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First molecular identification of multiple tick-borne pathogens in livestock within Kassena-Nankana, Ghana

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Abstract

The risk of pathogen transmission continues to increase significantly in the presence of tick vectors due to the trade of livestock across countries. In Ghana, there is a lack of data on the incidence of tick-borne pathogens that are of zoonotic and veterinary importance. This study, therefore, aimed to determine the prevalence of such pathogens in livestock using molecular approaches. A total of 276 dry blood spots were collected from cattle (100), sheep (95) and goats (81) in the Kassena-Nankana Districts. The samples were analyzed using Polymerase Chain Reaction (qPCR) and conventional assays and Sanger sequencing that targeted pathogens including *Rickettsia*, *Coxiella*, *Babesia*, *Theileria*, *Ehrlichia* and *Anaplasma*. An overall prevalence of 36.96% was recorded from the livestock DBS, with mixed infections seen in 7.97% samples. Furthermore, the prevalence of infections in livestock was recorded to be 19.21% in sheep, 14.13% in cattle, and 3.62% in goats. The pathogens identified were *Rickettsia* spp. (3.26%), *Babesia* sp. Lintan (8.70%), *Theileria orientalis* (2.17%), *Theileria parva* (0.36%), *Anaplasma capra* (18.48%), *Anaplasma phagocytophilum* (1.81%), *Anaplasma marginale* (3.26%) and *Anaplasma ovis* (7.25%). This study reports the first molecular identification of the above-mentioned pathogens in livestock in Ghana and highlights the use of dry blood spots in resource-limited settings. In addition, this research provides an update on tick-borne pathogens in Ghana, suggesting risks to livestock production and human health. Further studies will be essential to establish the distribution and epidemiology of these pathogens in Ghana.

Keywords Livestock, *Rickettsia*, *Babesia*, *Theileria*, *Ehrlichia*, *Anaplasma*

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Introduction

In Africa, the high demand for animal food products due to fast population development has spurred cross-regional livestock commerce leading to an increased risk of animal disease transmission (Volkova et al. 2010; Fèvre et al. 2006). Global transhumance is also associated with animal movements in Sub-Saharan Africa (Motta et al. 2018). It is common for herders to engage in cross-border migration with their livestock to efficiently use seasonal pasture resources (Lesse et al. 2016). Even though such activities could facilitate the spread of infectious pathogens, disease surveillance is poor or nonexistent near the



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borders of many Sub-Saharan African countries (Motta et al. 2017). The cross-border livestock trade has introduced exotic tick species and associated infections into naïve countries.

The situation in the Upper East Region of Ghana is somewhat similar, given the trade of livestock across the borders of Ghana and Burkina Faso, with no measures to prevent the spread of infectious diseases. The residents of this area are extensively involved in livestock rearing, which provides a food source and income. However, because zoonotic diseases frequently afflict people who interact with animals, these animals may act as amplifying hosts for zoonotic pathogens, placing owners at risk of becoming infected (Al-Tayib 2019). Animals are the source of various new and emerging pathogenic zoonotic diseases (Daszak et al. 2001; Taylor et al. 2001), with human activities serving as the primary drivers of their spread (Jones et al., 2013; Karesh et al., 2012). More importantly, increased livestock production, especially near wildlife habitats, has allowed disease interchange, with livestock serving as reservoirs for human zoonoses (Jones et al., 2013; Karesh et al., 2012). Furthermore, the rapid transmission of infections over long distances can be aided by animal movements inside countries and across borders (Dean et al. 2013; Fèvre et al. 2006).

In Sub-Saharan Africa, the health and productivity of livestock are greatly affected by ticks and tick-borne diseases (Jongejan and Uilenberg 2004). Babesiosis, anaplasmosis, theileriosis and ehrlichiosis are the most common tick-borne diseases in Sub-Saharan Africa, harming livestock health and incurring production losses (Morrison 2015). Climate change and the global trade of animals, according to studies, aid tick species invasion and the spread of infectious pathogens into new locations (Byamukama et al. 2021). This will most certainly increase tick-borne diseases in livestock and make pathogen transmission between wildlife and domestic animals

easier (Espinaze et al. 2018; Hassell et al. 2017; Olwoch et al. 2008). It is important to note that *Babesia*, *Theileria* and *Anaplasma* have been identified to infect livestock in Ghana (Ayeh-Kumi et al. 2022; Beckley et al. 2016).

