Genetic Detection and Phylogenetic Relationship of *Babesia* Species Infecting Domestic Dogs from Select Regions in Kenya

Ismail Thoya Ngoka (Dr.Ismailthoya@gmail.com)
Jomo Kenyatta University of Agriculture & Technology
Kevin Mbogo
Jomo Kenyatta University of Agriculture & Technology
Martina Kyallo
Biosciences Eastern & Central Africa Hub
David Obiero
Maasai Mara University
Roger Pelle
Biosciences Eastern & Central Africa Hub

Research Article

Keywords: Domestic dog, Babesia parasites, zoonosis, wildlife, human-livestock-wildlife interaction

Posted Date: August 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-95605/v2

Abstract

The *Babesia* genus has more than 100 species which are transmitted by ticks and infects humans, livestock and wildlife, some of which are zoonotic. New species continue being discovered which are poorly characterized. Locally, *Babesia* species occur in wildlife and livestock. Published literature on the species infecting dogs is limited.

Local management practices enable close interaction between wildlife, livestock and humans. The societal role of dogs enable them serve as conduits for pathogens.

Canine babesiosis causes a severe disease in dogs which can be fatal. Treatment required is lengthy and expensive. Current control methods rely on acaricide use. A vaccine against the disease is needed. Genetic characterization of local canine *Babesia* species would lay foundations for such development and assess any zoonotic potential.

Molecular and bioinformatic methods i.e. DNA extraction (143 dogs sampled), PCR, sequencing and bioinformatic analysis were used in the study.

13 samples were positive for *Babesia canis;* prevalence 9.0%, 95% confidence interval, (0.0437 to 0.1381).From the 13 positive samples, 2 were identified as *Babesia canis vogeli;* prevalence 1.4%, 95% confidence interval, (0.0138 to 0.142).While 11 were identified as *Babesia canis rossi;* prevalence 7.69%, 95% confidence interval, (0.033 to 0.12).*Babesia rossi* and *Babesia vogeli* were 84.6% and 15.4% of cases respectively.

Phylogenetic analysis revealed the Kenyan *B.rossi* sequences to be closely related to *B.rossi* sequences from black-backed jackals. The *B.vogeli* sequences were closely related to a *B.vogeli* sequence obtained from a pet cat in China.

Babesia rossi is known to cause the most severe form of canine babesiosis, 84.6% of the cases were positive for this parasite which requires immediate and aggressive medical intervention. The role of wildlife in the maintenance of the parasites especially *B.rossi* was noted, control measures would of necessity have to incorporate this component of the parasite lifecycle.

Introduction

The genus *Babesia* comprises parasites belonging to the order Apicomplexa which infect vertebrate red blood cells and are transmitted by ticks (1). There are more than 100 species in this genus, based on their microscopic appearance; they are classified as either small or large piroplasms. *Babesia* species can infect livestock, wildlife, human beings, and occur worldwide (1).

Babesiosis is considered a disease of increasing public health importance – an emerging zoonosis (24). The following *Babesia* species have been shown to infect and cause Babesiosis in the domestic dog;

B.canis, *B. rossi, B.vogeli, B. gibsoni, B. conradae*, and *B. vitallii* (21, 24). However, there are other yet to be characterized *Babesia* species that can infect dogs (24).

Babesia species of veterinary and medical significance include; *B.divergens* a zoonotic parasite, which infects cattle, humans, non-human primates, and other wildlife; *Babesia microti* whose lifecycle revolves around its' tick vector and rodent hosts is also zoonotic (1). *B.bovis* and *B.bigemina* are of major importance in the cattle production industry (4) and also infects wildlife such as the white-tailed deer (*Odocoileus virginianus*) and the African Buffalo-*Syncerus caffer* (1). Other *Babesia* species include *B.venatorum*, which infects sheep but has also been implicated in a zoonotic event involving a dog in a report from China (7); *B.caballi* infects horses, donkeys, and mules and *B.motasi* which infects sheep and goats (1).

Infections by *Babesia* parasites are known to occur in Kenya and have been documented in dogs, cattle, and wild felids (2, 3, 4, 34). However, published studies on the *Babesia* species infecting dogs in Kenya are limited. Similar studies have been reported from other African countries (15,16,17,18,19,20).

Canine babesiosis causes a debilitating illness in dogs characterized by fever, hemolytic anemia, jaundice, anorexia, vomiting, and lethargy (24).Factors determining the clinical severity of the disease include the *Babesia* species causing infection with *B.rossi* known to cause the most severe disease (24). The disease compromises the welfare of dogs and renders them unable to carry out their prescribed role in society (3).

Drugs used for the medical management of canine babesiosis include imidocarb dipropionate and diminazene aceturate. However, the veterinary treatment costs borne by the dog owners are high and the recovery period from the disease lengthy (24).

Dogs in Kenya serve a variety of purposes, including; livestock guarding and herding, providing security for business premises and homesteads as well as serving as companion animals (5). The role the dog plays in society, coupled with the local livestock keeping practices promotes an intimate interaction between dogs, humans, livestock and wildlife (6). This provides an opportunity for the spread of parasites and diseases between these entities.

The objective of this study is to identify the *Babesia* species circulating among dogs from select regions of Kenya and determine their phylogenetic relationship. This would enable a more tailored clinical intervention in dogs suffering from the disease as well as lay the foundation for the development of a much-needed vaccine against the disease (8). Current control measures against the disease rely on the use of acaricides against the tick vectors (17). The shortcomings of these methods include; poor owner compliance with the required periodic acaricide applications, toxic nature of the chemicals used and development of resistance in the target tick populations (25).Species identification and phylogenetic characterization of the *Babesia* parasites circulating among dogs will also inform on any existing zoonotic potential.

Materials And Methods

1.2.1) Study Areas & Description

The study was carried out in 3 counties in Kenya; Nairobi, Mombasa, and Nakuru.

