Quantitative Real Time PCR (qPCR) Screening Confirms *Babesia Bovis* Infections in Cattle in Kenya

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Research Article

Keywords: Babesiosis, Rhipicephalus microplus, Tick-borne diseases, Transboundary diseases, Ticks, Babesia bigemina, Animal disease diagnostics

Posted Date: October 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-979686/v1

Abstract

Background

Among protozoan parasites in the genus *Babesia*, *Babesia bigemina* is endemic and widespread in the East African region while the status of the more pathogenic *Babesia bovis* remains unclear despite the presence of the tick vector, *Rhipicephalus microplus* which transmits both species. Recent studies have confirmed the occurrence of *R. microplus* in coastal Kenya and although *B. bovis* DNA has previously been detected in cattle blood in Kenya, no surveillance has been done to establish its prevalence. This study therefore investigated the occurrence of *B. bovis* in cattle in Kwale County, Kenya, where *R. microplus* is present in large numbers.

Method

A quantitative species-specific multiplex Taqman PCR assay targeting two *Babesia bovis* genes, 18S ribosomal RNA and mitochondrially-encoded cytochrome *b* and *Babesia bigemina* cytochrome *b* gene was used to screen 506 cattle blood DNA samples collected from Kwale County for presence of *Babesia* parasite DNA.

Results

A total of 131 animals (25.8%) were found to have bovine babesiosis. Of these, 87 (17.2%) were positive for *B. bovis* while 70 (13.8%) had *B. bigemina* while 26 (5.1%) were observed to be co-infected with both *Babesia* species. A total of 61 animals (12.1%) were found to be infected with *B. bovis* parasites only, while 44 animals (8.7%) had *B. bigemina* only. *B. bovis* infections were detected in 11 sites while *B. bigemina* was detected in 9 out of the 12 sites sampled in three Kwale sub-Counties.

Conclusion

These findings reveal high prevalence of pathogenic *B. bovis* in a Kenyan area cutting across a busy transboundary livestock trade route with neighboring Tanzania. The *Babesia* multiplex assay used in this study is specific and can detect and differentiate the two *Babesia* species and should be used for routine *B. bovis* surveillance to monitor the spread and establishment of the pathogen in other African countries where *B. bigemina* is endemic. Moreover, these findings reveal a potential threat of fatal babesiosis in the absence of endemic stability in the local cattle populations.

Introduction

Bovine babesiosis, the most economically important vector-borne disease of livestock globally is caused by protozoan parasites *Babesia bovis* and *Babesia bigemina* that are transmitted by tick species in the sub-genus *Boophilus* [1]. The disease causes major economic losses through animal mortality, poor growth rates, reduced milk yields in sick or recovered animals, and direct costs of tick control and disease treatment [2]. In Africa, *B. bigemina* is endemic and currently more widespread than *B. bovis* reflecting the distribution of the indigenous tick vector *Rhipicephalus decoloratus* which is more widely distributed in Africa, although *Rhipicephalus evertsi evertsi* is also a known vector [3]. *Rhipicephalus microplus* which is an efficient vector of *B. bovis* has become established in mainland Tanzania with evidence of displacement of the native *R. decoloratus* [4]. The tick has only been recorded in a small foci in coastal Kenya [5, 6]. However, in the past 15 years, *R. microplus*, which is the definitive host for both *B. bovis* and *B. bigemina* has been confirmed to occur in several countries in West Africa [7–9] Central Africa [10, 11] and recently in East Africa [12–16]. The impact of this dispersal on the occurrence of *B. bovis* and the risk of pathogenic babesiosis is currently unknown.

Clinical bovine babesiosis presents with significant haemolysis of the red blood cells, continuous fever, anaemia and often haemoglobinuria, which colours the urine reddish brown giving the disease the common name ‘red water’. Infections associated with *B. bovis* are often acute or subacute, have a shorter time course with more severe nervous symptoms rapidly leading to death, or a protracted recovery rate in non-fatal cases [17]. Acute disease can cause nervous symptoms such as ‘pedalling’ movements and aggressive behaviour. In dairy cows, abortion and reduced or complete loss of milk (agalactia) are early signs of *Babesia* infection. Redwater causes high mortality and morbidity in susceptible livestock, especially in exotic and cross breed cattle. Mortality rates of 30% for *B. bigemina* and 70-80% for the more pathogenic *B. bovis* infections have been observed. Indigenous breeds of cattle can also be greatly affected by the less pathogenic *B. bigemina* under conditions of poor health or nutrition, a situation that is common in many vast areas of Africa, including Kenya [2]. Babesiosis caused by *B. bigemina* is characterized by low parasitemias of less than 1%. In contrast, *B. bovis* infection has a high parasitemia of greater than 10%, frequently with sequestration of infected red blood cells in cerebral capillaries resulting in symptoms which are often fatal. Cattle that recover from primary acute babesiosis, either naturally or after treatment, remain persistently infected and serve as a source of future transmission [17, 18].

There is paucity of data on the status and occurrence of *R. microplus* outside the coastal counties in Kenya. Therefore, *R. decoloratus* which is widely distributed in all agriculturally productive areas of eastern, central, Rift Valley and western Kenya [19] has been regarded as the major vector of bovine babesiosis in Kenya. Previously, McLeod and Kristjanson [20] predicted that 70% of Kenyan cattle were at risk from babesiosis and anaplasmosis with estimated annual economic losses amounting to $6.9 million per year. Co-infestation of animals with multiple tick species is typical, so multiple co-infection with tick-transmitted pathogens and significant disease burdens are frequently detected.