In resource-limited settings, the use of dry blood spots (DBS) in surveillance efforts to diagnose infectious diseases comes with some advantages, including the non-invasive collection of blood, less volume of blood required, easy transport, and the long-term storage of the blood spots (Grüner, Stambouli, and Ross 2015; Gupta and Mahajan 2018). Even though some factors such as the method of sample preparation and storage conditions could influence the results obtained using DBS (Lim 2018), studies have successfully identified tick-borne pathogens in livestock DBS (Tigani-asil et al. 2021; Roy et al. 2018). This study employed the use of DBS to determine the prevalence of tick-borne pathogens of zoonotic and veterinary importance in livestock. There is limited information on the distribution and epidemiology of these pathogens in Ghana. Findings from this study will be essential in formulating control measures, especially with the constant movement of livestock and humans.

Results

A total of 276 DBS were collected from physically healthy livestock across the Kassena-Nankana district. The livestock sampled were Cattle ($n=100$; 36.23%), Sheep ($n=95$; 34.42%) and Goats ($n=81$; 29.35%). These livestock were sampled from Navrongo ($n=246$), Nakong ($n=10$) and Pungu ($n=20$). Overall, 102 (36.96%) livestock were infected with tick-borne pathogens. Furthermore, the prevalence of tick-borne infections in livestock was recorded in sheep (19.21%), cattle (14.13%), and goats (3.62%). Also, all the sampled locations recorded at least one tick-borne pathogen, with the most diverse pathogens recorded from the Abattoir (Tables 1 and 2). No *Coxiella burnetii* or *Ehrlichia* pathogens were detected.

Table 1 Livestock characteristics and parasitic tick-borne pathogens prevalence

Characteristics	Total livestock (%)	Parasites				
		<i>Babesia</i> sp. Lintan	<i>P</i> -value	<i>Theileria orientalis</i>	<i>P</i> -value	<i>Theileria parva</i>
Age						
< =1 year	164 (59.42)	4 (2.44)	<i>p</i> < 0.001	5 (3.05)	0.422	1 (0.61)
2–5 years	68 (24.64)	17 (25.00)		1 (1.47)		0
= > 6 years	44 (15.94)	3 (6.82)		0		0
Sex						
Female	194 (70.29)	8 (4.12)	<i>p</i> < 0.001	6 (3.09)	0.107	1 (0.52)
Male	82 (29.71)	16 (19.51)		0		0
Animal host						
Cattle	100 (36.23)	21 (21.00)	<i>p</i> < 0.001	0	0.003	0
Goat	81 (29.35)	0		0		0
Sheep	95 (34.42)	3 (3.16)		6 (6.32)		1 (1.05)

Table 2 Livestock characteristics and bacterial tick-borne pathogens prevalence

Characteristics	Total livestock (%)	Bacteria									
		<i>Rickettsia</i> spp	P-value	<i>Anaplasma capra</i>	P-value	<i>Anaplasma ovis</i>	P-value	<i>Anaplasma phagocytophilum</i>	P-value	<i>Anaplasma marginale</i>	P-value
Age											
	<= 1 year	164 (59.42)	4 (2.44)	0.648	32 (19.51)	0.664	14 (8.54)	0.363	0	9 (5.49)	0.042
	2–5 years	68 (24.64)	3 (4.41)		13 (19.12)		5 (7.35)		2 (2.94)	0	
	= > 6 years	44 (15.94)	2 (4.55)		6 (13.64)		1 (2.27)		3 (6.82)	0	
Sex											
	Female	194 (70.29)	8 (4.12)	0.214	39 (20.10)	0.285	8 (4.12)	0.002	1 (0.52)	8 (4.12)	0.214
	Male	82 (29.71)	1 (1.22)		12 (14.63)		12 (14.63)		4 (4.88)	1 (1.22)	
Animal host											
	Cattle	100 (36.23)	4 (4.0)	0.731	16 (16.00)	$p<0.001$	5 (5.00)	$p<0.001$	5 (5.00)	0	0.044
	Goat	81 (29.35)	3 (3.70)		4 (4.94)		0		0	3 (3.70)	
	Sheep	95 (34.42)	2 (2.11)		31 (32.63)		15 (15.79)		0	6 (6.32)	

Prevalence of tick-borne pathogens

An overall prevalence of 3.26% was recorded for *Rickettsia* DNA in the livestock examined. Prevalence rates were recorded as 1.49% in cattle, 1.12% in goats, and 0.75% in sheep. However, the positive samples could not be characterized to determine the specific *Rickettsia* species. This could be due to insufficient DNA in the PCR products that were sequenced.