1.2.1.1) Nairobi

It is a highly urbanized county and the commercial and administrative capital of Kenya (Fig.1). It has an area of 696.3km^2 with a high human population density of 4800/km^2 (16). The county lies at an altitude of 1669m with a warm and temperate climate, which is considered Cfb according to the Koppen-Geiger climate classification system. The average temperature is 19°C; annual rainfall is 869mm(37). There are significant levels of farming in the peri-urban zones. These include semi-nomadic pastoralism of cattle, sheep, and goats, and intensive rearing of dairy cattle, and poultry (9). Some districts within the county have free-roaming populations of goats, sheep, chicken, and other poultry e.g. ducks. Nairobi National park, located within the county is home to a large and diverse wildlife population such as the Cape buffalo, Maasai giraffe, Grants zebra, African leopard, lion, eland, impala, cheetah, etc (10, 34). Sections of the park borders are porous allowing constant interaction between wildlife, livestock (including dogs), and human beings (34). Anecdotal reports and published studies indicate that there is a large population of dogs, both stray and owned within Nairobi County. Some of the owned dogs are not kept under confinement and are allowed to roam freely. The dog population comprises both exotic and indigenous breeds with the majority being the latter (13).

1.2.1.2) Mombasa

The county is situated along the Indian Ocean coast of Kenya and consists of Mombasa Island and the surrounding mainland areas (Fig.1). It has an area of 294.9km^2 of which 65km^2 is water. Mombasa lies at an altitude of 23m above sea level and has a tropical climate which is classified as As by the Koppen-Geiger system. The average temperature is 26.7°C and the annual rainfall 1196 mm (37). The County has a human population of about 1.2 million. It is a highly urbanized county and is the 2nd largest city in Kenya. Farming activities are carried out in the peri-urban zones and include intensive poultry (broilers & layers) and dairy farming, free-roaming cattle, sheep, goats, and chickens are also observed in the suburban areas (9). There is a large population of dogs, both owned and stray, a large proportion of the owned dogs are allowed to freely roam around (11).Commercial/semi-commercial and subsistence fishing is carried out in the Indian Ocean waters around Mombasa. Mombasa also serves as a landing and processing zone for commercial fishing vessels (9).

1.2.1.3) Nakuru

The county is located in the Rift valley region of Kenya (Fig.1). It lies at an altitude of 1871m with a mild climate which is generally warm and temperate. Under the Koppen-Geiger system, the climate is classified as Csb, where the average temperature is 17.5°C and annual rainfall around 895mm (37). The study area

comprised of Naivasha town and its surroundings which has a human population of 181,966. Naivasha area is dominated by major geographical features such as Lake Naivasha, a freshwater lake, and Mt. Longonot with an elevation of 2,776m which is part of and surrounded by the Mt. Longonot National Park. The Park hosts a variety of wildlife including buffaloes, giraffes, plains zebra, Thomson's gazelle, and hartebeest (12). Naivasha is an important livestock farming area that includes intensive dairy cattle & poultry rearing, there are commercial beef cattle ranches as well. There is a significant population of free-roaming sheep, goats, chickens, and pigs. Nomadic pastoralism of cattle, sheep, and goats is also common in the county. There are significant fishing and related activities at L.Naivasha which is also an important wildlife area with hippopotamus and numerous bird species (9). There is a large population of dogs, both stray and owned especially in the peri-urban areas where a significant proportion of the owned animals are allowed to roam freely (13).

1.2.2) **Sample Size Determination**

The study objective was to determine which *Babesia* species are found circulating among dogs within the study locations. Literature review from similar studies in the region as well as local estimates based on clinical diagnosis of canine babesiosis indicated that prevalence for *Babesia* species infection among dogs was low (3,15,16).

The following formula was therefore used to determine sample size (38). **See formula in the supplementary files section.**

The minimum number of samples provided for by this formula is 30.I therefore collected a minimum of 30 blood samples from different dogs in each county. However, I managed to collect a larger number of samples than the minimum required in all the counties. The total number of samples collected was 143.

1.2.2.1) **Sampling**

The study employed a descriptive design, where sampling was opportunistic. A total of 143 dogs were sampled (Fig.2). In all counties, samples were collected from dogs presented at the shelter facilities of the Kenya Society for the Protection & Care of Animals® (KSPCA). Samples were collected from outwardly healthy dogs as well as individuals clinically symptomatic from a variety of ailments.

The dogs were of diverse backgrounds including stray, loosely owned, surrendered, and confiscation cases. The dogs were also of varied ages, sex, and breed (14).Fig.2 below presents the numbers in each demographic category used to classify the sampled canine population.

Age assessment was carried out using a combination of criteria, including teeth characteristics, size of the dog, hair coat, and state of ocular lens (26, 27). Sex was determined by visual inspection of the external genitalia (28). The breed was established by visually comparing the dog's physical attributes against established breed standards.

Approximately 2ml of blood was collected from the cephalic vein of each dog and loaded into 4ml EDTA tubes and refrigerated at 4°C as previously described (35).

1.2.3) **DNA extraction and characterization**

Total DNA was extracted using the TanBead™ Automated DNA extractor. For extraction process optimization, two protocols were used; the Viral DNA™ extraction protocol and the Optipure™ protocol for blood DNA. Six samples were used for optimization process. For each protocol, DNA was extracted from 300µl of whole blood sample. 10µl of Proteinase K was then added into each of the samples and mixed before transfer to the TanBead™ extractor for total DNA extraction.DNA quality and quantity was assayed through agarose gel electrophoresis and Nanodrop Spectrophotometry, a higher quantity and quality were obtained via the Optipure™ protocol. Thereafter total DNA extraction for the rest of the blood samples (142) was carried out using the Optipure™ protocol.

1.2.3.1) **DNA extraction from a Canine Babesiosis +ve dog**

To optimize PCR conditions for diagnostic PCR, total DNA was extracted according to the manufacturer's instructions (using QIAGEN DNeasy kit™ for blood and tissue using the nucleated tissue protocol) from blood drawn from a dog clinically diagnosed with babesiosis, the protocol was altered after adding lysis buffer, where the incubation temperature was set at 56°C at 1000rpm for 12hrs. The extracted DNA sample was then diluted to 25ng/µl using triple distilled Milli-Q water (35).

The extracted DNA was stored at -20°C (35).

1.2.4) **Gradient Polymerase Chain Reaction (PCR)**

Gradient PCR was used to determine optimum diagnostic PCR conditions for *Babesia* DNA. Total DNA from a dog positively diagnosed with Babesiosis after clinical examination (as described under section 1.2.3.1) was used for optimization via gradient PCR. The conditions were as follows; 95° C for 3min,94° C for 45sec,52° C to 62° C range with 12 intervals and a median of 57° C each for 1 min,72° C for 1min,72° C for 10min, 15° C ∞ . A 1.5% gel was prepared to run the PCR products at 135V for 30min. The results were visualized using a gel documentation system and used to establish optimum PCR conditions (35).

