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BRIEF REPORT

Molecular detection and phylogenetic analysis of tick-borne pathogens in cattle from southern Malawi

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Abstract

Introduction Tick-borne diseases (TBDs) pose a major hindrance to livestock production in countries with limited resources. Effective prevention and management of TBDs require a thorough understanding of disease vectors and pathogens. However, there is limited information on studies of bovine tick-borne pathogens (TBPs) using molecular methods in Malawi. This study aimed to detect TBPs of cattle populations in southern Malawi, which has the largest cattle population in the country.

Methodology A total of 220 blood samples from apparently healthy cattle were collected in six districts, and were screened for selected TBPs using polymerase chain reaction (PCR).

Results The overall detection rate of TBPs was 72.3%. Among the detected pathogens, *Babesia bigemina* had the highest detection rate (34.5%), followed by *Anaplasma marginale* (23.2%), *Anaplasma phagocytophilum* (22.3%), *Theileria taurotragi* (22.3%), *Theileria parva* (15.5%), *Anaplasma bovis* (9.6%), *Babesia bovis* (7.3%), *Theileria mutans* (4.1%), and *Babesia naoakii* (2.7%). Among the positive samples, 64.2% were found to be co-infected with two or more TBPs, with the highest number of seven pathogens detected in a single sample. The study documents the existence of A. *phagocytophilum*, B. *bovis*, and B. *naoakii* in Malawian cattle for the first time.

Conclusion The findings herein demonstrate a significant burden of TBPs on cattle in Malawi, which gives a challenge in combating TBDs. The high TBP burden, along with the high co-infection frequencies in Malawian cattle necessitates the urgency to implement effective control strategies to enhance cattle production in the country.

Keyword Cattle · Malawi · PCR · Phylogenetic analysis · Tick-borne pathogens

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Introduction

Tick-borne pathogens (TBPs), transmitted by vectors, account for a substantial number of infections in humans and animals (Parola and Raoult 2001). Globally, 80% of the bovine population is greatly afflicted by TBDs, resulting in decreased livestock production and loss of resources in fighting the diseases (Mangold et al. 1998). It is a key burden on livestock farming, and has a substantial economic impact on rural people, disturbing their food accessibility and agricultural undertakings (Minjauw and McLeod 2003). Farmers are subjected to economic losses due to huge cattle deaths, poor body condition of the animals, reduced milk production, and huge amounts of costs used in dealing with the tick burden and diseases they transmit (Uilenberg 1992). In Africa, anaplasmosis, babesiosis, ehrlichiosis, and theileriosis are the main health and management drawbacks for cattle and small ruminant production (Ocaido et al. 2009).

Bovine babesiosis is transmitted by *Rhipicephalus microplus* ticks and is caused by *Babesia* species that include *B. bigemina, B. bovis* and *B. naoakii*. However, *B. bovis* is more virulent among the pathogens, with catastrophic consequences in cattle (Woodford et al. 1990). It is clinically manifested as pyrexia, hemoglobinuria, anemia, and jaundice (Bock et al. 2004). Similarly, *Babesia naoakii*, which can cause bovine clinical babesiosis, is a recent challenge in Asian countries, causing mortalities in the cattle population (Sivakumar et al. 2020). Meanwhile, *Anaplasma marginale*, which causes bovine anaplasmosis, is marked by anemia, jaundice, pyrexia, cachexia, abortions, and stupor. In the worst cases, it can lead to death (Mohanta et al. 2023a). On the contrary, *A. phagocytophilum* causes bovine granulocytic anaplasmosis, also known as tick-borne fever. It can cause clinical disease in humans and animals with depression, loss of appetite, and fever as clinical symptoms (Woldehiwet 2009), although most of the human cases have been reported in Europe (Dugat et al. 2015). Despite *Ixodes* ticks being responsible for the transmission of *A. phagocytophilum*, mechanical transfer of infected blood via hematophagous arthropods, fomites and transplacental transmission during pregnancy have also been reported (Jurković et al. 2020).

