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REGULAR ARTICLES

Occurrence of serum antibodies to *Toxoplasma gondii* and associated risk factors in donkeys from central Kenya

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Abstract

Toxoplasma gondii is an intracellular parasite of zoonotic concern and economic importance in humans and animals, respectively. This study was conducted to determine the occurrence of *T. gondii* and associated risk factors in domestic donkeys from Kirinyaga and Meru counties in Kenya. Blood samples were collected from 363 randomly selected donkeys for detection of antibodies to *T. gondii* using a commercial kit ID Screen® Toxoplasmosis Multi-species indirect enzyme-linked immunosorbent assay (ELISA). The data on risk factors were collected by interviewing donkey owners using epidemiological questionnaire. Serum antibodies to *T. gondii* were detected in 26.4% (95% CI: 22.2–31.3) of the donkeys. The analysis showed that age of donkeys (OR = 2.484, 95% CI: 1.315–4.693; $p = 0.005$) was associated with increased risk for *T. gondii* seroprevalence while county of origin of donkeys (OR = 0.182, 95% CI: 0.083–0.400; $p = 0.000$), residential place of donkeys (OR = 0.301, 95% CI: 0.136–0.665; $p = 0.003$), rearing chicken (OR = 0.203, 95% CI: 0.064–0.644; $p = 0.007$), and donkey production system (OR = 0.644, 95% CI: 0.456–0.909; $p = 0.012$) were associated with reduced risk of *T. gondii* seroprevalence. This is the first report to provide epidemiological information on *T. gondii* infection among donkeys in Kenya. The presence of antibodies to *T. gondii* in donkeys suggests the high potential of transmission to other animals and humans. Regular monitoring and control of *T. gondii* infection in donkeys were recommended in the study area.

Keywords Donkeys · Indirect ELISA · Occurrence · Risk factors · *Toxoplasma gondii*

Introduction

Toxoplasma gondii (*T. gondii*), the etiological agent of toxoplasmosis, is an intracellular protozoan parasite (Dubey, 2010a) with a worldwide distribution with the exception of Artactica (Li et al., 2020; Al-Malki et al., 2021). The definitive hosts for *T. gondii* are cats and other felids (Dubey, 2010a). The parasite has a broad range of intermediate hosts (Markovic et al.,2014) comprising mammals including humans and birds which harbor the cysts stage in their tissues (Foroutan et al., 2019; Opsteegh et al., 2016). Among the food-borne infections of parasitic origin, *T. gondii* infection has been ranked fourth worldwide (Torgerson et al., 2015) with a third of the human population being estimated to have had exposure to the parasite (Zhou et al., 2018). In humans, infection with *T. gondii* occurs through ingestion of oocysts shed by cats (Wang et al., 2017), consumption of bradyzoites in raw or undercooked meat from intermediate hosts (Tenter et al., 2000; Weiss and Dubey, 2009), congenital infection, blood transfusion, and organ transplant (Morris et al., 2010). The main sources of *T. gondii* infection for domestic animals including donkeys are water or feed contaminated with sporulated oocysts and probably from mother to fetus (Dubey, 2010a). In livestock, especially small ruminants, *T. gondii* is mainly involved in abortions (Hussein et al., 2011), reproductive failure (Fernández-Escobar et al., 2020), loss of milk production (Ali et al., 2021), and production of weaker offsprings (Khan and Noordin, 2020). Generally, *T. gondii* infection in equines is considered to be asymptomatic (Dubey, 2010a; Zeybek et al., 1998) but infected equines may display unusual clinical signs (Miao et al., 2013).

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In Kenya, the donkey population is estimated at 1.8 million out of which two-thirds plays a role in the economy by providing employment opportunities and income (Maichomo et al., 2019). Donkey meat was legalized in 1999 (GoK, 2012) in order to curb back yard slaughter, improve food security, and increase donkey production in response to availability of market. An increase on global demand for donkey meat led to establishment of commercial donkey slaughter houses (Maichomo et al., 2019). Most of the meat and skin from the slaughterhouses is exported to Asian countries especially China and reports indicate that donkey meat could locally be sold fraudulently as beef (Gichure et al., 2020). Kenya exported total of 16,544 tonnes of donkey meat valued at Ksh 1.76 billion between 2016 and 2018 (KNBS, 2019; Maichomo et al., 2019). Additionally, donkey meat and milk are consumed by some communities such as the Turkana and Maasai (Rono et al., 2018; Fernando and Starkey, 2004). Infection with *T. gondii* has been reported in donkeys worldwide (Zhang et al., 2017; Dubey et al., 2020). Although infected equines remain asymptomatic, *T. gondii* DNA has been isolated from donkey milk (Mancianti et al., 2014) and viable *T. gondii* has been isolated from donkey tissue (Gennari et al., 2015). Although Kenya is a major donkey breeding area with a well-developed donkey industry, there is scarcity of information on *T. gondii* seroprevalence in donkeys. Therefore, the current study was carried out to determine the occurrence of antibodies to *T. gondii* and evaluate potential risk factors associated with seroprevalence in domestic donkeys in Kirinyaga and Meru counties of Kenya.