1.2.4.1) **Conventional PCR**

The master mix was set as follows 2x AccuPower™ master mix 5µl, pF 0.33µl, pR 0.33µl, g DNA 2.5µl, double distilled water 1.84µl for a total volume of 10.0µl. Diagnostic PCR conditions used were; 95°C for 5min, 94°C for 30sec, 52°C for 1min, 72°C for 1min, 72°C for 10min,15°C ∞ . A 1.5% gel was prepared to run the PCR products at 135V for 30min. The results were visualized using a gel documentation system (35). The positive PCR products were purified using the QIAGEN quick gel purification kit™ according to the manufacturer's instructions and the products submitted for sequencing. The primers used were as outlined in table 1.

Table 1.*Babesia* genus common primers used in PCR.

Primer	Oligo Sequence	Target Species
18S_rDNA_BTH_F	5'-CCT GMG ARA CGG CTA CCA CAT CT-3' (23mer)	*Babesia* genus
18S_rDNA_BTH_R	5'-TTG CGA CCA TAC TCC CCC CA-3'(20mer)	*Babesia* genus
18S_rDNA_GF1	5'-GTC TTG TAA TTG GAA TGA TGG-3'(21mer)	*Babesia* genus
18S_rDNA_GR2	5'-CCA AAG ACT TTG ATT TCT CTC-3'(21mer)	*Babesia* genus

1.2.5) **DNA Sequencing & Analysis**

Sequencing was done using the Sanger Dideoxymethod at the International Livestock Research Institutes' Segoli Lab (35).The obtained sequences were analyzed using the CLC Genomics Workbench version 20.0.2 where the sequences were trimmed, conflicts resolved using the forward and reverse sequences, and a consensus sequence generated (35). The consensus sequence was analyzed using the BLASTn program of the National Center for Biotechnology Information to identify closely related sequences on GenBank (32). Multiple sequence alignment and a phylogenetic tree were constructed using Geneious Prime 2020.1.2, Build 2020-04-07 08:42, Java version 11.0.4+11 (64bit) using the MUSCLE program version 3.8.425 by Robert C.Edgar and Geneious tree builder. The genetic distance model used was Jukes-Cantor, while the tree build method was Neighbor-joining; the number of bootstrap replicates was 1000 (36).

The following sequences from GenBank with accession numbers, *Tryps simiae* Muhak AJ404608,*Theileria parva* South Africa HQ895984,*B.duncani* USA HQ289870,*B.vogeli* Spain AY150061,*B.vogeli* Brazil KT333456,*B.vogeli* Texas EU084681,*B.vogeli* Zambia LC331058,*B.vogeli* China KJ939326,*B.vogeli* Nigeria AB303076,*B.vogeli* Egypt MN625891,*B.vogeli* Japan AY077719,*B.rossi* Zambia LC331056,*B.rossi* South Africa KY463434,*B.rossi* Sudan DQ111760,*B.rossi* Nigeria JN982353, *B.rossi* China MH143395,*B.rossi* Turkey MF040149,*B.rossi* Texas HM585429, *B.coco* USA AY618928,*B.gibsoni* India KF928958,*B.venatorum* China KU204792 were used to analyze the Kenyan *Babesia canis* sequences. (32)

Results

1.3.1) **Sampling**

All the samples were collected from the shelter facilities of the Kenya Society for the Protection & Care of Animals of the respective counties. A total of 143 dogs were sampled as described under materials and methods.Fig.2 below depicts the demographic structure of the dogs sampled.

1.3.2) **DNA Extraction Protocol Optimization**

Two protocols, the Optipure™ protocol and the Viral blood™ DNA protocol were used in optimizing DNA extraction as described under materials and methods. Assessment of the two extraction protocols used was done through agarose gel electrophoresis and Nanodrop Spectrophotometry. These clearly indicated the superior DNA yields of the Optipure™ protocol. Thereafter 142 out of the 143 samples had their DNA extracted using the Optipure™ protocol. One sample was extracted using Qiagen DNeasy kit™ (covered on 1.2.3.1)

The ideal thermal cycler conditions were thus established to be as follows, 95 C for 5min, 94 C for 0.30sec, 53 C for 1 min, 72 C for 1min, 72 C for 10min, 15 C for ∞.

1.3.4) **Diagnostic PCR for all the Extracted DNA samples**

Using the established PCR conditions, all the DNA samples were screened for *Babesia* species DNA.13 of the 143 samples were positive for possible *Babesia* species DNA after processing and visualization via agarose gel electrophoresis and gel documentation by forming a band at the expected 500-700bp range.

1.3.5) **DNA Submission for Sequencing & Bioinformatic Analysis**

The 13 positive samples were purified and submitted for sequencing via the Sanger Dideoxy method at the International Livestock Research Institutes' Segoli lab. The raw sequences were then analyzed using bioinformatic software and methods including CLC genomics workbench, NCBI-BLAST and Geneious prime software as described under materials and methods.

Using NCBI-BLAST, two of the sequences were identified as *Babesia canis vogeli*, while the remaining 11 sequences were identified as *Babesia canis rossi* on GenBank.

The two *Babesia canis vogeli* sequences were submitted to and assigned the following GenBank accession numbers MT740261 and MT740272.

Likewise the eleven sequences identified as *Babesia canis rossi* sequences were submitted to and assigned the following GenBank accession numbers MT740262, MT740263, MT740264, MT740265, MT740266, MT740267, MT740268, MT740269, MT740270, MT740271, and MT740273.

Additionally 21 sequences from GenBank were used to analyze the Kenyan *Babesia canis* sequences as described under materials and methods.

Geneious prime software was used in the construction of a phylogenetic tree.

The Kenyan *Babesia canis rossi* sequences clustered closely around each other. The 2 *Babesia rossi* sequences from Nakuru formed a sub-cluster that was in close association with the other Kenyan *B.canis rossi* (Fig.6).

On NCBI-BLAST other *B.rossi* sequences closely related to the Kenyan sequences included KY463431.1 from black-backed jackals in South Africa with a P.I (percentage identity) of 99.52 % (40), DQ111760.1 from dogs in Sudan with a P.I of 99.52 % (33), and LC331056.1 from dogs in Zambia with a P.I of 99.36% (50).

