: donkey, equine piroplasmosis, health, tick, Kenya, serology

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Introduction

Man's relationship with donkeys spans over six millennia, the equid's domestication for purposes of work is believed to have been triggered by the desertification of the Sahara over 7000 years ago (Beja-Pereira *et al*., 2004; Starkey and Starkey, 2004; Rossel *et al*., 2008). The current global donkey population is estimated at 44 million and is predicted to increase in sub-Saharan Africa, Latin America and Asia in tandem with human population growth (Fernando and Starkey, 2004; FAO, 2009; FAO, 2014). As their use increases in developing countries, it is prudent that likely barriers to their optimal use be elucidated. Among others, equine piroplasmosis poses a significant threat and is reported to be the most important tick borne disease in this species (Kumar *et al*., 2009).

Equine piroplasmosis is an acute, sub-acute or chronic tick borne disease of members of the taxonomic family Equidae (including mules, horses, donkeys and zebras). The causal organisms are the obligate intraerythrocytic haemoprotozoans *Theileria equi* and *Babesia caballi*. Transmission of these pathogens can be via the bite of competent tick vectors. Some of the tick vectors that have been implicated in transmission belong to the genera *Dermacentor, Rhipicephalus, Amblyomma* and *Hyalomma* (de Waal, 1992; Melhorn and Schein, 1998; Rampersad *et al*., 2003; Wise *et al*., 2013). Other viable options for transmission include iatrogenic means (Scoles *et al*., 2011) and transplacental transmission, reported in *T. equi* infections (Philips and Otter, 2004; Georges *et al*., 2011; Chhabra *et al*., 2012). Clinical presentation of overt disease is commonly associated with *T. equi* infections. Infections with *B. caballi* often remain latent but can be sometimes exhibited as anaemia accompanied with other clinical signs associated with the disease. Per-acute, acute, sub-acute and chronic forms of the disease have been described. Clinical signs of the disease may include varying degrees of inappetence, pyrexia, dependent oedema, haemoglobinuria, anaemia, reduced work efficiency, weight loss and abortion in mares (de Waal, 1992; Hailat *et al*., 1997; Allsopp *et al*., 2007; Anzuino, 2008; Zobba *et al*., 2008; Garba *et al*., 2011).

Equine piroplasmosis is known to occur worldwide with tick vector distribution accurately dictating disease abundance (de Waal, 1992). Several European countries have reported the disease. Infections have also traversed Africa, Asia, South and Central America and Madagascar (Friedhoff, 1982; Garcia-Bocanegra *et al*., 2013). Data from the World Organisation for Animal Health (OIE) between 2012 and 2013

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indicate that up to 33 countries reported the disease (WAHID, 2013). In Africa, the disease has been reported in horses and donkeys from Sudan (Salim *et al*., 2008; Salim *et al*., 2013), donkeys from Ethiopia (Gizachew *et al*., 2013) and horses from South Africa (Bhoora *et al*., 2010a), Tunisia (Ros-Garcia *et al*., 2013) and Nigeria (Garba *et al*., 2011). In Kenya, equine piroplasmosis was suspected but not confirmed according to the reports submitted to the OIE in 2005. The disease has not been among the afflictions affecting domestic and or wild animals in Kenya reported to the OIE since then (WAHID, 2012). Disease occurrence presents an obstacle to international trade in horses. This has prompted movement restrictions by countries such as USA, Canada, Australia, Japan, Mexico and Brazil pending a negative result on serological testing (Knowles, 1996).

The bulk of published literature describes endemicity in horses leaving the epidemiology of piroplasmosis in donkeys poorly understood despite the species' carrier state potential (Chahan *et al*., 2006; Kumar *et al*., 2009; Machado *et al*., 2012; Gizachew *et al*., 2013, Hawkins *et al*., 2015). Studies on equine piroplasmosis in Kenyan donkeys have been previously conducted by Vranova *et al*. (2011) and Hawkins *et al*. (2015). Kenya recorded a 64% increase in the national donkey population between the years 1999-2009. From the current official tally of 1.8 million, close to 38 % inhabit the arid and semi-arid parts of the country (KNBS, 2011). In this region, 90% of the people's livelihoods is directly associated with livestock (MoLD, 2008). Within these dry areas donkeys play a pivotal role in a household's subsistence system (Blench, 2004; Fernando and Starkey, 2004). The study area, Mwingi District, boasts an estimated 55,000 donkeys and is situated in semi-arid Kenya (Rubyogo *et al*., 2005). This investigation seeks to unravel the extent to which donkeys have been exposed to the haemoparasites *T. equi* and *B. caballi* the causatives of equine piroplasmosis. Despite their physiological adaptation to thrive in such an environment devoid of rich flora (Blench, 2004) they often become subjected to overworking especially during periods of prolonged drought (personal observation, D.O.O) putting them at risk of developing potentially fatal diseases (Hailat *et al*., 1997; Anzuino, 2008; Ros-Garcia *et al*., 2013).