Diagnosis of redwater in Kenya is mainly through observation of clinical signs and because of the characteristic haematuria, microscopic examination of blood smears for *Babesia* parasites is usually not performed. Given the limited records of *R. microplus* in Kenya to date, bovine babesiosis has always been attributed to the more ubiquitous *B. bigemina* vectored by *R. decoloratus*. However, the recent confirmation of the occurrence of *R. microplus* in coastal Kenya by Kanduma et al. [13] portends the existence of *B. bovis*. *Babesia bovis* DNA has been reported previously in cattle blood in Kenya [21, 22]. The use of molecular tests increases the sensitivity for detection and enables differentiation of *B. bovis* from other *Babesia* parasites. Kim *et al.* [23] and Zhang *et al.* [24] previously developed and validated highly sensitive quantitative qPCR Taqman probes for detecting, quantifying and differentiating *B. bovis* from *B. bigemina*. In this study, we used these probes to investigate the occurrence of *B. bovis* in Kenya considering the recent reports of *R. microplus* in the local cattle populations.

Materials And Methods

Study site

A cross-sectional study was conducted in May 2019 in 12 sites in Kwale County, Kenya (Figure 1). The County is situated along the Kenyan coastline neighbouring the Indian Ocean on the East and South East and Tanzania on the South West. The County has a tropical type of climate with an average temperature of 23ºC with a high of 25ºC in March and a low of 21ºC in July. Annual precipitation is less than 800mm with the coastal parts of the County receiving greater than 1000 mm of precipitation per year, while a majority of the central to west areas receive around 500-750 mm. Rainfall is bi-modal with a short rain season from October to December and a long rain season from April to July. Detailed geo-climatic characteristics of the County have previously been described [13].

Cattle blood sampling

A total of 506 adult cattle were randomly sampled across 12 sites located in three sub-Counties namely, Matuga, Msabweni and Lunga Lunga (Figure 1). Blood samples were collected in EDTA and vacutainer plain tubes by jugular vein puncture, transported in cool boxes to the regional laboratory in Kwale and refrigerated at 4°C. The samples were later transported to the International Livestock Research Institute (ILRI) Laboratories in Nairobi for analysis and long-term storage. Sera was collected in 2 ml cryotubes and stored at -20ºC for subsequent serological detection of circulating tick-borne pathogen antibodies. Two (ml) aliquots of the EDTA blood were preserved at -80^{o}C for future use. The remaining EDTA blood sample was stored at -20^{o}C for use in molecular detection of tick-borne pathogens.

Whole blood genomic DNA isolation

Whole cattle blood collected in EDTA tubes was thawed and thoroughly mixed by gentle rocking. Whole blood genomic DNA was isolated from 300µl of whole cattle blood using the Promega Wizard® genomic DNA purification kit (Promega Corporation, Wisconsin, USA) by following manufacturer's protocol. DNA quantity and purity were measured with a nanodrop spectrophotometer "Nanodrop2000" (Thermo-Scientific, United States of America). The DNA was stored at -20°C until use.

Detection and quantification of Babesia DNA

A multiplex quantitative Taqman probe assay targeting two *B. bovis* and one *B. bigemina* genes was used to detect *Babesia* DNA. One *B. bovis* primer and probe set targeted the nuclear 18S rDNA [23] while the other targeted the mitochondrial cytochrome *b* gene [24]. The *B. bigemina* primers and probe were derived from the cytochrome *b* gene. Details of the primer and probe sequences used in the multiplex assay are listed in Table 1. The qPCR was conducted in a QuantStudio™ 5 detection system (Thermo Fisher Scientific, Massachusetts, USA). The 20 µL reactions included 10 µL of 2× Universal Probe qPCR Master Mix (New England Biolabs, Massachusetts, USA), 0.8 µL of each oligonucleotide primer, 0.2 µL of the fluorescence-labelled probe, and 3 µL of genomic DNA template. Temperature cycling parameters were 95.0°C for 3 min followed by 45 cycles of 95 ◦C for 10 s and 54 ◦C for 25 s. Each PCR run included a *B. bovis* and *B. bigemina* positive control sample and a no-template PCR grade water sample as negative control. The cycle quantification (Cq) scores corresponding to the PCR cycle number at which the amplification curve of each sample intersected the threshold line was recorded for each sample.

Standardization and construction of qPCR calibration curves

To evaluate the performance of the three primer sets, 3 µL of *B. bovis* control DNA sample (48ng/µl) isolated from an *in vitro* culture and a *B. bigemina* positive control DNA sample (17ng/µl) isolated from blood of an infected calf at the Tick Fever Centre (TFC), Queensland Department of Agriculture, Australia, were 10-fold serially diluted from 10^{-1} to 10^{-8}. The diluted DNA was then amplified using a multiplexed assay of three primer and probe sets (*B. bovis* 18S, *B. bovis* cytochrome *b* and *B. bigemina* cytochrome *b*; Table 1). Resulting data was analysed using the QuantStudio design and analysis software version 2.6.0. To generate the calibration curves, the cycle quantification (Cq) scores for the diluted samples were determined based on a default threshold of 0.02 ΔRn then plotted against corresponding DNA dilution. The efficiency (E) of the primer sets expressed as a percentage of each of the individual qPCR assay was calculated from the slope of the respective standard curve. The coefficient of correlation (R^2) of each standard curve was also determined using the QuantStudio analysis software.

Data analysis

The data was exported to Ms Excel where the mean and range of Cq scores were calculated. Paired nonparametric Wilcoxon signed rank test was applied in GraphPad Prism (V 9.2.0) to compare the Cq levels detected by the two *B. bovis* genes employed. Statistical significance was determined when $P < 0.05$. Kappa coefficient was calculated in Excel to evaluate the degree of agreement between the 18S and cytochrome *b* genes in *B. bovis* detection. A XY scatter plot of Cq values detected was generated using GraphPad Prism. A bar graph of distribution of positive cases across sampled sites was constructed.

Results

Babesia primer statistical parameters, standard curves and efficiency

A summary of the statistical parameters, standard curves and efficiencies of the primers sets used in the multiplex assay are shown in Table 2 and Figure 2. The lowest Cq value detected by the *B. bovis* 18S primer pair at the lowest serial dilution of 10^{-1} was 23.096 while the cytochrome *b* detected a Cq of 16.879. The *B. bigemina* cytochrome *b* primers detected a Cq of 23.537 at the lowest dilution of 10^{-1}. The *B. bovis* 18S primers returned a high Cq value of 44.872 at 10^{-7} dilution while the cytochrome *b* returned a high of 34.847 at a dilution of 10^{-6}. The *B. bigemina* cytochrome *b* primers returned a high of 33.387 at a dilution of 10^{-4}. Cq values obtained with the *B. bovis* cytochrome *b* primers were significantly lower (Wilcoxon matched pairs signed rank test $p < 0.0001$) than corresponding values obtained with18S (Table 2).