The most divergent *B.rossi* sequence within the Kenyan cluster was F12 (MT740273.1) from Nairobi County with a P.I ranging between 97.88% for MT740267.1 (Nvsa_B) to 99.39% for MT740262.1 (Nvsa) from Nakuru County (Fig. 6).

The 2 *Babesia canis vogeli* samples detected from Nairobi County, clustered closely to each other and other *Babesia canis vogeli* from Africa on the phylogenetic tree. On NCBI-BLASTn, *Babesia canis vogeli* identified in samples from Nairobi County were closely related to *B.vogeli* from Nigeria AB303076.1 with a percent identity (P.I) of 98.89% (36), *B.vogeli* from Egypt MN625891.1 with a percent identity of 99.42% (52). Other closely related sequences include *B.vogeli* from Japan AY077719.1. The 2 B.vogeli sequences obtained from this study, “B.vogeliNrb and B.vogeliNrbB” had a 99.0% percent identity (P.I) to a *B.vogeli* sequence from a pet cat in China (42).

The overall prevalence of the 13 sequences identified as *Babesia canis* translates to 9.0% with a 95% confidence interval of 0.0437 to 0.1381.

More specifically, the detected *Babesia canis rossi* sequences had a prevalence of 7.69% and 95% Confidence Interval of 0.033 to 0.12 while *Babesia canis vogeli* had a prevalence of 1.4% and 95% Confidence interval of 0.0138 to 0.0142

The characteristics of the dogs, samples, and species of *Babesia* identified are presented in table 2.

Table 2: Distribution *of Babesia* species based on the population characteristics of age, sex, breed and county

		Age			Totals	Sex		Totals	Breed		Totals
		J	A	O		M	F		L	E	
Nairobi	***B.canis rossi***	2	5	1	8	5	3	8	6	2	8
	B.canis vogeli	-	2	-	2	1	1	2	2		2
Nakuru	***B.canis rossi***	-	2	-	2	1	1	2	2	-	2
	B.canis vogeli	-	-	-	-	-	-	-	-	-	-
Mombasa	***B.canis rossi***	-	1	-	1	-	1	1	1	-	1
	B.canis vogeli	-	-	-	-	-	-	-	-	-	-

J	Juvenile	≤ 1yr old
A	Adult	> 1yr ≤ 5yrs old
O	Old	> 5yrs old
M	Male	-
F	Female	-
E	Exotic breed	-
L	Local breed	-

Notes

84.6% of the *Babesia canis* species positive samples were *Babesia canis rossi*, while 15.4% were *Babesia canis vogeli.*

Out of the 13 positive cases, 23.08% were juveniles <1year old, while 69.23% were adult animals >1year ≤5 years old and 7.69% were older dogs > 5years old.

Based on the sex of dogs sampled, 53.85% were males and 46.15% were females. Sequence analysis of the positive cases indicated a county distribution of *Babesia canis rossi* as Nairobi having 8 cases with Nakuru and Mombasa counties having 2 cases and 1 case respectively

Discussion

The result from the current study reveals that Kenyan dogs from regions sampled are infected with at least two *Babesia canis spp. Babesia canis rossi* and *Babesia canis vogeli* were detected at a prevalence of 7.69% and 1.4% respectively. These results are in agreement with findings from similar studies in other African countries, Oyamada et al (2005) determined the prevalence for both *B.canis vogeli* and *B.canis rossi* in a study from South Sudan to be 9.0% which is comparable to the results obtained from this study (table 2, 33). The prevalence of *B.vogeli* and *B.rossi* in the South Sudan study was 2.6% and 6.4% respectively (15). In Uganda, *B.rossi* was detected at a prevalence of 7.8 % (16), while in Zambia; *B.rossi* was detected at a prevalence of 8.0% (17).In Nigeria, *B.rossi* and *B.vogeli* were detected at a much lower prevalence of 2.0% and 0.3% respectively (18). All these studies compare well with the current study where the prevalence of *Babesia canis vogeli* was also relatively lower than that of *Babesia canis rossi*. Studies from African countries reported *B.vogeli* prevalence ranging between a low of 1.4% to a high of 5.8% (19, 20). The prevalence result from the current study was 1.4% which is within the range of earlier studies (19, 20)

The presence of competent tick vectors is an important factor determining infection by and therefore prevalence of *Babesia* parasites (1). Lower levels of tick vector infestation probably accounted for the

reduced prevalence numbers observed in the Nigerian study (18).

Generally, *B.rossi* has been detected at a higher prevalence compared to *B.vogeli* from similar studies in African countries. *B.rossi* is mainly transmitted by *Haemophysalis leachi* while *B.vogeli* is by *Riphicephalus sanguineus*, the degree of infestation by competent tick vectors probably accounts for the observed pattern where the former *Babesia* parasite occurs at a higher prevalence compared to the latter (1).

Clinically, canine babesiosis in Kenya is associated with severe morbidity and high mortality especially for cases that go untreated (3). This finding concurs with the current study findings where *Babesia canis rossi,* which is known to be the most virulent species, (24) was observed in 84.6% of *Babesia canis* positive cases while the less virulent *Babesia canis vogeli* was observed in only 15.6% of cases. *Babesia canis rossi* has routinely been detected in studies from various African countries including South Sudan (15), South Africa (8, 21), Nigeria (18), Zambia (17), and Uganda (16). Countries it has not been detected in include Angola and Namibia, but the non-detection could be attributable to sample size and study design for the Namibia case where only 1 dog was sampled (19). While in Angola, the geographical limitation of the samples used in the study probably excluded the parasites' detection (20).

Canine *Babesia* species not detected in this study but observed in similar studies from African countries include *B.gibsoni* observed in Zambia (17), Angola (20) and South Africa (21). Reports indicate that *B.gibsoni* is mainly transmitted by *Rhiphicephalus sanguineus* ticks which are also the main vectors for *B.vogeli*.