East Coast Fever (ECF), also known as theileriosis, is a disease caused by *Theileria parva,* which is transmitted by *Rhipicephalus appendiculatus* ticks (Walker et al. 2003). It leads to acute lymphoproliferative diseases, usually leading to death in most affected cattle populations (Olds et al. 2018). Moreover, *T. mutans* might cause severe illness in cattle, with clinical symptoms that may be confused with a mild form of *T. parva* in cattle populations (Chaisi et al. 2013). However, *T. mutans* is transmitted by ticks belonging to the genus *Amblyomma* (Walker et al. 2003). Furthermore, *T. taurotragi*, causes mild and cerebral theileriosis in cattle and elands. It is associated with bovine cerebral disease known as turning disease, thereby making it an economic disease of veterinary importance (De Vos et al. 1981).

The livestock subsector makes a significant contribution to Malawi's economy and food security. It contributes approximately 10.5% to the national income and roughly 37.4% to the agricultural gross domestic product (MoAIWD 2022). About 60% of Malawians, mostly smallholder farmers, own various livestock species. Malawi has a cattle population of about 1,959,101, of which 92% is composed of the indigenous Malawi zebu, and 8% dairy crossbreds and exotic breeds (MoAIWD 2022). Ticks belonging to the genera *Amblyomma*, *Rhipicephalus*, and *Hyalomma* have been reported in Malawi (Chikufenji et al. 2024; Berggren 1978). On the contrary, TBPs of genera *Theileria*, *Anaplasma*, and *Babesia* have been reported mostly in central and northern Malawi where ECF had been declared endemic (DAHLD 2006; Chatanga et al. 2022). Less sensitive and conservative diagnostic methods, such as the use of blood smears, have been used in Malawi. Nevertheless, information on the detection of TBPs in cattle in Malawi is scarce. This study, therefore, aimed to investigate the prevalence of TBPs in cattle in southern Malawi using molecular methods.

Materials and methods

Sampling locations, sample collection, and DNA extraction

Two hundred and twenty blood samples were collected from randomly selected, and communally grazed local heads of cattle in six districts of southern Malawi (SI Fig. 1) (Blantyre: n=47; Chikwawa: n=43; Chiradzulu: n=37; Mulanje: n=28; Thyolo: n=29 and Zomba: n=36), between October and December 2021. Approximately 2–5 mL of blood was obtained from each animal into an ethylenediaminetetraacetic acid (EDTA) containing tube (BD Bioscience, Bergen County, NJ, USA). After collection, blood samples were briefly kept on ice in the field, and at -20°C in the laboratory. DNA from the collected samples was extracted as described by Ringo et al. (2022).

Molecular detection of tick-borne pathogens and sequencing

Selected tick-borne pathogens were screened from the DNA samples using the primers listed in SI Table 1. The PCR assays were performed as described by Chikufenji et al. (2024). The PCR bands were cut from the gel and purified using Nucleospin® Gel and PCR Clean-up kit (Macherey–Nagel, Düren, Germany), following the manufacturer's instructions. The purified PCR products were directly sequenced using a

Table 1 Detection rates of tick-borne pathogens based on sampling locations

Pathogen	Study sites						
	Chiradzulu	**Thyolo**	**Mulanje**	**Blantyre**	**Chikwawa**	**Zomba**	**Overall (n = 220)**
	(n = 37)	(n = 29)	(n = 28)	(n = 47)	(n = 43)	(n = 36)	
T. parva	12 (32.4%)**	2 (6.9%)	5 (17.9%)**	3 (6.4%)	9 (20.9%)**	3 (8.3%)	34 (15.5%)
T. mutans	3 (8.1%)	0	0	3 (6.4%)	1 (2.3%)	2 (5.6%)	9 (4.1%)
T. taurotragi	8 (21.6%)	11 (37.9%)	5 (17.9%)	12 (25.5%)	8 (18.6%)	5 (13.9%)	49 (22.3%)
B. bigemina	16 (43.2%)*	8 (27.6%)*	11 (39.3%)*	12 (25.5%)*	22 (51.2%)*	7 (19.4%)*	76 (34.5%)
B. bovis	4 (10.8%)	0	1 (3.6%)	2 (4.3%)	7 (16.3%)	2 (5.6%)	16 (7.3%)
B. naoakii	1 (2.7%)	0	0	2 (4.3%)	3 (7.0%)	0	6 (2.7%)
A. marginale	12 (32.4%)	4 (13.8%)	7 (25.0%)	5 (10.6%)	14 (32.6%)	9 (25.0%)	51 (23.2%)
A. bovis	4 (10.8%)	6 (20.7%)	3 (10.7%)	2 (4.3%)	6 (14.0%)	0	21 (9.5%)
A. phagocytophilum	3 (8.8%)	5 (17.2%)*	8 (28.6%)*	7 (14.9%)*	17 (39.5%)*	9 (25.0%)*	49 (22.3%)