The *B. bovis* 18S primer set had an efficiency of 96.7%. Its standard curve had a slope of -3.404 and a correlation coefficient (R^2) of 0.982. The Y-Intercept was 19.89. The *B. bovis* cytochrome *b* primers had an efficiency of 90.3%, a slope -3.579 and a R^2 of 1.0 and a Y-Intercept of 13.19. The *B. bigemina* set had an efficiency of 96.7%, a slope of -3.404 and a R^2 of 0.997 and a Y-Inter:20.102. The standard curve, slope, R^2 and efficiencies of each primer set as determined from the Cq values of the serially diluted standard solutions are shown in Figure 2. The *B. bovis* 18S and cytochrome *b* which were labelled with different fluorescence dyes were not detected in the *B. bigemina* control and neither was the *B. bigemina* cytochrome *b* detected in the *B. bovis* control included in each PCR run. Thus, the primer sets employed were species-specific for *B. bovis* and *B. bigemina* as no cross-reaction was observed with the respective controls. In all the PCR reactions carried out, a no-template water sample was included as a negative control to rule out false positives due to cross-contamination.

Detection and quantification of B. bovis and B. bigemina DNA

The qPCR primer sets employed in this study allowed for species-specific detection and quantification of *B. bovis* and *B. bigemina* DNA present in the screened blood samples. Since the efficiency parameters of the primers were within the recommended limits (Table 2 and Figure 2), all samples that returned Cq values of <45 for the *B. bovis* 18S primers with a good quality amplification plot were regarded as positives since the corresponding Cq values obtained with the cytochrome *b* primers for the same samples were lower (Additional file Table S1). For the *B. bovis* and *B. bigemina* cytochrome *b* primer sets, all samples that returned Cq values of <42 with a good quality amplification plot were also regarded as positives. A total of 131/506 returned Cq values that met these criteria and were thus classified as positive for either *B. bovis* or *B. bigemina* or both. Detectable amplifications with the *B. bovis* 18S primers were observed in 39 samples, while a total of 87 samples resulted in detectable amplicons using the *B. bovis* cytochrome *b* primers. A list of the 131 field samples and their corresponding Cq values is shown in Additional file Table S1. The range distribution of the Cq levels detected by the three assays is shown in Figure 3. The highest Cq value obtained with the field samples using the *B. bovis* 18S was 44.68 and the lowest was 12.95 with a mean Cq value of 36.55 (SD ± 5.995). The *B. bovis* cytochrome *b* returned a high Cq value of 41.158 and a low of 29.914 with a mean Cq value of 35.37 (SD ± 2.997). The *B. bigemina* cytochrome *b* primers returned a high of 41.38 and a low of 20.956 with a mean of 33.152 (SD ± 3.766). A comparison of 33 pairs of *B. bovis* Cq values obtained with both 18S and cytochrome *b* primers using Wilcoxon matched pairs

signed rank test revealed that the cytochrome *b* values were significantly lower than corresponding values obtained with18S ($p < 0.0001$) (Table 2). No detectable amplification was observed in 375 of the screened DNA samples.

Prevalence of B. bovis **and** B. bigemina **infection**

A total of 506 cattle blood samples were screened for presence of both *B. bovis* and *B. bigemina*. The distribution of the number of positive cases across the sampled sites is shown in Table 3 and Figure 4. The overall bovine babesiosis prevalence rate was 25.8% (131/506). Of the 131 *Babesia* positive animals, 87 were found to be infected with *B. bovis* indicating a prevalence of 17.2% (87/506), while 70 were positive for *B. bigemina* giving a prevalence of 13.8% (70/506) and a further 26 (5.1%) had mixed infections of both *B. bovis* and *B. bigemina*. Using the *B. bovis* 18S and cytochrome *b* primers employed in this study, a total of 61 animals (12.1%) were found to have *B. bovis* infection while 44 animals (8.7%) were positive for *B. bigemina* only. The 18S *B. bovis* primers detected 39 positive cases (7.7%) while the *B. bovis* cytochrome *b* primers detected 81 (16%) positive cases. There was a weak agreement between the *B. bovis* 18S and cytochrome *b* detection assays (Kappa = 0.36).

Babesia bovis infections were detected in 11 sites while *B. bigemina* was detected in 9 out of the 12 sites sampled. The highest *B. bovis* prevalence was 5.3% (27/506) observed in Vanga (Lunga Lunga sub-County) while the lowest was 0.5% (3/506) observed in Kiwambale (Lunga Lunga sub-County) and Tangini (Msambweni). The highest prevalence of *B. bigemina* was 3.4% (17/506) observed in Miaji Farm. There were no cases of either *B. bovis* or *B. bigemina* in Mwanjaba (Msambweni sub-County.

Discussion

Following from the recent demonstrations of *R. microplus* in southern-eastern Kenya, this study confirmed the presence of *B. bovis* DNA in cattle blood from the same region. This was necessary to instigate monitoring and control measures because the economic burden of ticks and tick-borne diseases in Africa [2] and also globally is high [1]. Climate change has contributed to expanded range of tick vectors increasing the risk of tick-borne diseases in Africa [25]. Bovine babesiosis is one of the four major livestock diseases that is prevalent in Kenya [21, 26–30]. Until recently, bovine babesiosis in Kenya was presumed to be caused by *B. bigemina*, transmitted by *R. decoloratus* which is endemic in many regions in Kenya [19]. Recent recordings of *R. microplus* in Kwale [13], indicated an urgent need for surveillance of *B. bovis* to update epidemiological data on bovine babesiosis.