Materials and methods

Study area

The study was conducted in Kirinyaga and Meru counties (Supplementary Fig. S1) that lie in central and upper eastern regions of Kenya, respectively. The two counties were purposively selected based on the high population of donkeys in these areas (Maichomo et al., 2019; Chege et al., 2015). Kirinyaga County (latitudes 001′ and 00 40′ S and longitudes 37° and 38° E) lies on the southern slopes of Mount Kenya and southeastern slopes of the Aberdare Ranges. The altitude ranges from 1158 to 5380 m in the south and at the peak of Mt. Kenya, respectively. The county has a tropical climate and an equatorial rainfall pattern with two rainy seasons, the long rains which average 2146 mm and short rains which average 1212 mm. The amount of rainfall declines from the high-altitude slopes of Mt. Kenya towards the semi-arid zones. Temperature ranges from a mean of 8.1 °C in the upper zones to 30.3 °C in the lower zones during the cold and hot seasons, respectively (GoK, 2018a, b).

Meru County (longitudes 0° 6′ N and 0° 1′ S and between latitudes 37°W and 38°E) covers the northern to eastern slopes of Mount Kenya. The climate is warm and temperate. Rainfall ranges from 300 mm in the lower midlands in the north to 2500 mm in the southeast. Other areas receive on average 1250 mm of rainfall annually. Temperatures range from 8 to 32 °C during the cold and hot seasons, respectively (GoK, 2018a, b).

Study design and sampling

This was a cross-sectional epidemiological study that was carried out between September, 2019 and December, 2020. The units of study were donkeys within the households that owned them. Since no data was available to estimate the distribution of donkeys in Kirinyaga and Meru counties, it was not feasible to design a sample that would reliably represent the donkey population in these two counties. Administrative regions (sub-counties) and donkey owner households were purposively selected based on availability of donkeys under the guidance of chairpersons of donkey owner's association and local administrators. In Kirinyaga County, sampling was done in Mwea West and Mwea East sub-counties while in Meru County, sampling was done in Tigania East, Igembe Central, Imenti Central, Imenti South, and Buuri sub-counties (Fig. 1). Blood samples from donkeys were collected through systematic random sampling. However, in Imenti Central, Imenti South, and Buuri sub-counties where the population of donkeys was low, all the available donkeys were sampled. The sample size was calculated assuming a prevalence of 50%, using the formula $n = Z\alpha^2 pq/L^2$ (Dahoo et al., 2009) where n is the sample size, $Z\alpha$ is the accepted error at 5% level of significance, p is the estimated prevalence, $q = (1-p)$, and L is the allowable error of estimation. *Toxoplasma gondii* seroprevalence of 50% was assumed due to the fact that no study has been conducted in donkeys in Kenya. Therefore, with a desired absolute precision of 5% and 95% level of confidence, blood samples were required from at least 384 donkeys. However, samples were analyzed from 363 donkeys comprising of 177 and 186 donkeys from Kirinyaga and Meru counties respectively.

Collection and handling of blood samples

All the procedures involving the collection of blood samples followed the national and international protocols for research in Veterinary Medicine. Blood samples were collected from donkeys raised by residents of Kirinyaga and Meru ccounties (from September, 2019 to December, 2020). Venipuncture of the jugular vein was used to obtain 5 ml of blood from each animal by use of sterile 10-ml tubes (Vacutainer® Beckton-Dickinson, USA) without EDTA (anticoagulant). Blood samples were labeled and stored in icebox during the day