On the phylogenetic tree (Fig.6), the Kenyan *Babesia rossi* sequences although also associating with sequences from other African countries clustered closely together indicating the possible existence of a local sub-population. The 2 *Babesia rossi* sequences from Nakuru County also formed a sub-cluster indicating a sub-population from that region. The black-backed jackal (*Canis mesomelas*) is known to be the reservoir host for *Babesia rossi* (22). The *B.rossi* sequences from Kenya, South Sudan, and Zambia were identical to a *B.rossi* sequence obtained from a Black-backed Jackal (*Canis mesomelas*) in South Africa (22).*B.rossi* is widely considered to originate from and occur only in Africa. Countries outside the African continent where it has been detected include Turkey (30), USA (31) and China. In the American study, *B.rossi* was detected in a dog recently imported from South Africa (31). Surprisingly, the Turkish study detected *B.rossi* circulating in the tick species, *Haemophysalis parva* found infesting wild animals such as boars and hares, the tick vector in Africa is *Haemophysalis leachi* (22*)*. Further research into the Turkish *B.rossi* is necessary to determine its relationship if any to the Africa originating parasite as well as other aspects such as pathogenicity to domestic dogs.

B.canis vogeli is global in distribution (1), the 2 Kenyan sequences identified as *B.canis vogeli* clustered closely to each other and to *B.vogeli* sequences from other African countries including South Sudan (15), Nigeria (18), and Egypt (29) on the phylogenetic tree. Of note, *B.canis vogeli* although primarily known as

a dog parasite has been detected in felids such as domestic cats in China (23) and Lions in Zimbabwe (33).In Kenya, species classified as part of *the B.canis* complex have been identified in wild felids such as lions (*Panthera leo*) from the Nairobi National Park (34).

In this study, 69% of *Babesia canis* positive cases were in adult dogs between 1 and 5yrs old followed by Juvenile dogs less 1yr old at 23%. While the proportion of infected dogs older than 5yrs was 7%.The results are in agreement with findings from a Zambian study; dogs between 2 and 5yrs old comprised 29.47% of positive cases, while dogs between 0.6 and 1yr old comprised 22.11%, dogs older than 5yrs had the smallest proportion of cases (17). Similar findings were observed in a Nigerian study where 66.7% of *Babesia canis* positive cases were adult dogs between 1 and 3yrs old, and 33% of cases were less 1yr old (18). Sampling bias probably undermined findings as the majority of dogs sampled in these studies are adult dogs between 1 and 5yrs old.

Co-infections between different *Babesia* species although not common were observed in the studies from Zambia; *B.rossi* & *B.gibsoni* (17), in South Africa; *B.rossi* & *B.vogeli* (21).Mixed infections between a *Babesia* species and parasites from different genera were more frequently observed, in South Sudan, Angola (15,20).This study was specifically designed to detect parasites belonging to the *Babesia* genus. No cases of co-infections between different species of the *Babesia* genus were observed.

The public health importance of the *Babesia* parasites is increasing. They are considered an emerging zoonosis. New *Babesia* species continue being discovered and many of the known *Babesia* species are still poorly characterized in many aspects such hosts, vectors, geographic range and pathogenicity. E.g. of these *Babesia* species include, *B.divergens* like (MO1), *Babesia* sp CA.1 & WA1 (1). Poorly characterized *Babesia* sp known to infect dogs include *B.microti*-like from Spain and *Babesia* sp CA.1 (24).

The recent implication of a dog in the transmission of the zoonotic *B.venatorum* (previously known to infect sheep) to a human being has served to reinforce the need to fully characterize canine *Babesia* species.

The two *Babesia* species detected in this study are well characterized and pose little zoonotic risk.

Conclusions

This study has identified *B.canis rossi* and *B.canis vogeli* infecting dogs from the study areas at a prevalence of 7.69% & 1.4% respectively. 84.6% of the *B.canis* spp identified were *B.canis rossi* which are known to cause the most severe form of canine babesiosis.

The identified *B.canis rossi* sequences were phylogenetically closely related to *B.rossi* sequences detected in black-backed jackals (*Canis mesomelas*).The identified *B.canis vogeli* sequences were phylogenetically closely related to *B.vogeli* sequences from disparate geographic regions as well to those detected in wild felids from Africa.

The Kenyan *B.rossi* and *B.vogeli* sequences were most closely related to each other and also to similar sequences detected in other African countries.

Declarations

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

Ismail Thoya Ngoka -conceived the study, collected the samples, extracted DNA, carried out laboratory experiments including PCR, submitted samples for DNA sequencing, analyzed the data and drafted the manuscript, Roger Pelle -supervised the lab work and analysis of the data, Martina Kyallo-participated in and supervised the lab work and analysis of the data, Kevin Mbogo -supervised the entire project as well as participated in the refining of the study idea and design, David Obiero -participated in refining the study idea and drafting of the manuscript, All the authors have read the manuscript and approved its publication.

Funding

This work was supported by the African Biosciences Challenge Fund Research Fellowships under the Biosciences Eastern and Central Africa Hub at the International Livestock Research Institute (BecA-ILRI).The sponsors had no role in conceptualizing, carrying out, analyzing, and interpreting the study results.

References

1. Schnittger L,Rodriguez A,Florian-Christian M,Morrison D. Babesia: A world emerging Infection, Genetics, and Evolution,12(2012)1788-1809
2. Mbugua S,Gakombe J,Warimwe G.Study on the Prevalence of Clinical Canine Babesiosis and Ehrlichiosis in Nairobi,Kenya.Inter J Vet Sci,2015,4(2):60-62
3. Mbindyo S,Gitau G, Mulei C,Mbugua S. Etiological Factors Associated with Disease Morbidity and Mortality of Adult Dogs in Kennels in Nairobi,Kenya.Inter J Vet Sci 2016,5(3)164-170
4. Moumouni P,Aboge G, Terkawi M,Masatani T, et al. Molecular Detection and Characterization of *B.bovis*, *B.bigemina, Theileria spp*, and *Anaplasma marginale* Isolated from Cattle in Kenya. Parasites& Vectors(2015)8:496
5. Muriuki J, Thaiya A, Mbugua S et al. Dog Populations and Dog Keeping Practices in Relation to Rabies Control in Kisumu and Siaya Counties, Kenya. Inter J Vet AgricSci Vet Med 2016:4:3
6. Gakuya F, Ombui J, Heukebach J,Maingi N et al. Knowledge of Mange among Maasai Pastoralists in Kenya. PLoS ONE 7(8)43342