Screening of the cattle samples with two *B. bovis* and one *B. bigemina* specific probes, we observed that *B. bovis* was present in 17.2% (87/506) of the animals while 13.8% (70/506) of the samples were positive for *B. bigemina*. Co-infections with the two *Babesia* spp were observed in 26 (5.1%) of the animals screened (Table 3). This is the first study to report significant occurrence of the pathogenic *B. bovis* in Kenya and East African region. The findings indicate that *B. bovis* infections do occur in significant numbers in this region but are probably disregarded in most studies screening for tick pathogens because of the belief that only the endemic *B. bigemina* is present.

In this study, the highest prevalence of *B. bovis* was observed in Vanga located within the Lunga Lunga sub-County which borders Tanzania. There is a livestock market in Tanga, a port city in Northeast Tanzania and at the Kenya border post at Lunga Lunga where cattle from Tanzania are purchased by Kenyans from neighbouring counties. There is a holding area at Lunga Lunga and another in Msambweni in Kwale along the major highway from the border for keeping recently acquired animals that are in transit to neighbouring counties. The Lunga Lunga market is frequented by Kenyan traders from as far as Tana River County while some are from Somalia. The extensive movement and transborder trade of infected and tick-infested animals is regarded as the major driving factor that is responsible for introduction of tick vectors and *Babesia* spp into new areas [17]. A similar pattern of transboundary cattle trade contributing to the spread of *R. microplus* and possibly its associated pathogens has been reported in Central and West Africa where animal movement across national boundaries is common [11]. The finding of high prevalence of the pathogenic *B. bovis* reported in this study indicates a possible threat of fatal bovine babesiosis across eastern Africa countries where most cattle would be lacking endemic stability to the disease. These observations together with the recent confirmation of occurrence of *R. microplus* in Kenya provide a rationale to implement urgent surveillance to determine and monitor the spread of *B. bovis*. Effective measures should also be instituted to control the spread of *R. microplus* to limit establishment of *B. bovis* babesiosis.

The molecular tests used have added both reliability and sensitivity to the detection of *B. bovis* infections in Kenya. Previously, for an active *B. bovis* infection, a diagnosis was confirmed by the presence of two pairs of small pear-shaped bi-lobed parasites in giemsa-stained blood smears[17]. However, the presence of *B. bigemina* in a blood smear may not necessarily indicate clinical babesiosis, as symptoms can be due to resurgence of a chronic infection. In Kenya, diagnosis of redwater is confirmed by observation of the characteristic red urine and therefore microscopic examination of blood smears is seldom performed. In national diagnostic and research laboratories, detection of circulating *B. bigemina* antibodies is used for disease surveillance [26, 27, 31]. Molecular PCR techniques have been used to detect and

differentiate *Babesia* parasites with high sensitivity and specificity [32–34]. Based on PCR amplification and sequencing of the *B. bovis* spherical body protein-4 (SBP-4) gene, Moumouni et al. [22] previously reported a *B. bovis* prevalence of 12.3% and 23.7% in Kajiado and Machakos Counties, respectively. *Babesia bigemina* was found to be more prevalent in Kajiado. Using the reverse line blot (RLB) assay targeting the V4 hypervariable region of *Babesia* rRNA, Njiiri et al., [21] reported a *B. bovis* prevalence of 2.2 % in Busia County, Western Kenya. In this study we used a well validated Taqman probe multiplex assay based on *B. bovis* 18S [23], *B. bovis* cytochrome *b* [24] and *B. bigemina* cytochrome *b* [24] that can detect, differentiate *B. bovis* from *B. bigemina* and has the potential to quantify levels of infection. High specificities and efficiencies of above 96% were observed with these primer and probe sets (Figure 2) confirming the efficiency and usefulness of these assays in detecting, quantifying, and discriminating *B. bovis* infections (Figure 2, Table 2). In our study, a total of 61 animals (12.1%) were found to have *B. bovis* parasites only using both the 18S and cytochrome *b* primers while 44 animals (8.7%) had *B. bigemina* only. There was a weak agreement between the two *B. bovis* detection assays (Kappa = 0.36). In 33 blood samples where *B. bovis* was detected by both probes used, the 18S gene was detected in significantly lower copies compared cytochrome *b* (Wilcoxon matched pairs signed rank test ($p < 0.0001$). The difference in sensitivities between the *B. bovis* 18S and cytochrome *b* primers may be due to differences in abundance of the two gene products. The *B. bovis* genome has been shown to contain three rRNA operons [35]. Although information on the number of mitochondria in *Babesia* parasites is lacking, some apicomplexan parasites, such as *Toxoplasma gondii* and *Plasmodium falciparum* have been reported to have only a single mitochondrion per parasite [36]. The mitochondrial DNA of *Plasmodium falciparum* comprises approximately 20 copies of a 6 kbp linear genome per cell [37, 38] encoding three protein coding genes including cytochrome *b* [35]. Therefore, the quantities detected by different genes could reflect the gene copies per individual organisms as well as level of parasitaemia which was not determined in this study. More *B. bovis* positive samples were detected with the cytochrome *b* primers compared to the 18S indicating the higher sensitivity obtained by using this gene as compared to the nuclear-encoded 18S rRNA gene. Therefore, based on the data obtained in this study and theoretical predictions, the *B. bovis* cytochrome *b* primers would be the best target for a routine field diagnostic assay. However, it would appear from all these studies that *B. bovis* is well established in Kenya, while its biological vector, *R. microplus*, has also been confirmed to occur [13].

Animals that recover from babesiosis become carriers of babesia parasites for life and can develop the disease again if they undergo physiological stresses such as nutritional restriction or co-infection with another infection. Babesiosis is therefore a costly chronic animal disease in endemic areas, because of frequent resurgence, especially if the animals are experiencing stress [2]. Factors such as animal breed, type of agro-ecological zone (AEZ) and livestock production systems are important risk factors associated with babesiosis infections [27]. Majority of farmers in Kwale County which has an estimated 190,988 zebu cattle and 5,475 dairy crosses [39] practice open grazing system which has previously been shown to be significantly associated with high prevalence of *B. bigemina* infections in Murang'a County in Kenya [27]. Kwale County, which is a gateway to mainland Kenya for livestock purchased from the Tanzanian border market, could be acting as focal source of *B. bovis* infections to the rest of the country.