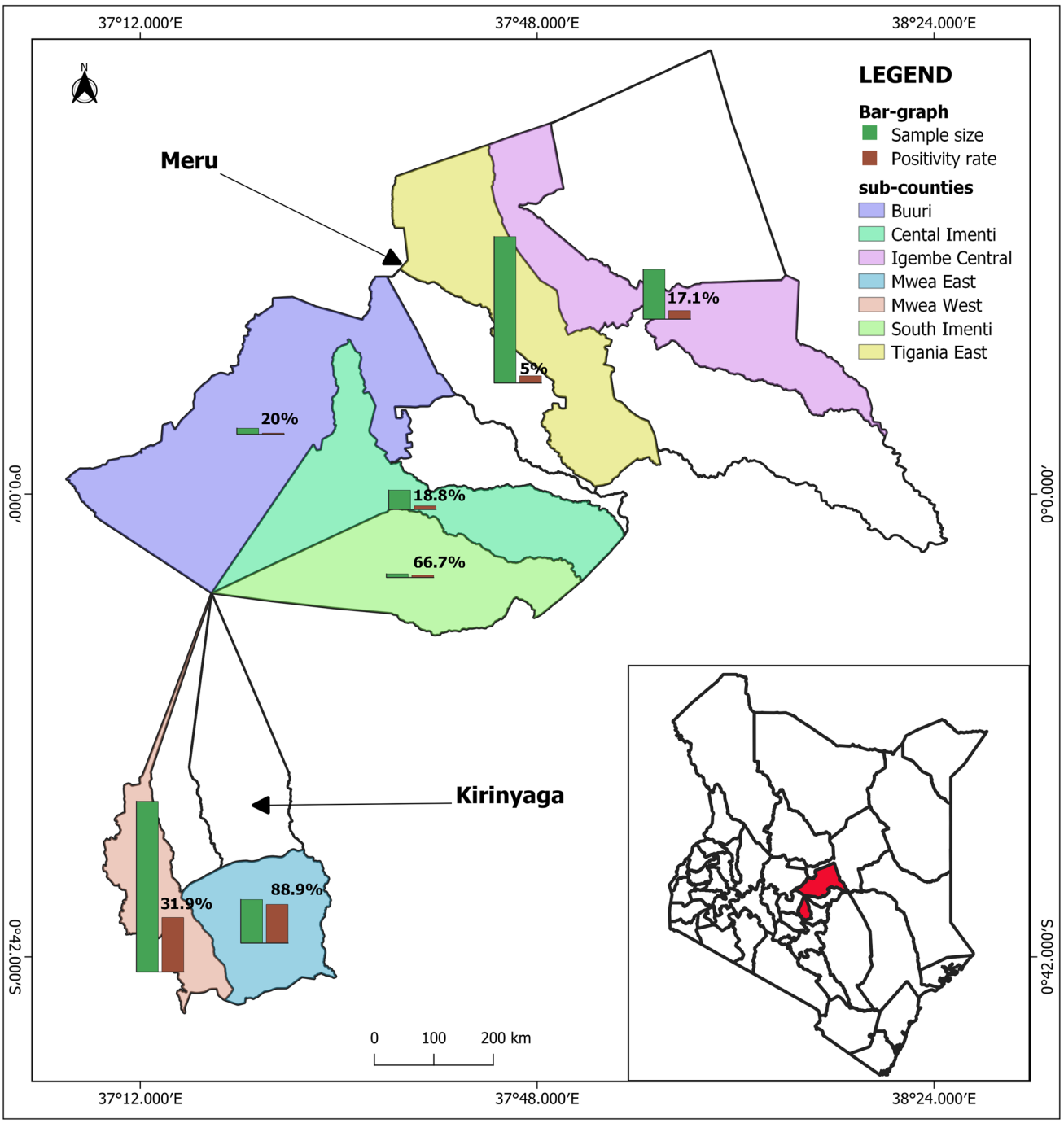

Fig. 1 Seroprevalence of *Toxoplasma gondii* infection in donkeys from different sub-counties of Kirinyaga and Meru counties

and transported to the laboratory at Meru University of Science and Technology where vacutainer tubes were removed from the icebox and blood allowed to clot. After staying overnight at room temperature, the samples were centrifuged at 3000 × *g* for 10 min to separate the serum. The serum samples (approximately 1 ml per sample) were transferred into clean 1.5-ml tubes (Eppendorf Tube®) and stored at − 20 °C awaiting further analysis for antibodies to *T. gondii*.

Serological detection of *T. gondii* antibodies

Serum samples from donkeys were tested for *T. gondii* IgG antibodies using commercial kit ID Screen® Toxoplasmosis Indirect Multi-species Indirect ELISA (ID Vet, Montpellier, France). The kit detects specific immunoglobulin G (IgG) antibodies following infection with *T. gondii* by use of P30 *T. gondii* protein as a substrate and a multi-species

peroxidase as a conjugate. The kit has been used to test for *T. gondii* antibodies in horse sera (Ouslimani, 2019; Paștiu et al., 2015). Positive and negative control sera were provided in the kit by the manufacturer. The test was done following the manufacturer's instructions. The optical density (OD) of ELISA results was read at 450 nm. The percentage sample (*S*) to positive (*P*) ratio (S/P %) for each of the test serum samples were calculated according to the following formula: $S/P\% = \frac{\text{ODsample}-\text{ODnegativecontrol}}{\text{ODpositivecontrol}-\text{ODnegativecontrol}} \times 100$.

The serum samples with *S*/*P*% values greater than 50% were considered to be positive, those between 40 and 50% were classified as doubtful, and measurements less than or equal to 40% were considered to be negative as per the manufacturer. Borderline results were considered positive to achieve a dichotomous outcome.

Epidemiological questionnaire

After the collection of blood samples, the donkey owners were interviewed to answer semi-structured questionnaire composed of semi-open/objective questions, to obtain information on possible risk factors for *T. gondii* infection. Information collected in the questionnaire was related to demographic information (age, sex, and marital status, level of education, occupation, and area of residence/location of the farm) of donkey keepers. The questionnaire also contained questions regarding herd structure (number of donkeys kept, age, sex, body condition score, and contact with cattle, sheep, goats, pigs, poultry, dogs, and cats). Information about feeding and management practices (production system, feeding type, provision of feed, source of drinking water, and how the water and feed are provided) was also collected. Questions on farm biosecurity (housing system, provision of bedding, frequency of changing the bedding, source of replacement stock, presence of cats, presence of rodents, contact with wild animals, and rodent control) were also included in the questionnaire.