7. Yi Sun, Shao-Gangili, Jia-Fu Jiang et al. *Babesia venatorum* Infection in Child in China. Emerging Infectious Diseases, vol.20, no.5 May 2014
8. Collet G. Survey of Canine Babesiosis in South Africa. JSAfr Vet Association 2000 Sep; 71(3):180-6
9. https://www.knbs.or.ke/. Nairobi City County Statistical Abstract 2015
10. Reid R, Gichohi H, Said M, Nkedianye D et al. Fragmentation of a Peri-urban Savannah, Athi Kapiti Plains, Kenya. Fragmentation in Semi-Arid & Arid Landscapes: Consequences for Human & Natural Systems,195-224 ,2008 Springer
11. https://kspca-kenya.org/ .Sourced on 21/11/2020.
12. Ogutu J, Kuloba B, Piepho H, Kanga E. Wildlife Population Dynamics in Human Dominated Landscapes under Community Based Conservation; The Example of Nakuru Wildlife Conservancy Kenya. PLoS ONE 12(1): e0169730(1) Jan 2017
13. https://www.anaw.org/ .Sourced on 18/11/2020.
14. Taherdoost H. Sampling Methods in Research Methodology: How to Choose a Sampling Technique for Research. International Journal of Academic Research in Management (IJARM) vol.5, no.2,2016 page 18-27
15. Oyamada M, Davoust B, Boni M et al. Detection of *Babesia canis rossi, Babesia canis vogeli*, and *Hepatozoon canis* in Dogs in a Village of Eastern Sudan by using a Screening PCR and Sequencing Methodologies. Clinical& Diagnostic Laboratory Immunology Nov.2005,p1343-1346
16. Proboste T, Kalema-Zikusoka G, Atet L et al. Infection and Exposure to Vector-Borne Pathogens in Rural Dogs and their Ticks, Uganda. Parasites & Vectors(2015) 8:306
17. Nalubamba K, Mudenda N, Namwila M, Mulenga C, et al. A Study of Naturally Acquired Canine Babesiosis Caused by Single and Mixed Babesia Species in Zambia: Clinical Pathological Findings and Case Management. Journal of Parasitology Research, vol 2015, article id 985015,p9
18. Sasaki M, Omobowale O, Tozuka M, Ohta K et al. Molecular Survey of *Babesia canis* in dogs in Nigeria. J.Vet.Med.Sci 69(11):1191-1193,2007
19. Penzhorn B, Vorster I, Redecker G, Oosthuizen M.Confirmation of Occurrence of *Babesia vogeli* in a dog in Windhoek, Central Namibia. Journal of the South African Veterinary Association 87(1),a1427
20. Cardoso L,Oliveira A, Granada S,Nachum-Biala Y.Molecular Investigation of Tick-Borne Pathogens in Dogs from Luanda,Angola.Parasites& Vectors(2016) 9:252
21. Matjila P,Leisewitz A,Jongejan F,Penzhorn B.Molecular Detection of Tick-Borne Protozoal and Ehrlichial Infections in Domestic Dogs in South Africa. Veterinary Parasitology 155(2008) 152-157
22. Penzhorn B,Vorster I, Harrison-White R,Oosthuizen M. Black-backed Jackals (*Canis mesomelas*) are Natural Hosts of *Babesia rossi*, the Virulent Causative agent of Canine Babesiosis in Sub-Saharan Africa.Parasites&Vectors,2017 Mar 13;10(1):124
23. Zhang X.First Molecular Evidence of Babesia in Pet Cat in Mainland China. College of Life Sciences and Engineering, Foshan University,Guangyun Road No.33, Foshan, Guangdong 528231,China.

24. Koster S, Lobetti G, Kelly P: Canine Babesiosis: a perspective on clinical complications, biomarkers & treatment. Vet Med: Res Reports 2015,6:119-128
25. Coles T, Dryden M. Insecticide/acaricide resistance in fleas & ticks infesting dogs &cats. Parasites & Vectors 7,8(2014)
26. Abe B. Age Determination in Dogs. Iowa State University Veterinarian; vol.2:Iss 1, article 6
27. Tobias G, Tobias T, Abood S, Hamor R, Ballam J. Determination of Age in Dogs and Cats by Use of Changes in Lens Reflections &Transparency. Am J Vet Res.1998 Aug;59(8):945-50
28. Christensen B. Disorders of Sexual Development in Dogs &Cats. Veterinary Clinics of North America: Small Animal Practice vol42,issue3, May 2012, pg 515-526
29. Abdullah H, Raoult D, Mediannikov O: Molecular Characterization of Multiple Vector-borne diseases in some Animals Including Potential New Genotypes in Egypt.30th/10/2018
30. Omer O, Zafer K: Molecular Characterization of Babesia species in wild animals and their ticks in Turkey. Infect Genet Evol.2017 Nov; 55:8-13.
31. Allison R, Yeagly T, Levis K, Reichard M: Babesia canis rossi infection in a Texas dog. Vet Clin Pathol.2011 Sep;40(3):345-50.
32. Nucleotide [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] –. Accession No.MH143395.1 [2020/12/15]. Available from: https://www.ncbi.nlm.nih.gov/nuccore/MH143395.1/
33. Kelly P et al: Molecular detection of tick-borne pathogens in captive wild felids, Zimbabwe. Parasites & Vectors 2014, 7:514.
34. Githaka N et al: Molecular detection and characterization of potentially new Babesia and Theileria species/variants in wild felids from Kenya. Acta Trop.2012 Oct; 124(1):71-8.
35. Skilton R et al: Introduction to Molecular Biology and Bioinformatics Training Workshop, BecA-ILRI Hub Nairobi, Kenya, May 5-16,2014
36. O'Halloran D.A. A Practical Guide to Phylogenetics for Non-experts. J.Vis Exp(84),e50975,doi: 10.3791/50975(2014)
37. https://en.climate-data.org/africa/kenya. Sourced on 15/12/2020.
38. Dell R, Holleran S, Ramakrishnan R: Sample Size Determination. ILAR J.2002; 43(4): 207-213. doi: 10.1093/ilar.43.4.207