n:number of samples collected, *: $p < 0.05$ **: $p > 0.01$

BigDye™ Terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Codon Code Aligner version 9 software (Codon Code Corporation, Centerville, MA, USA) was used for trimming, assembling, and generating the consensus sequences for the reads. The BLASTn analysis was performed to confirm the identity of the sequences.

Phylogenetic analysis and accession numbers assigned

The nucleotide sequences obtained from the TBPs in this study were assembled by using Clustal W multiple alignments in MEGA XI software (Tamura et al. 2021), and the maximum likelihood (ML) method was used to construct phylogenetic trees for the sequences generated, with those previously deposited in the GenBank. The sequences obtained were submitted to the GenBank of the National Center for Biotechnology Information (NCBI) through BankIt and GenBank for DNA sequences and ribosomal RNA sequences, respectively, and the following accession numbers were assigned: OP839189–OP839190 for *B. bovis*; OP854628–OP854629 for *B. naoakii*; OP866967–OP866971 for *B. bigemina*; OP868837–OP868840 for *A. marginale*; OP824619–OP824620 for *A. phagocytophilum*; and OP824766 for *A. bovis*; OP866888–OP866892 for *T. parva*; OP821414–OP821415 for *T. mutans*; OP824492–OP824498 for *T. taurotragi*.

Results

Tick-borne pathogen detection rates

A total of 159 (72.3%) samples were positive for at least one of the screened TBPs. The pathogens detected were *B. bigemina* (34.5%), *A. marginale* (23.2%), *A. phagocytophilum* (22.3%), *T. taurotragi* (22.3%), *T. parva* (15.5%), *A. bovis* (9. 5%), *B. bovis* (7.3%), *T. mutans* (4.1%), and *B. naoakii* (2.7%) (Table 1). A significant difference in the detection rate of *B. bigemina* was observed among the study locations, while a significantly higher detection rate of *T. parva* was observed in Chiradzulu (32.4%), Mulanje (17.9%) and Chikwawa (20.9%). In terms of infection rate by sex, 41 males (47.7%) and 118 females (88.1%) were infected with at least one of the examined pathogens. Significantly higher detection rates for *T. taurotragi* (26.9%; $p < 0.05$) and *B. bovis* (10.4%; $p < 0.05$) were observed in females than males (Table 2). *Coxiella burnetii, E. ruminantium, A. platys, T. orientalis*, and *Rickettsia* spp., were not detected in any of the screened samples.

Co-infections were observed in 64.2% of the positive samples, with up to septuple different pathogens simultaneously detected in one sample. Co-infections with double, triple, quadruple, quintuple, hextuple, and septuple pathogens were observed in 44.0% (70/159), 15.1% (24/159), 1.9% (3/159), 1.3% (2/159), 1.3% (2/159), and 0.6% (1/159) of the TBP-positive samples, respectively (SI Table 2).

Gene sequence analysis

The *groEL* gene sequences of *A. marginale* (OP868837–OP868840) were well conserved among themselves, with high identity values of 97.2–99.6%. The identity values of these sequences ranged from 99.1–100% when compared with *A. marginale* sequences from Cattle in Benin (KX685364) and Tanzania (OP414689).