Statistical analysis

The serological results and questionnaire data were entered into Excel 2010 (Microsoft, Sacramento, California, USA), coded, and exported to the Stata® 15.1 statistical software (StataCorp LLC, College Station, Texas, USA) for analyses. Descriptive statistical analysis was used to summarize data and determine the occurrence of *T. gondii* in collected blood samples. For categorical variables, proportions were obtained while mean, standard deviation, and median were calculated for continuous variables. The seroprevalence (%) of antibodies to *T. gondii* was determined from the proportion of seropositive donkeys to the total number of donkeys examined and multiplying by hundred.

Initially, univariate multilevel mixed-effects logistic regression model was used to determine the unconditional relationship between the predictors and the seroprevalence of the animal and variables with $p \leq 0.20$ were offered for multivariable analysis. The significant variables by univariate analysis were used for multivariate analysis. Multilevel mixed-effects logistic regression analysis that accounted for the effects of clustering within herds at the household was used to evaluate the relationship between categorical and continuous variables and *T. gondii* seroprevalence. Subsequently, a final multivariable multilevel mixed-effects logistic regression model ($p \leq 0.05$) was built using a backward selection. Potential confounding was evaluated and considered if the coefficients of the variables in the model were altered by more than 30% when the non-significant explanatory variable was removed (Dahoo et al., 2009). Two-way interactions between the explanatory variables in the final model were tested, and an interaction term was retained if the interaction term's *p*-value was ≤ 0.05. The overall performance of the model was assessed by use of area under the curve (AUC) of the receiver operating characteristics. Epidemiological maps of the distribution of sampled donkeys and seroprevalence from different areas of Kirinyaga and Meru counties were constructed using Arc Gis 10.1 (ESRI).

Results

Socio-demographic characteristics of donkey keepers in Kirinyaga and Meru counties

Overall, 84.2% of the respondents were male while 15.8% were female (Supplementary Table S1). In terms of gender distribution, Kirinyaga County had the highest number of male respondents (53.0%) and Meru had the least (47.0%). Most of the donkey owners were married (90.6%) with a few being single (10.3%) and 0.7% were divorced. Regarding the level of education, most of the donkey owners (67.6%) had attained primary school education with a few attending high school (23.0%) and tertiary institutions (2.2%). Majority (40.3%) of the donkey owners relied on farming as source of income, 36.7% relied on farming and business, while 19.4% obtained their income from business alone. A large proportion (43.9%) of the respondents had kept donkeys for over 10 years with some respondents keeping donkeys between 5–9 years (33.8%) and 1–4 years (20.9%). Majority (94.2%) of the respondents kept at least one animal species apart from donkeys. Ninety-six farms (69.1%) reared both donkeys and cattle. Besides rearing cattle, there were other animal species kept including sheep (45.3%), goats (45.3%), poultry (92.8%), pigs (13%), dogs (53.2%), and cats (49.5%).

Herd structure

The mean number of donkeys kept was 3.2 with a range of 1 to 12. The age of the donkeys ranged between 4 months and 24 years (mean = 5.6 years, median = 6.0 years, standard deviation = 3.5 years). One hundred and fifty-three (42%) of the donkeys were female while 210 (58%) were male.

Seroprevalence of *T. gondii* in donkeys

Of the 363 donkeys examined from Kirinyaga ($n = 177$) and Meru ($n = 186$) counties, 96 (26.4%; 95 CI: 22.2–31.3) were seropositive for *T. gondii* antibodies (Supplementary Table S2). The seroprevalence of *T. gondii* in donkeys from Kirinyaga and Meru counties were 43.5% (77/177) and 10.2% (19/186), respectively. There was a significant difference in seroprevalence of *T. gondii* between the two counties ($p = 0.000$). In Kirinyaga County, a higher seroprevalence (88.9%) was recorded in donkeys from Mwea East sub-county than in donkeys from Mwea West sub-county (31.9%). Seroprevalence was significantly associated with the differences in locality of donkeys between two sub-counties ($p = 0.003$). In Meru County, the highest seroprevalence was recorded in donkeys from Imenti South sub-county (66.7%) while the lowest seroprevalence (5.0%) was recorded in donkeys from Tigania East sub-county (Fig. 1). Igembe Central, Imenti Central, and Buuri sub-counties recorded seroprevalence rates of 17.1%, 18.8%, and 20.0% respectively (Supplementary Table S2). Seroprevalence of *T. gondii* within the five sub-counties in Meru County was significantly associated with locality of the donkeys ($p = 0.003$).

The occurrence of *T. gondii* antibodies in male and female donkeys was 32.4% and 18.3%, respectively, with a significant difference between the sexes ($p = 0.003$) (Supplementary Table S3). In both counties, a higher seroprevalence of *T. gondii* was recorded in male donkeys from Kirinyaga (45.0%) and Meru (12.3%) counties than in female donkeys whose seroprevalence was 39.6% and 8.6% from the same counties, respectively. Male donkeys from Kirinyaga County exhibited higher seroprevalence (45.0%) than male donkeys from Meru County (12.3%). Also, female donkeys from Kirinyaga County recorded a higher seroprevalence (39.6%) than female donkeys from Meru County (8.6%). Seroprevalence in both counties was significantly associated with the sex of the donkey ($p = 0.003$).