Figures

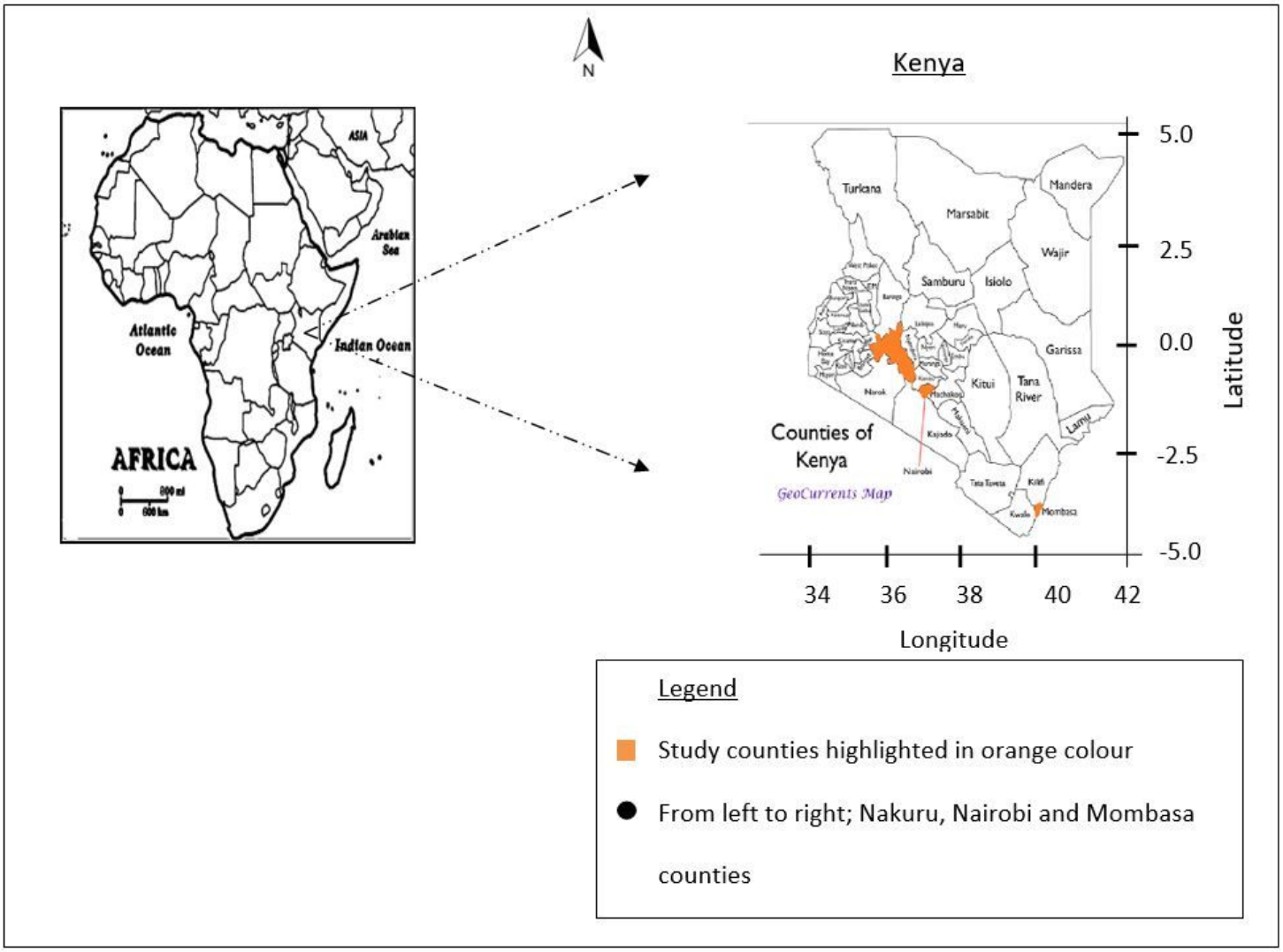

Figure 1

map of Africa showing the position of Kenya in East Africa and a map of Kenya where the 3 study counties are highlighted in orange color.

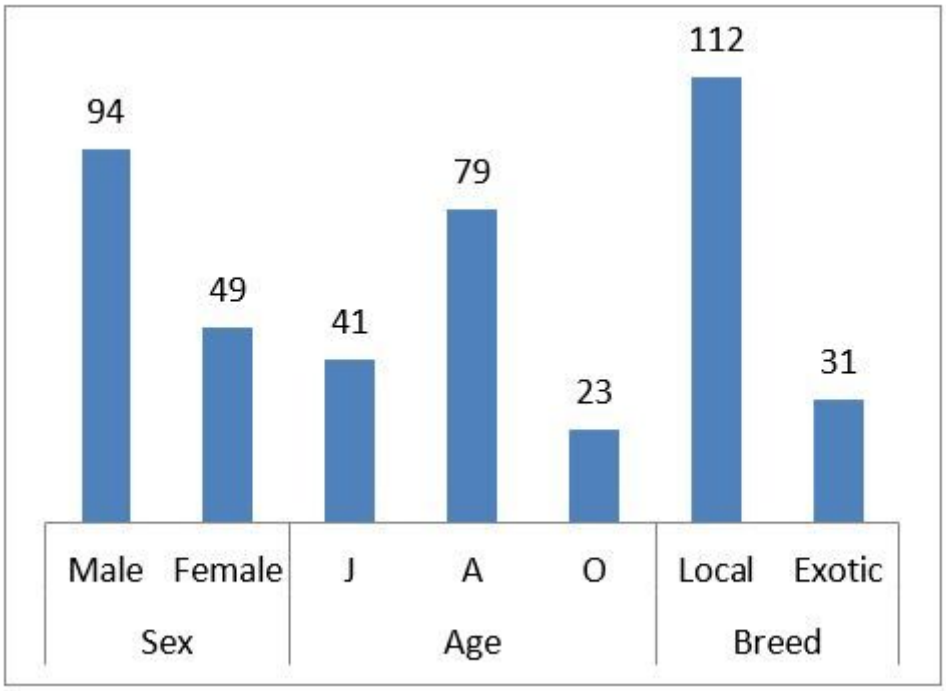

Figure 2

sampling was random; whole blood was collected from dogs presented at the shelter facilities of the Kenya Society for the protection and Care of Animals in Nairobi, Mombasa, and Nakuru. The bias observed in the sampling patterns is not due to deliberate collection from the population sub-groups but may indicate the underlying canine population structure.