Ticks and tick-borne diseases are the biggest threat to sustainable cattle production in Kenya which has close to 18 million cattle [40]. Although this study reports high prevalence of *B. bovis* in three sub-Counties in Kwale, the situation in the rest of the country is unknown. The spread and clinical consequences of *B. bovis* infections in Kenya could add severe burdens on survival and productivity of cattle enterprises, especially because of occurrence of multiple co-infections is common, thereby intensifying the impacts of other serious infections such as East Coast fever. Therefore, the demonstration of the parasite at the prevalence revealed indicate its establishment and local transmission. It is recommended that molecular diagnostics including the qPCR used in this study be added to routine surveillance to detect and differentiate the two *Babesia* parasites with the aim of obtaining a complete picture of the prevailing status of bovine babesiosis in the country. Such dataset is critical in designing disease and vector control programs both locally and for neighbouring countries.

Conclusions

This study found a high prevalence rate of *B. bovis* in Kwale County with potential to cause disease outbreaks in susceptible animal populations. The *Babesia* multiplex assay used in this study is specific and can detect and differentiate the two *Babesia* parasites. The *B. bovis* cytochrome *b* primers would be the best target for a routine field diagnostic assay. Urgent surveillance and mapping should be undertaken to determine and monitor the spread of *B. bovis* babesiosis and inform the design of effective control measures.

Abbreviations

EDTA: Ethylenediaminetetraacetic acid; Cq: cycle quantification; DNA: deoxyribonucleic acid; dNTPs: deoxyribonucleotide triphosphates; ILRI: International Livestock Research Institute; rDNA: ribosomal Deoxyribonucleic Acid; qPCR: Quantitative PCR; RLB: reverse line blot; TFC: Tick Fever Centre

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All the data sets supporting the conclusions of this article have been presented.

Competing interests

The authors declare that they have no competing interests.

Funding

The field sampling and laboratory analysis was supported by the CGIAR research program on Livestock (https://livestock.cgiar.org/). Training in qPCR diagnostics was supported by Australia Awards-Africa through a postdoctoral fellowship to EGK.

Authors' contributions

NG, EGK, EKN and RPB conceived the research proposal. NG, EGK, EKN and DE developed the research concept. NG and EGK coordinated and carried out field sampling. DE and JŠ designed and supervised the validation of the qPCR assay. EGK, NG and RPB drafted the manuscript. All authors reviewed the manuscript to its current form and approved the final manuscript.

Acknowledgements

We are very grateful for the permission, support, and facilitation of the Directorate of Veterinary Services, Department of Agriculture, Livestock and Fisheries, Kwale County, Kenya. Much appreciation to the Director, Dr. Mwalonya Hamisi and Sub-County Veterinary Officers; Dr. Mburu, Dr. Umlai M. and Dr. Nyamweya and Animal Health Assistants (AHAs) Emmanuel Nuamawi and Patience Salama who mobilized the farmers and coordinated blood sampling. We acknowledge the technical assistance with animal bleeding offered by Thomas Njoroge, (ILRI). We thank Prof Brian Cooke, James Cook University, Queensland, Australia for provision of *B. bovis* positive control DNA sample and the Tick Fever Centre, Biosecurity Sciences Laboratory, Department of Agriculture and Fisheries, State of Queensland for provision of *B. bigemina* infected blood for extraction of a positive control sample.

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Tables

Table 1. Details of qPCR primers used in detection of *Babesia* parasites

Target gene	Primer/probe name	Nucleotide sequence	Size	Reference
Babesia bovis 18S	Forward: S0934_BoF_18S	AGCAGGTTTCGCCTGTATAATG	22	[23]
	Reverse: S0933_BoR_18S	AGTCGTGCGTCATCGACAAA	20	
	Probe: S0935_BoP_Kim	FAM-CCTTGTATGACCCTGTCGTACCGTTGG-BHQ1	27	
Babesia bovis Cytochrome *b (cytb)*	Forward: S0936_bovisF160_cytb	ATATGTTTGCATTTGCTG	18	[24]
	Reverse: S0937_bovisR249_cytb	CTCCAAACCAATATGAAAG	19	
	Probe: S0938_bovisPb_cytb	JOE- CAAACCATAAAGTCATCGGTATATCCTAC-BHQ1	29	
Babesia bigemina Cytochrome *b (cytb)*	Forward: bigemF295	GGTCTATTTGGTGGAGTT	18	[24]
	Reverse: bigemR413	ACAAGACCAAATGCAATT	18	
	Probe: bigemPb	TAMRA-CAATTGTTCTTGGAGCAGCT- BHQ1	20	

Table 2. Summary details of the statistics and efficiency parameters of the three primer sets