A higher seroprevalence of *T. gondii* infection was recorded in donkeys older than 6 years and the lowest was recorded in donkeys aged less than 6 years. There was a significant difference ($p = 0.031$) among different ages of donkeys in Kirinyaga and Meru counties (Supplementary Table S3). Donkeys aged more than 6 years from Kirinyaga County exhibited a higher seroprevalence (56.1%) than donkeys from Meru County (14.0%). Similar results were recorded for donkeys aged less than 6 years where higher seroprevalence (37.5%) was recorded for donkeys from Kirinyaga County than 6.5% for Meru County. In Kirinyaga County, a higher seroprevalence (56.1%) was recorded in donkeys older than 6 years than in donkeys aged less than 6 years (37.5%). Also, in Meru County, higher seroprevalence (14.0%) was recorded in donkeys aged more than 6 years than in donkeys aged less than 6 years (6.5%). Seroprevalence was not significantly associated with age between the two counties ($p = 0.198$).

Risk factors associated with *T. gondii* seroprevalence

In the univariate analysis (Table 1), 15 variables were significantly found to be associated with *T. gondii* seroprevalence in donkeys including the county of origin of donkeys (OR = 0.157, CI: 0.084–0.259), residence place of donkeys (OR = 0.160, CI: 0.093–0.275), sex of the donkeys (OR = 0.468, CI: 0.283–0.772), age of the donkeys (OR = 2.100, CI: 0.951–1.084), rearing sheep (OR = 0.527, CI: 0.324–0.858), rearing goats (OR = 0.421, CI: 0.260–0.683), keeping dogs (OR = 0.337, CI: 0.208–0.546), rearing chicken (OR = 0.210, CI: 0.091–0.486), keeping of cats (OR = 0.474, CI: 0.294–0.766), type of production system (OR = 0.411, CI: 0.246–0.689), main way of feeding the donkey (OR = 8.056, CI: 3.026–21.442), feeding donkeys on something else apart from the main feed (OR = 2.759, CI: 1.671–4.555), source of drinking water (0.164, CI: 0.080–0.337), how water is provided to animals (OR = 0.538, CI: 0.326–0.890), and contact of donkeys with wild animals (OR = 0.437, CI: 0.219–0.871). Factors which were not significantly associated with *T. gondii* infection were rearing cattle (OR = 0.731, CI: 0.440–1.214), rearing pigs (OR = 1.300, CI = 0.659–2.564), source of feed for the donkeys (OR = 1.250, CI: 0.901–1.735), and presence of rodents in the farm (OR = 1.098, CI: 0.662–1.820).

In the multivariate analysis (Table 2), the age of the donkey had significant effect on *T. gondii* seroprevalence (OR = 2.484, 95% CI: 0.083–0.400, $p = 0.005$). The odds to test seropositive were 2.5 times higher in donkeys sampled from those aged more than 6 years than those aged less than 6 years. Keeping donkeys under extensive production system (OR = 0.644, 95% CI: 0.456–0.909, $p = 0.012$), donkeys residing in the urban area (OR = 0.301, 95% CI: 0.136–0.665, $p = 0.003$), rearing chicken on the farm (OR = 0.203, 95% CI: 0.064–0.644, $p = 0.007$), and donkeys originating from Meru County (OR = 0.182, 95% CI: 0.084–0.259, $p = 0.000$) were shown to have protective effect on *T. gondii* seroprevalence in donkeys.

The area under the curve (AUC) of the receiver operating characteristics (ROC) was 0.85 implying that the observed data had a good overall goodness-of-fit (Fig. 2).

Table 1 Univariate analysis of risk factors associated with seroprevalence of *Toxoplasma gondii* in 363 donkeys from 139 farms in Meru and Kirinyaga counties, Kenya