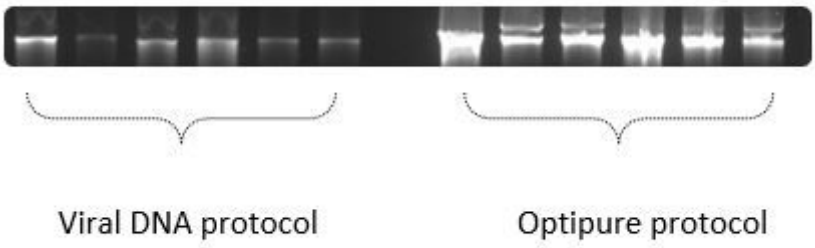

Figure 3

agarose gel electrophoresis image of DNA extraction optimization, Optipure™ protocol with higher DNA yield.

Gradient PCR annealing temperature of 53°C at which band size and intensity was most distinct

Figure 4

Optimum annealing temperature observed at the 53° C mark

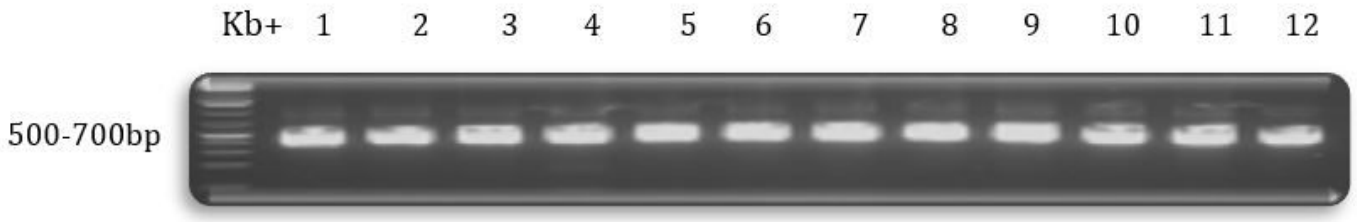

Figure 5

all the positive samples from the initial mass screening of the extracted total DNA underwent Re–PCR to increase the DNA yield to facilitate further processing of the positive samples through sequencing. The positive samples generated a band at the expected 500-700bp position on agarose gel electrophoresis.

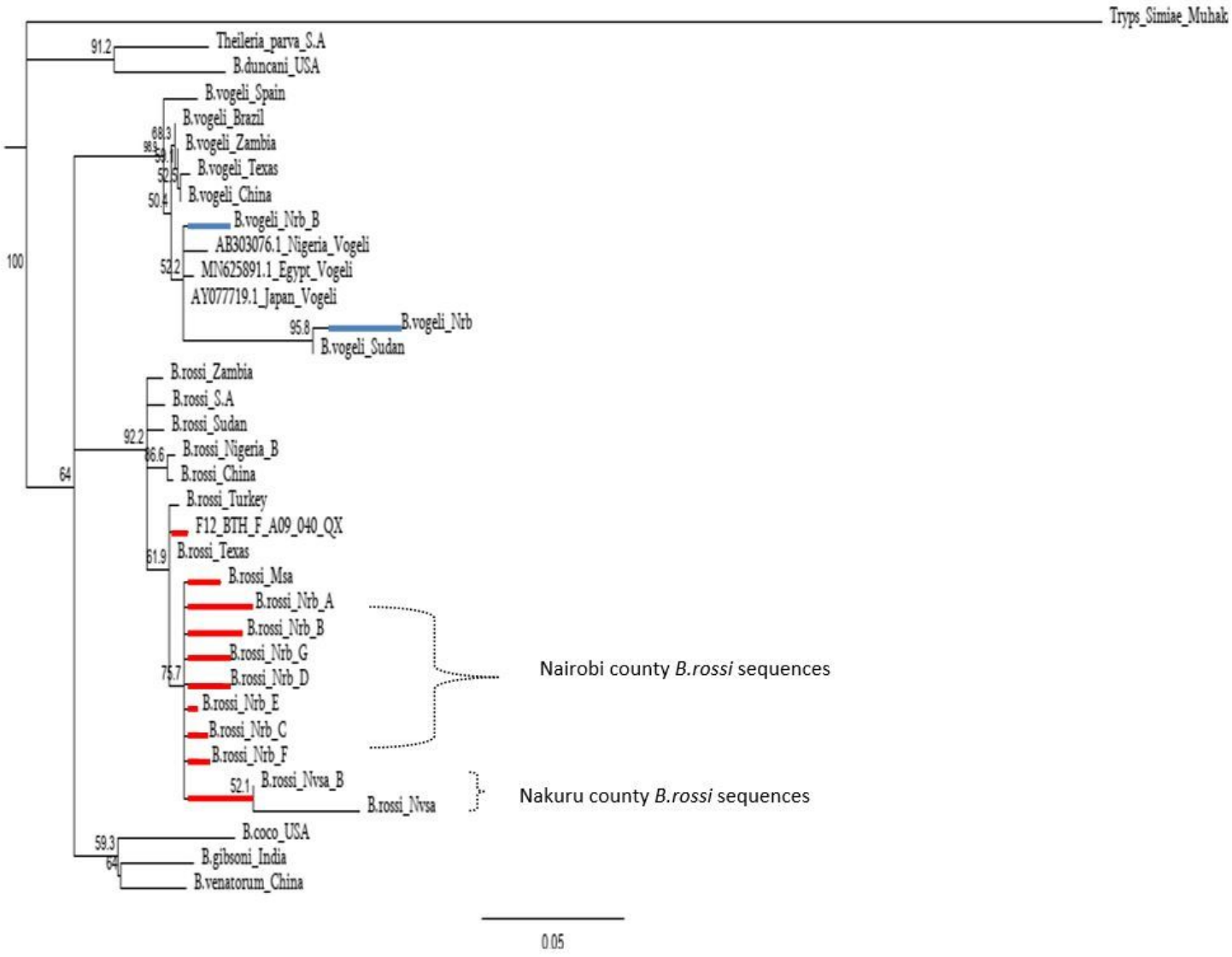

Figure 6

Phylogenetic Tree, the Kenyan B.rossi samples highlighted in red. B.rossi_ Msa; B.rossi_Nrb_A; B.rossi_Nrb_B; B.rossi_Nrb_G clustered around each other, the 2 positive samples from Naivasha formed a separate sub-cluster. The detected B.rossi was most closely related to samples from Turkey, China, Nigeria, Sudan, South Africa, and Zambia. The two B.vogeli samples highlighted in blue were closely related to samples from Sudan, Japan, Egypt, and Nigeria.