Sample Name	Serial dilution level	DNA concentration	Gene	CT	Y-Intercept	Slope	Efficiency
Bbov1	10^{-1}	14.4 ng	*B. bovis* 18S	23.096	19.887	-3.404	96.686
Bbov2	10^{-2}	1.44 ng	*B. bovis* 18S	26.650	19.887	-3.404	96.686
Bbov3	10^{-3}	144 pg	*B. bovis* 18S	30.442	19.887	-3.404	96.686
Bbov4	10^{-4}	14.4 pg	*B. bovis* 18S	34.005	19.887	-3.404	96.686
Bbov5	10^{-5}	1.44 pg	*B. bovis* 18S	37.187	19.887	-3.404	96.686
Bbov6	10^{-6}	144 fg	*B. bovis* 18S	39.268	19.887	-3.404	96.686
Bbov7	10^{-7}	14.4 fg	*B. bovis* 18S	44.872	19.887	-3.404	96.686
Bbov8	10^{-8}	1.44 fg	*B. bovis* 18S	Undetermined	19.887	-3.404	96.686
Bbov1	10^{-1}	14.4 ng	*B. bovis* Cytochrome *b*	16.879	13.187	-3.579	90.289
Bbov2	10^{-2}	1.44 ng	*B. bovis* Cytochrome *b*	22.327	13.187	-3.579	90.289
Bbov3	10^{-3}	144 pg	*B. bovis* Cytochrome *b*	23.886	13.187	-3.579	90.289
Bbov4	10^{-4}	14.4 pg	*B. bovis* Cytochrome *b*	27.370	13.187	-3.579	90.289
Bbov5	10^{-5}	1.44 pg	*B. bovis* Cytochrome *b*	30.973	13.187	-3.579	90.289
Bbov6	10^{-6}	144 fg	*B. bovis* Cytochrome *b*	34.847	13.187	-3.579	90.289
Bbov7	10^{-7}	14.4 fg	*B. bovis* Cytochrome *b*	Undetermined	13.187	-3.579	90.289
Bbov8	10^{-8}	1.44 fg	*B. bovis* Cytochrome *b*	Undetermined	13.187	-3.579	90.289
Bbig1	10^{-1}	51 ng	*B. bigemina* Cytochrome *b*	23.537	20.012	-3.404	96.700
Bbig2	10^{-2}	5.1 ng	*B. bigemina* Cytochrome *b*	26.746	20.012	-3.404	96.700
Bbig3	10^{-3}	510 pg	*B. bigemina* Cytochrome *b*	29.944	20.012	-3.404	96.700
Bbig4	10^{-4}	51 pg	*B. bigemina* Cytochrome *b*	33.387	20.012	-3.404	96.700
Bbig5	10^{-5}	5.1 pg	*B. bigemina* Cytochrome *b*	Undetermined	20.012	-3.404	96.700
Bbig6	10^{-6}	510 fg	*B. bigemina* Cytochrome *b*	Undetermined	20.012	-3.404	96.700
Bbig7	10^{-7}	51 fg	*B. bigemina* Cytochrome *b*	Undetermined	20.012	-3.404	96.700
Bbig8	10^{-8}	5.1 fg	*B. bigemina* Cytochrome *b*	Undetermined	20.012	-3.404	96.700

Table 3. Distribution of *Babesia* positive blood across the study sites

Sampling site			Total number of *Babesia* positive samples by gene						
Sub-County	***Sampling site***	Number of animals sampled	*B. bovis* positive by 18S (including mixed infection)	*B. bovis* positive by cytochrome *b* (including mixed infection)	*B. bovis* positive by both 18S & cytochrome *b* (including mixed infection)	*B. bovis* (Single infection) by both18S & cytochrome *b*	*B. bigemina* including mixed infection	*B. bigemina* (Single infection)	Mixed infection
Matuga	***Matuga***	13	2	4	5	2	6	3	3
Matuga	***Miaji farm***	40	1	4	4	2	17	15	2
Matuga	***Kipabane***	43	3	8	8	5	4	1	3
Matuga	***Kichaka Simba***	107	0	17	17	17	0	0	0
Msambweni	***Tangini***	59	3	2	3	2	10	9	1
Msambweni	***Majiboni***	32	1	3	3	3	0	0	0
Msambweni	***Ukunda***	28	1	1	1	0	3	2	1
Msambweni	***Mwanjaba***	10	0	0	0	0	0	0	0
Lunga Lunga	***Shimoni Kidimu***	34	4	9	10	4	10	4	6
Lunga Lunga	***Kiwambale***	33	3	2	3	2	2	1	1
Lunga Lunga	***Shimoni***	32	5	6	6	5	3	2	1
Lunga Lunga	***Vanga***	75	16	25	27	19	15	7	8
Total		**506**	**39 (7.7%)**	**81 (16%)**	**87 (17.2%)**	**61 (12%)**	**70 (13.8%)**	**44 (8.7%)**	**26 (5.1%)**