Variable	Categories	No. of samples tested	No. positive (% prevalence)	OR	*p*-value	CI (95%)	
						LCL	UCL
County of origin of donkeys	Kirinyaga	177	77 (43.5)	0.157	0.000*	0.084	0.259
	Meru	186	19 (10.2)				
Residence of the donkey	Rural	78	45 (57.7)	0.160	0.000*	0.093	0.275
	Urban	285	51 (17.9)				
Sex of the donkey	Male	210	68 (32.48)	0.468	0.003*	0.283	0.772
	Female	153	28 (18.3)				
Age of the donkey	< 6 years	213	51 (23.9)	2.100	0.031*	0.951	1.084
	> 6 years	150	45 (30.0)				
Farmer rear cattle	No	100	31 (31.0)	0.731	0.226 ns	0.440	1.214
	Yes	263	65 (67.7)				
Farmer rear sheep	No	201	64 (31.8)	0.527	0.010*	0.324	0.858
	Yes	162	32 (19.8)				
Farmer rear goats	No	178	62 (34.8)	0.421	0.007*	0.260	0.683
	Yes	185	34 (18.4)				
Farmer keep dogs	No	156	60 (38.5)	0.337	0.001*	0.208	0.546
	Yes	207	36 (17.4)				
Farmer rear chicken	No	25	15 (60.0)	0.210	0.000*	0.091	0.486
	Yes	338	81 (24.0)				
Farmer rear pigs	No	318	82 (25.8)	1.300	0.449 ns	0.659	2.564
	Yes	45	14 (31.1)				
Farmer keep cats	No	174	59 (33.9)	0.474	0.002*	0.294	0.766
	Yes	189	37 (19.6)				
Production system used	Intensive	86	35 (40.7)	0.411	0.001*	0.246	0.689
	Extensive	277	61 (22.0)				
Main way of feeding the donkeys	Grazing	342	81 (23.7)	8.056	0.000*	3.026	21.442
	Stall feeding	21	15 (71.4)				
Source of feed for the donkeys	Agrovets	57	8 (11.0)	1.250	0.181 ns	0.901	1.735
	Own farm	122	39 (32.0)				
	Communal grazing	179	49 (27.4)				
Donkey being fed on anything else	No	170	28 (16.5)	2.759	0.000*	1.671	4.555
	Yes	193	68 (35.2)				
Source of drinking water for the donkeys	Tap water	84	32 (38.1)	0.164	0.000*	0.080	0.337
	Borehole water	142	13 (9.2)				
	Stream	137	51 (37.2)				
How water is provided to the donkeys	Individual water troughs	98	35 (35.7)	0.538	0.016*	0.326	0.890
	Communal watering points	265	61 (23.0)				
Rodents present in the farm	No	115	29 (25.2)	1.098	0.718 ns	0.662	1.820
	Yes	248	67 (27.0)				
Wild animals come in contact with donkeys	No	72	11 (15.3)	0.437	0.019*	0.219	0.871
	Yes	291	85 (29.2)				

OR odds ratio; *CI* confidence interval; *ns* not significant.

*Significant *p*-values < 0.05.

Discussion

This is the first epidemiological study of *T. gondii* infection and associated risk factors in donkeys in Kenya. Previously, *T. gondii* infection in Kenya has been detected in humans (Thiong'o et al., 2016; Mose et al., 2020), chicken (Dubey et al., 2005; Mose et al., 2016), and cats (Njuguna et al., 2017). In Kenya, donkeys are used as both companion and

Table 2 Multivariate analysis of risk factors associated with seroprevalence of *T. gondii* in 363 donkeys from 139 farms in Meru and Kirinyaga counties, Kenya

Variable	Category	OR	95% CI		*p*-value
			LCL	UCL	
County of origin of donkeys	Kirinyaga	Baseline			
	Meru	0.182	0.083	0.400	0.000
Age of donkeys	≤6yrs	Baseline			
	>6yrs	2.484	1.315	4.693	0.005
Residential place of donkey	Rural	Baseline			
	Urban	0.301	0.136	0.665	0.003
Keeping of chicken	No	Baseline			
	Yes	0.203	0.064	0.644	0.007
Production system	Intensive	Baseline			
	Extensive	0.644	0.456	0.909	0.012

OR odds ratio; *CI* confidence interval.

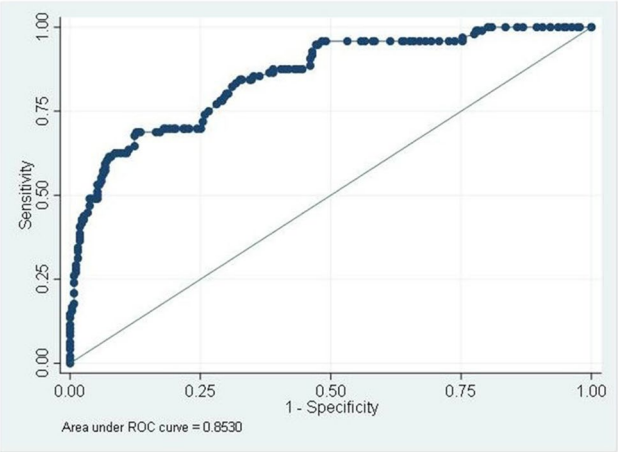

Fig. 2 Area under the curve graph indicating goodness-of-fit of the final model on risk factors of *Toxoplasma gondii* infection for 363 donkeys on 139 donkey farms in Kirinyaga and Meru counties, Kenya

working animals and in some communities, their meat and milk are used for human consumption.