Material and methods

Study Area

The study took place in an area known as Nuu division. Nuu division occupies an area of 1,324.4 km^2 (ALRMP, 2009) and has a population density of 17 persons per square kilometer (MoE, 2004). Eight similar administrative units to Nuu make up Mwingi district. Mwingi district is located in the Eastern province of Kenya which is located between latitudes 00 47' degrees and 380 57' east of Greenwich Meridian Time (ALRMP, 2009).

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It is classified as Low Midlands 5 agro-ecological zone, defined by red sandy soils, erratic rainfall and consequently, a 0.66 probability of crop failure (Rubyogo *et al*., 2005). The area experiences two rainy seasons from March to May and October to November. Its average annual temperature ranges between 26^0C-34^0C (MoE, 2004). The study spanned across nine sub-locations namely Mwangeni, Mutyangombe, Kaai, Kavindu, Ngangani, Mutulu, Mwambiu, Kathanze and Ngaani. These sub-locations constitute the greater part of Nuu division where donkeys are known to be kept and were selected based on this criteria.

Study Design

A stratified sampling approach was utilized, with the nine sub-locations representing nine donkey sub-populations. To facilitate this, a donkey census exercise was carried out from the 18th to 20th of July 2013. The exercise indicated the donkey population to be 1724. This represented the study population. The level of confidence desired was set at 95%, while the desired absolute precision was set at 0.05 and the expected prevalence set at 50% as prior evidence of a similar study in the area was absent. The sample size was set at 314 after adjusting for a finite population (Thrusfield, 2005).

Having stratified the study population by sub-location, proportional allocation was then utilized to determine the number of donkeys to be sampled per stratum (see Table 1). The test kits used in the study (VMRD®) have 100 % sensitivity and 100 % specificity (VMDR, 2013) it was therefore unnecessary to adjust the sample size to accommodate for test imperfections. The donkeys to be sampled were then selected randomly (Thrusfield, 2005). This was done by utilizing a list generated during the census exercise. This list indicated the names of farmers, the number of donkeys owned and sub-location where they resided.

Sample Collection and Serological Examination

The sampling exercise spanned from the 13th to 15th of November 2013. Prior to sampling, donkeys were appropriately restrained by their owners and aged as per the description by Crane (1997). The sex of the animals was noted and the donkeys were also examined for any signs of illness. Blood was then drawn from the selected donkeys via jugular venipuncture and collected in 4 ml serum tubes. The tubes were clearly labelled with a specific code and kept in a cooler box with ice bricks.

Upon completion of the exercise, the samples were transported to a designated laboratory in Nairobi about 170km from the study area by road. The samples made it to

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the laboratory within 12 hours of collection. At the laboratory samples were centrifuged at 402g for 10 minutes and serum harvested into labelled vials. The serum vials were then stored at -20^{0}C until when they were required (Cecchini *et al*., 1992). Collected samples were then tested using commercial cELISA kits (VMRD®) for detecting *T. equi* and *B. caballi*. The test quantifies a series of reactions expressing them as optical densities at 620-650nm. Positive test samples were those which yielded a percentage inhibition (PI) of ≥ 40 % while negative samples were those with a PI of < 40%.

Statistical Analysis

Epi Info version 3.5.4 (CDC, 2008) was used to calculate seroprevalence and associated 95% confidence intervals. Confidence Interval Analysis version 2.2.1 was used to generate the 95% confidence interval for *B. caballi* seroprevalence (Altman et al., 2000-2001). WinPepi software version 11.39 (Abramson, 2013) was used to determine the presence or absence of a statistical association between gender and seroprevalence.