Additionally, the percent identity values of *B. bigemina* (OP866967–OP866971) in this study ranged from 91.2–99.8% among themselves. The sequences had similarity scores ranging from 98.4–100% with the bovine *B.*

Table 2 Detection rates of tick-borne pathogens based on sex and age groups of cattle

Category	*T. parva*	*T. mutans*	*T. taurotragi*	*B. bigemina*	*B. bovis*	*B. naoakii*	*A. marginale*	*A. bovis*	*A. phagocytophilum*
Sex									
Male (n = 86)	10 (11.6%)	2 (2.3%)	13 (15.1%)	29 (33.7%)	2 (2.3%)	1 (1.2%)	15 (17.4%)	5 (5.8%)	14 (16.3%)
Female (n = 134)	24 (17.9%)	7 (5.2%)	36 (26.9%)	47 (35.1%)	14 (10.4%)	5 (3.7%)	36 (26.9%)	16 (11.9%)	35 (26.1%)
p-value	0.253	0.488	0.047*	0.885	0.031*	0.408	0.14	0.162	0.098
Age group									
Calves < 1.5 years (n = 37)	6 (16.2%)	2 (5.4%)	0	7 (18.9%)	2 (5.4%)	1 (2.7%)	12(32.4%)	0	7 (18.9%)
Adults ≥ 1.5 years (n = 183)	28 (15.3%)	7 (3.8%)	49 (26.8%)	69 (37.7%)	14 (7.7%)	5 (2.7%)	39 (21.3%)	21 (11.5%)	42 (23.0%)
p-value	0.808	na	na	0.036*	na	na	0.198	0.029*	0.67

n: number of samples, na: not analysed, *: $p < 0.05$

bigemina sequences from Kenya (KP347559) and South Africa (MK481015). On the other hand, the sequences of *B. bovis* sbp-4 gene (OP839189–OP839190) shared identity values ranging from 94.4–99.2% and showed higher identities of up to 99.8–100% with sequences OQ144958 (Bangladesh) and KF626632 (South Africa). Furthermore, two *B. naoakii* ama-1 sequences (OP854628 and OP854629) showed identity values of 99.5–100%. The sequences were identical to the sequences LC385804 (Sri Lanka) and OQ148404 (Bangladesh). In contrast, *p104* sequences of *T. parva* (OP866888–OP866892) shared percent identity between 93.4% and 99.6%. The sequences showed 96.3% and 100% identity with *T. parva* sequences from cattle in Cameroon (MK568804) and Tanzania (MZ798151 and OP390278), respectively. In addition, the percent identity of *T. taurotragi* (OP824492–OP824498) and *T. mutans* (OP821414–OP821415) in this study ranged from 97.5–99.6% and 99.2–100%, respectively.

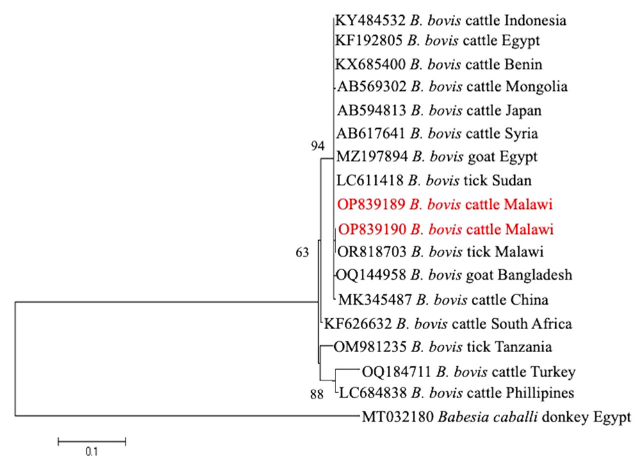

Fig. 1 Phylogenetic analysis of *Babesia bovis* identified in this study is based on the *SBP-4* gene. The tree was constructed by MEGA XI using the maximum likelihood method based on Kimura 2-parameter model. Numbers on the nodes indicate the percentage of 1000 bootstrap replicates. The sequences of this study are shown in red. *Babesia caballi* (MT032180) was used as an outgroup