Figures

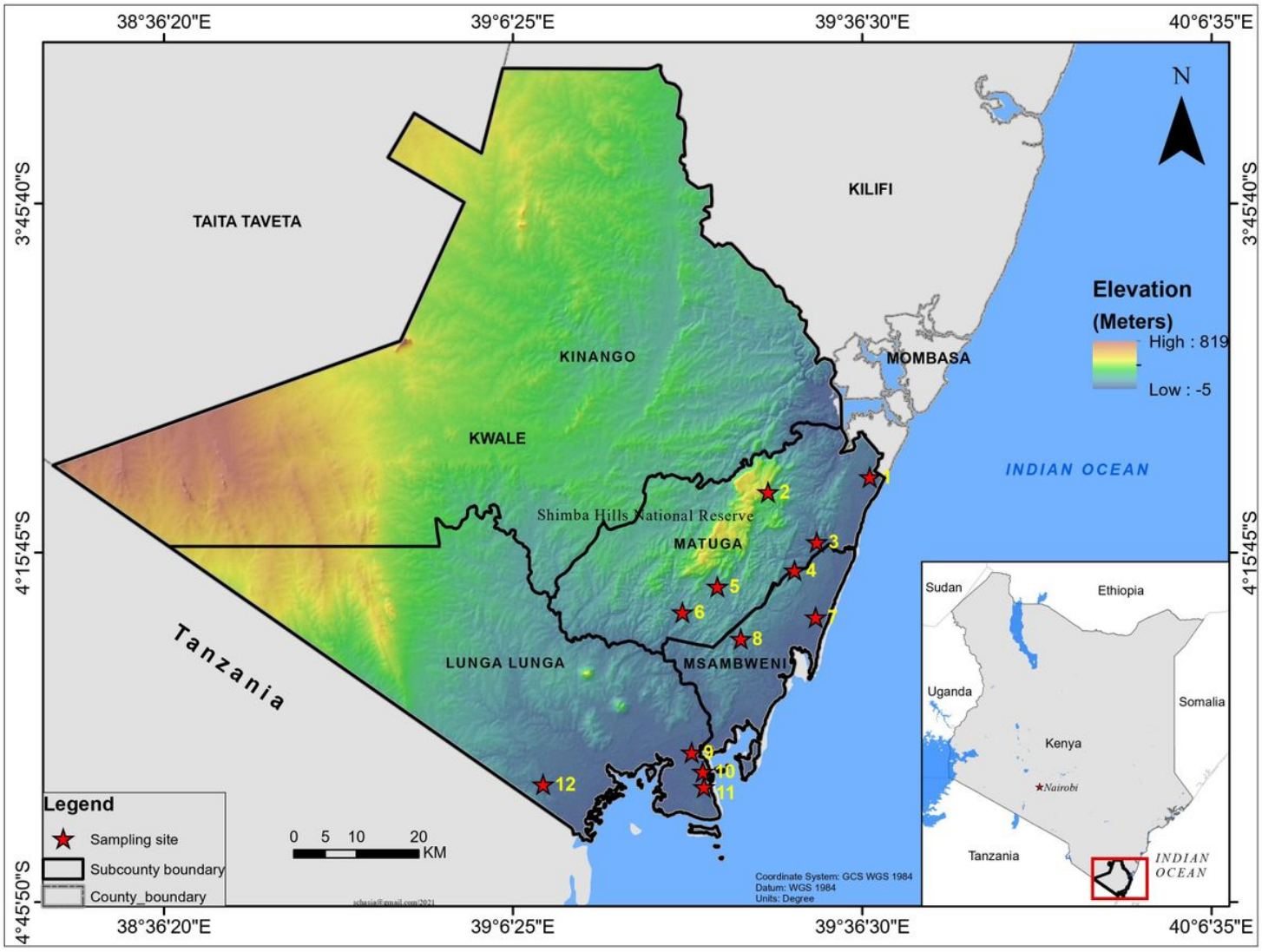

Figure 1

Map of Kwale County showing the 12 localities where 507 cattle were sampled (shown in numbers). The localities were: - 1). Miaji farm (n=40), 2). Matuga (n=13), 3). Ukunda (n=28), 4). Mwanjaba (n=10), 5). Kipabane (n=43), 6). Kichaka simba (n=107), 7). Majiboni (n=32), 8). Tangini (n=59), 9). Shimoni Kidimu (n=34), 10). Shimoni (n=32), 11). Kiwambale (n=33), 12). Vanga (n=75)

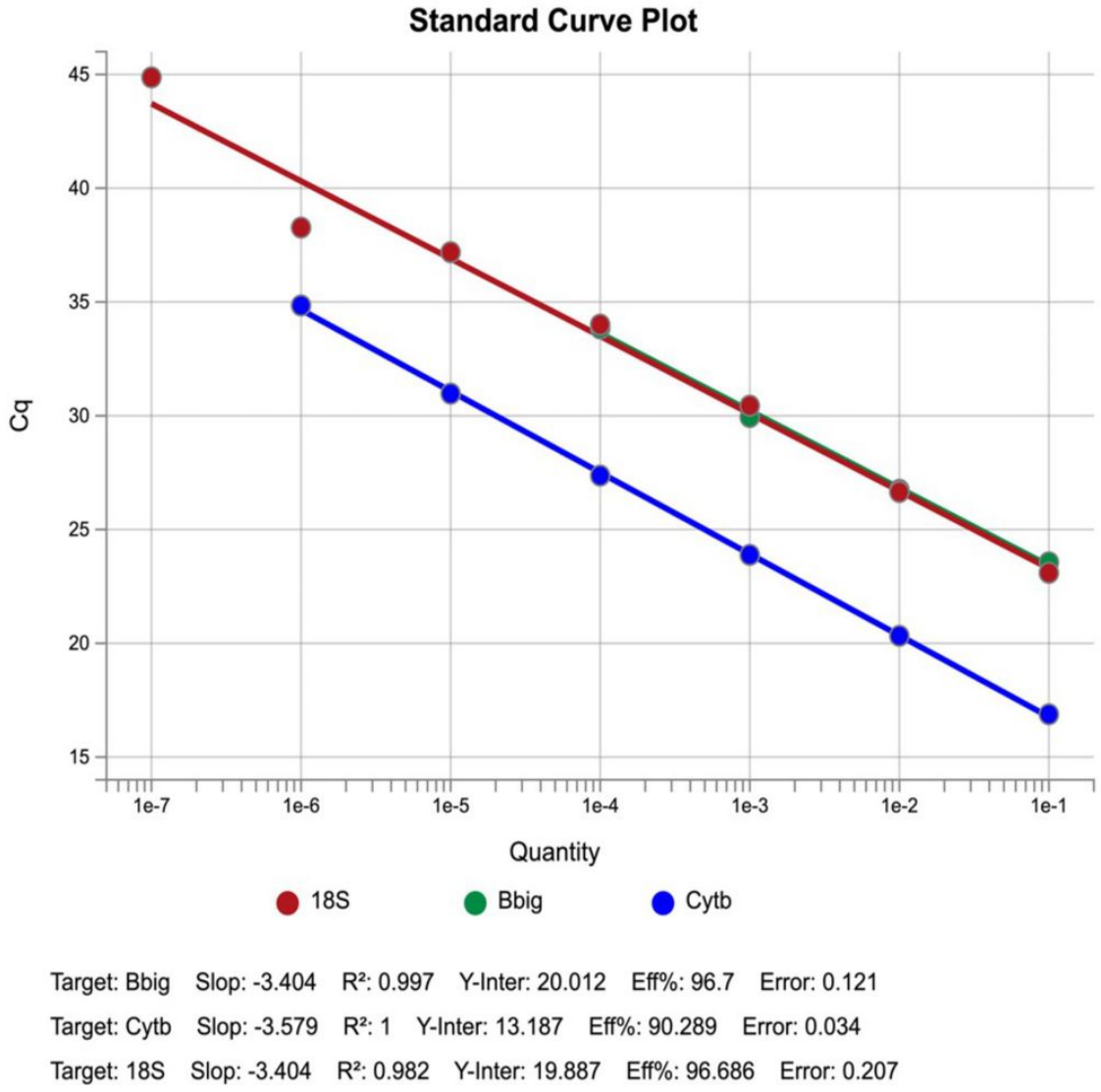

Figure 2

Standard curves of the three primer and probe sets used in the detection of Babesia parasites in the current study