Results

A total of 314 donkeys were sampled out of which 196 were female and 118 were male. None of the sampled donkeys displayed clinical signs associated with equine piroplasmosis. Tick infestation was however present in some of the sampled donkeys, the exact number of donkeys affected was not recorded. The overall seroprevalence of *T. equi* was 81.2% (95% CI: 76.4-85.4, *n*= 314). Antibodies against *B. caballi* were not detected by cELISA, *B. caballi* seroprevalence 0% (95% CI: 0-1.2, *n*=314).

The seroprevalence of *T. equi* in donkeys sampled in each of the 9 sub-locations of Nuu division and the 95% confidence interval for seroprevalence was computed. The results are summarized in Table 2. An overlap in the 95% confidence intervals for *T. equi* seroprevalence in the 9 sub-locations was demonstrated. This is indicative of an absence of difference in seroprevalence in these sub-locations.

To calculate *T. equi* exposure with age, the animals were grouped into age categories that adequately accommodate possible age estimates described by Crane (1997). The age groups used were < 1 year, 1-3 years, 3.5-5 years, 6-10 years, and 11-15 years. The results are summarized in Table 3.

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It is notable that the point estimate for seropositivity is highest at the age group < 1 year, it then falls at age group 1-3 years to steadily rise at age group 3.5-5 years. Overlaps of the 95% confidence interval for the point estimate of seropositivity in the different age groups are evident from the table. This denotes a lack of significant differences in seropositivity among age groups in this study. The crude prevalence ratio produced was 0.954, its 95% confidence level was 0.85-1.07 denoting a lack of relationship between sex and the prevalence of *T. equi* antibodies in the study population.

Discussion

Clinical signs that could be attributed to the disease were not observed in any of the sampled donkeys. The absence or rarity of clinical disease was consistent with the nature of the disease in an endemic setting, where equids over time have developed protective immunity (Barbosa *et al*., 1995; Kumar *et al.,* 2009). Nonetheless working equids in the developing world are commonly subjected to overworking and as a result suffer from chronic fatigue and depression (Swann, 2006; personal observation D.O.O). Overexertion is a major contributing factor to overt disease in donkeys (Anzuino, 2008; Kumar *et al.*, 2009). This, however, was not the situation as sampling was conducted in the month of November. Mwingi district experiences a spell of rain between October to November (MoE, 2004). This was the case during the sampling exercise. As donkeys in this area are primarily used for transporting water from rivers and boreholes to homesteads, when it rains, donkeys and their owners traverse shorter distances to achieve this. They generally make fewer trips to water sources as farmers usually trap rain water (personal observation, D.O.O). This could in addition help explain the apparently healthy animals sampled.

The overall seroprevalence of *T. equi* was found to be 81.2% (95% CI: 76.4, 85.4). Previous studies on donkeys have reported a wide range in the value of (sero)prevalence, this particular study was on the higher side of the range reported. Reported *T. equi* true (sero)prevalence in donkeys range from approximately 2 to 85% (Turnbull *et al*., 2002; Chahan *et al*., 2006; Acici *et al*., 2008; Machado *et al*., 2012; Garcia-Bocanegra *et al*., 2013; Gizachew *et al*., 2013; Salim *et al*., 2013) and was similar to the work of Hawkins *et al*. (2015) who found a prevalence of 72% (95% CI: 60.4–81.0%) using PCR. Scrutiny of prevalence values from previous studies is, however, limited due to their unique study designs and the effect of external factors such as husbandry (Kouam *et al*., 2010a; Garcia-Bocanegra *et al*., 2013). Comparing the area seroprevalence values, a uniformity was notable (see Table 2). A likely explanation for this is the relatively close proximity of the sampling sites to each other,

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the sites lie within an area 1710 km^2 (MoE, 2004) and the fact that they all lie within the same agro-ecological zone. This in turn subjects them to similar climatic conditions and vegetation cover. Ticks, which are the most likely agents of transmission of *T. equi* in the area are affected by climatic factors such as humidity, temperature and vegetation cover (Young and Leitch, 1981; Minshull and Norval, 1982; Chilton and Bull, 1994). The homogeneity of these factors in the sampling sites would dictate a similar tick distribution, infectivity, and consequently *T. equi* seroprevalence in the target hosts. Donkeys in this region are usually kept in a free range type of system with minimal input in terms of shelter and acaricide use (personal observation, D.O.O). Asgarali, *et al*. (2007) similarly reported a lack of association among area seroprevalence values for *T. equi* in Trinidadian horses. Statistical relationships between *T. equi* prevalence and geographical area have, however, been demonstrated by Karetepe, *et al*. (2009) and Steinman, *et al*. (2012). Though ticks were observed on donkeys during the current work, no identification work was undertaken.