Phylogenetic analyses

Babesia bovis SBP-4 sequences (OP839189 and OP839190) clustered in the clade with those from Bangladesh (OQ144958), Sudan (LC611418), Mongolia (AB569302), Syria (AB617641), Egypt (KF192805 and MZ197894), Japan (AB594481), Benin (KX685399), and Malawi (OR818703) (Fig. 1). In addition, the *B. naoakii ama-1* sequences (OP854628 and OP854629) clustered with LC506533 from Mongolia, LC486029 from Argentina, LC486011 from the Philippines, LC486017 from Vietnam, OQ148404 from Bangladesh, and OR601001 from Egypt (Fig. 2). Moreover, *Babesia bigemina Rap-1a* gene sequences (OP866967-OP866971) clustered in the same clade with isolates from Benin (KU042084), Tanzania (OP390284), Burkina Faso (OK323209), Kenya (KP347559), Bangladesh (OQ162126), Uganda (MG426198), South Africa (MK481015), Nigeria (OM406333), and Turkey (KT220512) (SI Fig. 2).

The *A. marginale* sequences (OP868837—OP868840) obtained herein, clustered in a clade with the sequences OQ148410 (Bangladesh), FJ226455 (Japan), OQ185223 (China), KC113455 (Philippines), OP414689 (Tanzania), OR767905 and LC664079 (Malawi), KY522983 (Uganda), MN870643 (Egypt), and KX685364 (Benin) (SI Fig. 3). The phylogeny inferred from 16S rRNA of *A. phagocytophilum* and for *A. bovis* resulted in clustering in the respective clades (Fig. 3). The *A. bovis* isolate had a close similarity to those deposited from Bangladesh (OQ135122), Malawi (OR823813), and Iran (KU242422). However, *A. phagocytophilum* isolates generated herein had similarities to that reported from South Korea (MF787269).

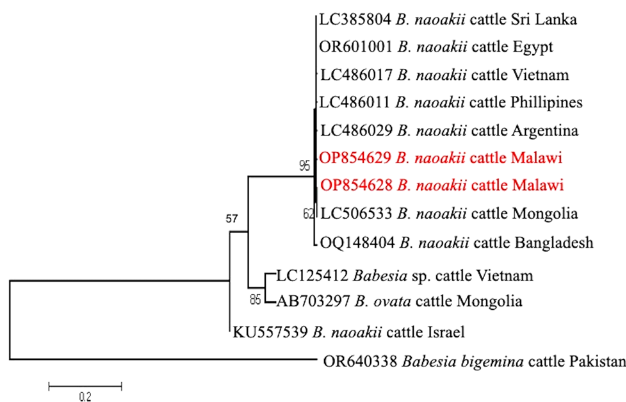

Fig. 2 Phylogenetic analysis of *Babesia naoakii* identified in this study is based on the AMA-1 gene. The tree was constructed by MEGA XI using the maximum likelihood method based on Kimura 2-parameter model. Numbers on the nodes indicate the percentage of 1000 bootstrap replicates. The sequences of this study are shown in red. *Babesia bigemina* (OR640338) was used as an outgroup

The *T. parva p104* gene sequences OP866888–OP866892 clustered together with MZ798149 from South Africa, MN810052 from Uganda, MK568804 from Cameroon, MZ798151 from Tanzania, KP347566 from Kenya, ON376062 from Mozambique, and EF469604 from Sudan (SI Fig. 4). The sequences of *T. mutans* (OP821414 and OP821415) were conserved and clustered in the same clade with those from South Africa (MH751463), Angola (MT898574), and Mozambique (FJ869899), whereas *T. taurotragi* sequences (OP824492-OP824498) formed a monophyletic clade with those previously obtained from Malawi (LC664058), South Africa (L19082), Tanzania (MN726635), Kenya (MT459438) and Zambia (MT814757) (SI Fig. 5).

Discussion

In this study, PCR was used to detect cattle TBPs from southern Malawi, and relatively high detection rates of TBP infections among cattle populations were recorded. The molecular techniques used in this study offer a more sensitive and dependable diagnostic tool than the conservative methods previously used in Malawi.