The *T. gondii* seroprevalence in donkeys in this study was 26.4%, which falls within the range reported for donkeys in Africa. The seroprevalence of *T. gondii* infection in donkeys varies widely between countries and ranges from as low as 17% in Nigeria (Bártová et al., 2017) to as high as 68.4% in Egypt (Younis et al., 2015). The variations in seroprevalence may be associated with the differences in sensitivity of the detection methods and the cutoff titer used in the interpretation of results, the number of donkeys, age of the animals, sanitation, environmental and climatic conditions, geographical area, feeding practices, and farm management systems (Meng et al., 2018; Dubey et al., 2014; Machacova et al., 2014; Tenter et al., 2000). The seroprevalence of *T. gondii* infections in donkeys reported in this study was lower than 44.3% with latex agglutination test (LAT) and 68.4% with ELISA (Younis et al., 2015), 45% (Haridy et al., 2010) and 28.8% (Fereig et al., 2016) with ELISA in Egypt, 30% with modified agglutination test (MAT) in Algeria (Mohamed-Cherif et al., 2015), 26.7% with LAT in Sudan (Shadia et al., 2013), and 27.4% with LAT in Nigeria (Ishaku and Kumra, 2019).. The seroprevalence is lower than 17% with indirect fluorescent antibody test (IFAT) in Nigeria (Bártová et al., 2017) and 23% with MAT in Senegal (Davoust et al., 2015).

The results of our study showed that there was a significant difference in the seroprevalence of *T. gondii* infections in donkeys between the two counties, i.e., *T. gondii* seroprevalence was significantly higher in donkeys from Kirinyaga County compared to Meru County. This indicates that climatic conditions (temperature and humidity) significantly influence the risk of *T. gondii* exposure. The warm and moist climatic conditions and high percentage of relative humidity in Kirinyaga County could possibly favor the survival and increase the chances of sporulation of oocysts (Dubey, 1998; Robert-Gangneux and Darde, 2012) hence higher frequency of infected donkeys. Meru County has variable climatic conditions ranging from relatively hot and dry in the north and east portions to cooler and moist in the central and western part of the county (CCAFS, 2016) which could affect the development of oocysts (Yan et al., 2016). It seems likely that donkeys from Kirinyaga County were more frequently exposed to water and feed contaminated with *T. gondii* oocysts than donkeys from Meru County. These oocysts were most likely to have spread in the environment via wind, rain, or surface water. Surface water may contain high levels of oocysts due to the fact that they remain viable in water over a long period of time (Tenter et al., 2000). Similar observations were made in studies conducted by Oliveira et al. (2013) in Brazil in which the locality of the donkeys influenced *T. gondii* seroprevalence.

There was a significant difference between the seroprevalence of *T. gondii* in donkeys and the sub-county of origin of donkeys. In Kirinyaga County, donkeys from Mwea East sub-county exhibited a higher seroprevalence than donkeys from Mwea West sub-county. Since the two sub-counties have similar climatic conditions, the differences in the seroprevalence could be related to the variation in the level of contamination of the environment by cats which are the definitive hosts of *T. gondii* and other mechanical carriers of oocysts such as rodents (Arruda et al., 2020). In Meru County, the lowest *T. gondii* seroprevalence was recorded in donkeys from Tigania East sub-county while the highest seroprevalence was recorded in donkeys from Imenti South sub-county. This could be due to the fact that Tigania East sub-county is located in the northern part of Meru which is relatively hot and dry with an annual precipitation of less than 750 mm and temperatures greater than 23 °C (CCAFS,

2016). These conditions are less favorable for the development and maintenance of *T. gondii* oocysts in the environment (Dubey, 2010a).

The results of this study showed that age was an important factor for *T. gondii* infection. Donkeys older than 6 years had significantly higher risk of *T. gondii* seroprevalence than those of aged less than 6 years which is in agreement with the previous reports by Ishaku and Kumra (2019) and Bártová et al. (2017) in Nigeria and Younis et al. (2015) in Egypt. This can be attributed to the fact that as the age of the donkeys increases, the likelihood of acquiring *T. gondii* infection from the environment increases due to greater exposure time (Ribeiro et al., 2016). Also, the lifelong persistence of antibodies once infected might add to the high seroprevalence in older donkeys. Generally, exposure to a broad range of intermediate hosts and highly contaminated environment with *T. gondii* oocysts results in a long-term exposure of animals to the infective stages of the parasite. This exposure is thought to have a direct relationship with the age of the animal (Stelzer et al., 2019). Our results are in contrast to reports by Meng et al. (2018) and Munhoz et al. (2019), where the age of the donkeys did not influence *T. gondii* seroprevalence.

The results of the current study showed that residential place of the donkey was a significant risk factor for *T. gondii* infection. Donkeys from urban areas had lower risk of *T. gondii* seroprevalence than those from rural areas. This could be explained by differences in the level of exposure of donkeys to the sporulated oocysts of *T. gondii*. Although keeping cats was not significantly associated with *T. gondii* infection in the final multivariate model, nearly half (49.6%) of the farms sampled owned cats. Cats have been reported to play a significant role in spreading *T. gondii* oocysts especially when they are confined indoors (Machacova et al., 2014). This is because cats shed oocysts in the environment and they are considered to be the main source of infection for herbivores such as donkeys because of their feeding habits (Dubey, 2010a; Munhoz et al., 2019). Donkeys from rural areas could have had a high level of exposure to the infective stages of *T. gondii* due to poor hygiene especially where the donkeys are kept and probably during feeding which are common in the rural areas compared to urban areas (Alvarado-Esquivel et al., 2012). Additionally, donkeys from rural areas may also be exposed to an environment that favors the development and survival of infective stages of *T. gondii* because of availability of a wide range of intermediate hosts (Opsteegh et al., 2016). This was similar to the observations made in equids from Mexico (Alvarado-Esquivel et al., 2012) where equines from rural areas had higher seroprevalence of *T. gondii* in comparison to those from urban areas.