A summative examination of the 95% confidence interval of the point estimate for seroprevalence in all the six age groups illustrated overlaps (see Table 3) thus depicting the absence of significant differences in *T. equi* infection among different age groups in the study population. This was consistent with observations by Shkap, *et al*. (1998), Acici, *et al*. (2008), Karatepe, *et al*. (2009), Grandi, *et al*. (2011), Mujica, *et al*. (2011), Cantu-Martinez, *et al*. (2012), Steinman, *et al*. (2012) and Farkas, *et al*. (2013). Reports by Asgarali, *et al*. (2007), Ruegg, *et al*. (2007) and (2008), Kouam, *et al*. (2010a) and Garcia-Bocanegra, *et al*. (2013) have, however, illustrated statistically significant cumulation of infection with progress in age. A possible explanation for the absence of differences in seropositivity among age groups in this study would be the presence of high transmission levels resulting in equids requiring shorter periods of exposure to become infected. This likelihood has been suggested in equid populations reared in endemic areas (Kumar *et al*., 2008; Kouam *et al*., 2010a). A likely explanation for the high seroprevalence in foals of less than a year old would be that the cELISA positives in this age group were mostly due to maternal antibodies acquired passively through colostrum as foals are born immunologically naive (de Waal and van Heerden, 2004; Kumar *et al*., 2008). The high seroprevalence of maternal antibodies in foals, approximately 85% and the high antibody prevalence in older animals, around 80-85% further suggest of a state of endemic stability (Ruegg *et al*., 2006). A generally high *T. equi* seroprevalence is demonstrated in this study across age groups, with seroprevalence persisting into old age. This suggests a lifelong carrier state (de Waal and van Heerden, 2004; Ruegg *et al*., 2008).

Upon exploring a possible statistical relationship between gender and seroprevalence of *T. equi* in this study, no association was found. This agreed with previous studies by Asgarali, *et al*. (2007), Karatepe, *et al*. (2009), Kouam, *et al*. (2010a), Grandi, *et al*.

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(2011), Mujica, *et al*. (2011), Cantu-Martinez, *et al*. (2012), Steinman, *et al*. (2012), Farkas, *et al*. (2013) and Gizachew, *et al*. (2013). The result, however, disagreed with reports by Shkap, *et al*. (1998) (*T. equi* seroprevalence 33.7%, 95% CI: 28.9-38.7, *n*=361) and Ruegg, *et al*. (2007) (*T. equi* seroprevalence 78.8%, 95% CI: 74.9-82.3, *n*=510). Their studies demonstrated significant differences in *T. equi* exposure between male and female animals. In both cases stallions were less likely to be infected with *T. equi* when compared to mares and geldings. The reason for this was that stallions in both study areas had the role of breeding and were thus kept in environments where they were less likely to come into contact with ticks. As mentioned earlier, donkeys in the study area are kept in a free-range type of system where both male and female animals forage together in communal grazing grounds and are thus similarly exposed to tick infestation (personal observation, D.O.O). This possibly contributed to the findings.

The survey did not detect *B. caballi* antibodies. One possibility that could explain this is a generally low prevalence or complete absence of *B. caballi* infective ticks in the population. Recent work in northern Kenya found no evidence of *B. caballi* infection in donkeys or zebras (Hawkins *et al*., 2015) using a nested PCR reaction targeting the 18s ribosomal (r)RNA gene. The other explanation would be a failure of the diagnostic test to detect the particular strain(s) of *B. caballi* in the sample. Diagnostic failure by the cELISA test that we used has previously been documented. For example, Bhoora *et al*. (2010b) in a study on South African horses found a seroprevalence of 0% despite an indirect fluorescent antibody test (IFAT) detecting a seroprevalence of 5.1% (95% CI: 3.9-6.5). This was associated with nucleotide heterogeneity in the rhoptry-associated protein 1 (RAP-1). Further work would be needed to confirm the presence/absence of *B. caballi* in the Mwingi donkey population. Reverse line blot hybridization and quantitative real-time PCR have reported successes in detecting *B. caballi* in chronic non clinical carriers with low parasitaemia (Nagore *et al*., 2004; OIE, 2008; Bhoora *et al*., 2009; Kouam *et al*., 2010b; Ros-Garcia *et al*., 2013).