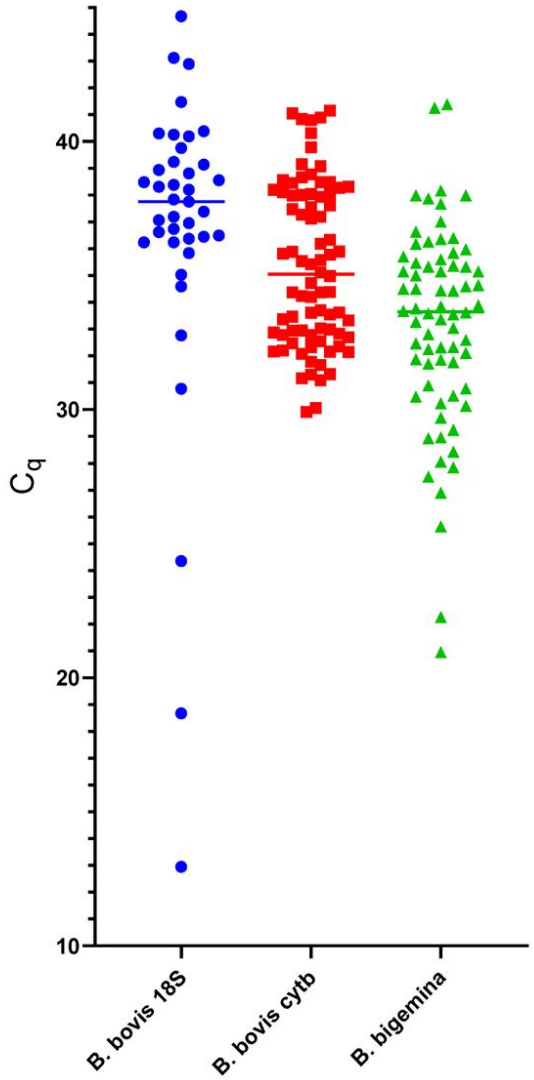

Figure 3

A scatter plot showing the range distribution of B. bovis and B. bigemina Cq values detected by the Taqman probes used in this study. A total of 39 and 81 blood samples were found to be positive for B. bovis by the 18S and cytochrome b assays respectively. The B. bigemina cytochrome b detected 70 positive bloods. The lowest Cq value was 12.95 and 29.91 for 18S and cytochrome b while the maximum was 44.68 and 41.16 respectively. The lowest Cq for B. bigemina was 20.96 and the highest was 41.38. Mean Cq value was 36.55 (SD ± 5.995) for 18S and 35.37 (SD ± 2.997) for B. bovis cytochrome b while it was 33.15 (SD ± 3.766) for B. bigemina.

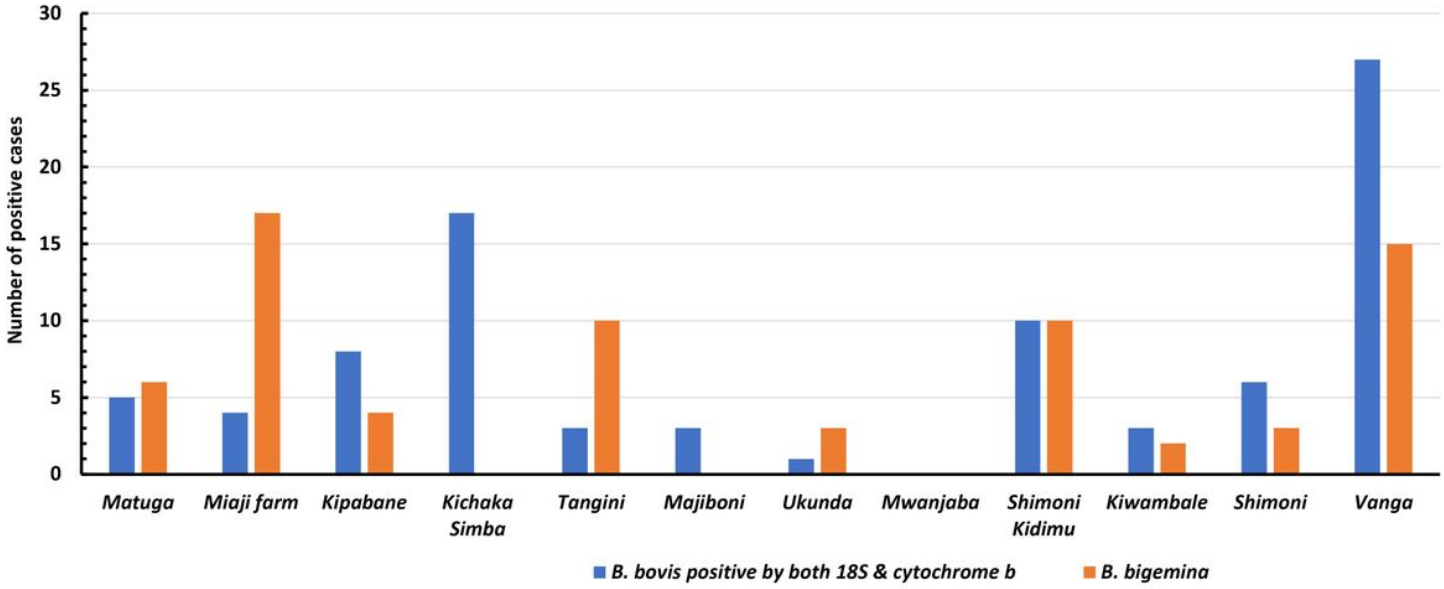

Figure 4

Distribution of Babesia bovis and B. bigemina positive blood across sampled sites

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalfileTableS1.docx
- B.bovisGraphicAbstract.pdf