Donkeys from farms where chickens were kept had lower odds of *T. gondii* seroprevalence than donkeys from farms that did not rear chicken. We could not give a plausible explanation for this finding. The presence of chicken as a protective factor for *T. gondii* infection in donkeys needs further investigation. Chickens have been reported to play a significant role in the epidemiology of toxoplasmosis (Hussain et al., 2017). Evidence suggests that back yard chickens and chickens kept under free range in large operations are capable of harboring viable *T. gondii* in tissues and they become infected by feeding from the ground which could be contaminated with *T. gondii* oocysts. Chickens can therefore be an indicator of contamination of the environment with *T. gondii* oocysts (Hill and Dubey, 2013). Furthermore, these chickens are slaughtered at home due to lack of slaughter facilities where the viscera are not disposed properly and may be accessed by scavengers (Dubey, 2010b). Poultry could act as reservoir for *T. gondii* in a farm (Stelzer et al., 2019).

The results of the present study indicated that the type of production (rearing) system had a significant influence on *T. gondii* seroprevalence. Donkeys kept under extensive production system (where donkeys graze freely in the field without supplementation) were less likely to be infected with *T. gondii* than donkeys kept under intensive production system (where a few donkeys are kept and they may be tethered or kept in stables where they are fed with enough quantities of hay, straw, vitamins, and concentrate rations). This might be related to poor hygienic levels of the farms where the donkeys are kept under intensive production system leading to contamination of feedstuff by cat feces or rodents (Dubey, 2004). Also, feedstuff such as hay may be poorly kept for prolonged periods of time under intensive production system increasing the presence of other intermediate hosts such as rodents in the storage facilities or on the farm resulting in the spread of infection. Additionally, feedstuff may be contaminated accidentally with sporulated oocysts especially when concentrates are stored on the farm as compared to extensive production system where feed storage facilities may be lacking. Donkeys reared under extensive system are pastured in large areas of grazing. These animals may be exposed to infective stages of *T. gondii* at a lower level compared to those kept under intensive production system. This is similar to the observation made in Mexico (Alvarado-Esquivel et al., 2012) where stall fed equines had higher seroprevalence than pastured equines. However, this observation is contrary to the observations made by Zhang et al. (2017) in China where *T. gondii* seroprevalence was found to be higher in cage-free donkeys than caged donkeys.

The current results showed that *T. gondii* infection is common in donkeys in Kirinyaga and Meru counties in Kenya, and the parasite is likely to prevail in the tissues of the animals lifelong. Although the consumption of donkey meat is not common in the study areas, infection from contaminated meat could act as a transmission route to humans. Antibodies to *T. gondii* have been detected in donkeys slaughtered

for human consumption (Arruda et al., 2020) with a possible link to human toxoplasmosis (Machacova et al., 2014).

In conclusion, the seroprevalence of antibodies to *T. gondii* in the current study indicate the circulation of the parasite in donkey populations in Kirinyaga and Meru counties of Kenya posing a risk for both animal and human health. The study confirmed that the county of origin of donkeys, age of donkeys, residence of donkeys, rearing chicken, and production system were the factors associated with *T. gondii* seroprevalence in donkeys from Kirinyaga and Meru counties in central Kenya. The factors associated with reduced *T. gondii* seroprevalence were donkeys originating from Meru County, donkeys residing in urban areas, donkeys kept under extensive production system, and donkeys from farms that reared chicken. Older donkeys were associated with a higher *T. gondii* seroprevalence. This is the first epidemiological report documenting *T. gondii* infections in donkey populations in Kenya. The current data could be used as baseline information for the prevention and control of *T. gondii* infection in donkeys raised under similar management systems. The findings in this study highlight the need to educate donkey owners to improve sanitation and hygienic conditions in the animal stables. Additional studies are required to determine the role of specific reservoirs and consumption of donkey products in the transmission of *T. gondii* to humans.

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Author contribution FOO, NM, SMG, and ENN conceived and designed the research. FOO collected data, conducted the experiments, and drafted the manuscript. FOO and PK analyzed and interpreted data. NM, SMG, and ENN critically revised the manuscript. All authors read and approved the final manuscript.

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Data Availability The data sets used and analyzed in this study are available from the corresponding author upon request.

Code availability Not applicable.

Declarations

Ethics approval This study was approved by the Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi, Kenya, reference number FVM BAUEC/2019/240, and informed consent was obtained before sampling at the household level from the donkey owner.

Consent to participate All authors have consented to participate.

Consent for publication All authors have approved to publish.

Conflict of interest The authors declare no competing interests.

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