This study reports *B. bovis* and *B. naoakii* for the first time in Malawi using molecular techniques. We report the detection rate of 7.3% for *B. bovis* in this study which was higher than 4.5% from Tanzania (Ringo et al. 2018). However, this was lower than 7.7% from Zambia (Tembo et al. 2018), 82% from Mozambique (Martins et al. 2008), and 12.3% from Kenya (Adjou Moumouni et al. 2015). The existence of *B. bovis* in Malawi was not surprising because the pathogen has already been reported from Zambia, Tanzania and Mozambique, the neighboring countries of Malawi. The detection of *B. bovis* was observed in all study locations except Zomba district. This observation could be due to differences in cattle-rearing systems among the study sites. Although the semi-intensive system is practiced in Zomba, the extensive system is used in other study areas which possibly exposed the animals more to tick vectors. The phylogenetic analysis of *sbp-4* gene sequences showed that the two sequences obtained in this study clustered in one clade along with those from Mongolia (AB569302), Egypt (MZ197894), Sudan (LC611418), Indonesia (KY484532), and Japan (AB594813). This result suggests that there might be one strain of *B. bovis* infecting cattle in southern Malawi.

We detected *B. naoakii* in 2.7% of the screened samples. This was similar to 1.09% reported from Bangladesh (Mohanta et al. 2023a). However, the detection rate in this study was lower than 9.6% from Vietnam (Sivakumar et al. 2020), 27.9% from Mongolia (Otgonsuren et al. 2020), and 11.3% from the Philippines (Sivakumar et al. 2020). The detection of this pathogen in Malawi was surprising because it has never been reported in any of the sub-Saharan African countries. However, it has been reported recently in Egypt (Sivakumar et al. 2020). The phylogenetic analysis of *B. naoakii ama-1* sequences clustered in one clade with previously reported isolates from Mongolia, Argentina, the Philippines, Vietnam, Bangladesh, and Egypt (Fig. 2). This finding indicates that *B. naoakii* isolates reported in Asian and other countries in the world is the same strain affecting cattle in Malawi. However, more studies on tick vectors of this pathogenic *Babesia* spp. and its impact on livestock in Malawi is urgently warranted.

We report a detection rate of 23.1% for *A. marginale* in Malawian cattle, which is consistent with a previous report (24.0%; Chatanga et al. 2022). However, a lower detection rate was recorded in other countries, 16% in Tanzania (Ringo et al. 2018), 7.9% in Kenya (Adjou Moumouni et al. 2015), and 10.51% in Bangladesh (Mohanta et al. 2023b). Extensive type of livestock rearing practice in the study locations herein might have contributed to the higher detection rates. We also report a detection rate of 22.3% for *A. phagocytophilum*, a zoonotic parasite causing acute and subclinical disease in the animal host (Inokuma et al. 2005). Compared to the present study, the lower detection rates of this zoonotic pathogen were reported in Ethiopia, 2.7% (Teshale et al. 2018), South Africa, 7.0% (Mtshali et al. 2016) and Bangladesh, 0.72% (Mohanta et al. 2023b). This variation might be a result of different sample sizes in these study locations.

Zoonotic cases of *A. phagocytophilum* have been reported in South Africa (Inokuma et al. 2005), and China (Cao et al. 2000) in acute and subclinical forms with fever, and central nervous system dysfunction (Inokuma et al. 2005), while anorexia and lameness in the animal hosts