Conclusions

The high *T. equi* seroprevalence in the young and old demonstrated in this study is suggestive of an endemically stable state (Ruegg *et al*., 2006). *T. equi* seropositivity within different geographical areas was uniform and the demographic factors: age and sex had no influence on seropositivity. This study, complements earlier ones by Vranova *et al*. (2011) and Hawkins *et al*. (2015) and would be relevant to the office of the Director of Veterinary Services (DVS). The DVS represents Kenya to the OIE and is responsible for submitting reports on various livestock diseases including equine piroplasmosis. The latest report submitted indicated a suspected occurrence of the disease in the year 2005 (WAHID, 2012). The knowledge of the equine piroplasmosis

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status of a country is important in the context of international trade in equines (OIE, 2008).

Bruning (1996), Kerber *et al*. (1999) and Norval and Horak (2004) recommend strategic tick control in equine piroplasmosis endemic areas as this reduces economic losses whilst still maintaining the host-environment-vector balance that ensures foals are exposed to infection early in life averting potentially fatal disease afterwards. In the study area, losses that could be attributed to the disease as per the authors' experience would include abortions, death and reduced capacity to work. As these negatively impact this arid community's livelihood, intervention after more comprehensive investigations is worthwhile. Research into risk factors, seasonal variations in equine piroplasmosis prevalence, entomological investigations and socioeconomic studies would complement this piece of work and may aid in forging a stronger evidence base for intervention (Pegram *et al*., n.d.) as based solely on the epidemiological picture in this study, tick control may not be necessary.

In the short term, however, non-governmental organizations working in this area on donkeys could be confidently informed of the exposure status of the population. This would appropriately modify the content of the training offered to local animal health practitioners (influencing the drugs sold at their drug stores) and also the training offered to farmers on donkey husbandry.

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Table 1: Determining the number of donkeys to be sampled from Nuu sub-locations based on an 18% (314/1724*100) proportion.

Sub-location	Donkey population	Number to be sampled
Mwangeni	177	314/1724*177 = 32
Mutyangombe	183	314/1724*183 = 34
Kaai	243	314/1724*243 = 44
Kavindu	155	314/1724*155 = 28
Ngangani	111	314/1724*111 = 20
Mutulu	119	314/1724*119 = 22
Mwambiu	243	314/1724*243 = 44
Kathanze	210	314/1724*210 = 38
Ngaani	283	314/1724*283 = 52
Total	1724	314

Table 2: Sub-location seroprevalence of *T. equi* in donkeys in Nuu division, Mwingi district, Kenya. The sample size per sub-location and the 95% confidence interval for seroprevalence are also indicated.

Sub-location	Number of donkeys sampled	Seroprevalence of *T. equi* (%)	95% confidence interval (%)
Mwangeni	32	78.1	60.0-90.7
Mutyangombe	34	85.3	68.9-95.0
Kaai	44	79.5	64.7-90.2
Kavindu	28	92.9	76.5-99.1
Ngangani	20	90.0	68.3-98.8
Mutulu	22	86.4	65.1-97.1
Mwambiu	44	70.5	54.8-83.2
Kathanze	38	71.1	54.1-84.6
Ngaani	52	86.5	74.2-94.4

Table 3: Representation of the population and seroprevalence of animals in different age groups

Age group of donkeys (years)	Number of donkeys	Seroprevalence of *T. equi* (%)	95% confidence interval (%)
< 1	27	85.2	66.3-95.8
1-3	43	67.4	51.5-80.9
3.5-5	10	80.0	44.4-97.5
6-10	43	81.4	66.6-91.6
11-15	87	85.1	75.8-91.8
≥ 16	104	82.7	74.0-89.4

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Highlights

- Serosurveillance of *Theileria equi* and *Babesia caballi* in donkeys is conducted by cELISA.
- *T. equi* piroplasmosis is endemic in donkeys from Eastern Kenya.
- No Statistical associations between seroprevalence and age, location or gender were established.
- *B. caballi* antibodies were not detected.

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