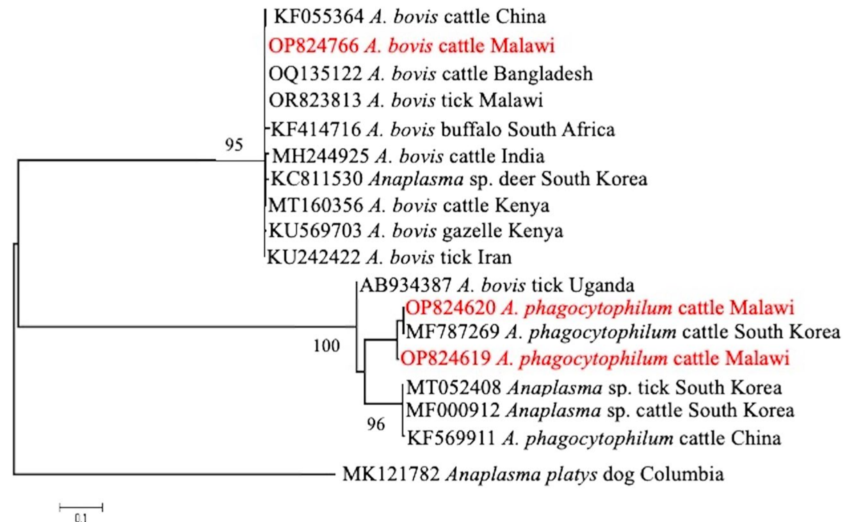

Fig. 3 The phylogenetic analysis of *A. phagocytophilum* and *A. bovis* identified in this study is based on the 16S rRNA gene. The tree was constructed by MEGA XI using the maximum likelihood method based on Hasegawa-Kishino Yano model. Numbers on the nodes indicate the percentage of 1000 bootstrap replicates. The sequences of this study are shown in red. *Anaplasma platys* (MK121782) was used as an outgroup

(Mtshali et al. (2016). This study reports the existence of *A. phagocytophilum* for the first time in Malawi.

The detection rate of 15.5% for *T. parva*, the causative agent for ECF, was found herein. The detection of this pathogen in southern Malawi suggests that the pathogen has geographically spread out in Malawi, and this may be attributed to uncontrolled animal movements from the endemic central and northern Malawi. The detection rate herein is lower than that previously reported from Malawi (33.0%, Chatanga et al. 2022), which could be a result of differences in sampling locations. In this study, sampling was done from the non-endemic region, while the previous study was done in the endemic central region. The significantly higher detection rates observed in Chiradzulu (32.4%), Mulanje (17.9%) and Chikwawa (20.9%) were due to the introduction of dairy animals from endemic regions of central and northern Malawi, and uncontrolled cattle movements from the neighboring Mozambique as well (MoAIWD 2022).

The detection rate of 4.1% for *T. mutans* found herein was notably lower than that previously reported from Malawi (73.8%, Chatanga et al. 2022), Tanzania (34.4%, Ringo et al. 2018) and Uganda (88.3%, Byaruhanga et al. 2016). The detection rates in different geographies might vary with tick activity in dry and wet or rainy seasons. In the present study, samples were collected during the dry season (October to December), while in the other studies, the sampling was done during or immediately after the rainy season.

The higher detection rates of co-infections (64.2%) with up to seven pathogens in this study and that reported previously in Malawi (79.6%, Chatanga et al. 2022) compared to those reported from neighboring Tanzania (44.8%, Ringo et al. 2022), and Mozambique (52%, Martins et al. 2008), indicate simultaneously multiple parasites in the same host, which further imply that the animals in the area are prone to multiple TBD outbreaks.

In conclusion, this study has revealed high frequencies of TBPs in cattle in southern Malawi. In addition, the detection of *A. phagocytophilum*, *B. bovis,* and *B. naoakii* for the first time in Malawi reveals the need for extra effort in dealing with TBDs, including zoonoses in Malawi. The findings herein provide critical information for the control clues against ticks and TBDs in Malawi.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s11259-024-10395-z.

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Data availability The data will be available on request from the corresponding authors.

Declarations

Ethical approval and consent to participate Authorization (permission ID number: DAHLD 002/2022) for sampling from cattle in the study locations was obtained from the Ministry of Agriculture Irrigation and Water Development (MoAIWD) through the Department of Animal Health and Livestock Development (DAHLD). Before sample collec-

tion, cattle owners were briefed on the significance of the activity and freely accepted to take part. Blood was collected by licensed veterinarians by following the ethical guidelines of Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan (animal experiment approval ID numbers: 22–23).

Consent for publication All authors have read and approved the final version of this paper and have agreed for its publication.

Competing interest The authors declare no competing interests.

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