Initial screening of the samples showed that 37 (13.41%) were positive for *Babesia/Theileria* with an infection rate of 24% (95% CI, 15.39–34.33) in cattle and 13.68% (95% CI, 7.09–22.79) in sheep. No infections were detected in goats. Sequencing of the positive PCR products resulted in identifying *Theileria orientalis* (2.17%), *Theileria parva* (0.36%) and an unclassified *Babesia* designated as *Babesia* sp. Lintan (8.70%). BLAST analysis showed that the *Babesia/Theileria* positive samples in this study were 82–94% similar to *Babesia* and *Theileria* isolated in China and East and Southern Africa. From the phylogenetic analysis, the samples LK-B41, LK-B42 and LK-B44 clustered with isolates from China (accession numbers KX698109.1 and MK962313.1) with 99% bootstrap support (Fig. 1a). However, all the samples LK-B50, LK-B51 and LK-B53 clustered with a bootstrap value of 100% (Fig. 1b).

From the 276 livestock samples examined, 85 (30.80%) were positive for *Ehrlichia/Anaplasma*. The highest prevalence rate was observed in sheep (18.84%), followed by cattle (9.42%) and goats (2.54%). It was observed that all positive samples were *Anaplasma* species after sequencing. The pathogens identified were *Anaplasma capra* (18.48%), *Anaplasma ovis* (7.25%), *Anaplasma marginale* (3.26%) and *Anaplasma phagocytophilum* (1.81%). BLAST analysis showed that the *Anaplasma* samples were 98–99% similar to isolates from Hungary, Panama, South Korea, and South Africa. Furthermore, from the phylogenetic analysis, all the *Anaplasma* samples (LK-A1, LK-A9, LK-A10 and LK-A15) clustered with isolates from France (accession number MF580636.1), Nigeria (accession numbers JF949765.1 and JF949768.1) and Burkina Faso (accession number MT259778.1) with 100% bootstrap support (Fig. 2).

Characteristics of tick-borne pathogens in sampled livestock

A significant association was observed between the parasitic pathogen *Babesia* sp. Lintan and the animal host, sex and age ($p < 0.001$). Male cattle between the ages of 2–5 years were the most infected with *Babesia* sp. Lintan (Table 1). Furthermore, bacterial pathogens *A. capra* and *A. ovis* were significantly associated with animal hosts ($p < 0.001$), with most infections recorded in sheep at 32.63 and 15.79%, respectively (Table 2).

Single bacterial infections were recorded in 30.8% livestock DBS, with 1.81% having coinfection. Additionally, 10.87% sampled livestock had one parasite infection without coinfections. In the Livestock, DBS, single bacterial or parasite infections were reported at 30.43%, with bacterial and parasitic coinfections at 6.52%. Sheep were the most often infected livestock host, and there was a strong correlation between bacterial infections and hosts ($p < 0.001$).

Additionally, it was discovered that parasitic infections had a highly significant ($p < 0.001$) correlation with age and the hosts. The majority of parasitic infections occurred in cattle (21%) between the ages of 2–5 years (26.47%) (Table 3). However, a substantial correlation was also found between disease with bacteria or parasites and the cattle hosts ($p < 0.001$).

According to the risk analysis, no livestock attribute (age, sex or animal host) was found to significantly increase the likelihood of contracting a bacterial or parasitic tick-borne infection (Table 4).

Discussion

Small-scale livestock producers in developing countries contribute significantly to agricultural production and rural development (McDermott et al. 2010). However, due to the cost of acaricides, tick infestations are not adequately controlled, especially during the rainy season (Achukwi et al. 2001), leading to an increase in tick-borne diseases infecting animals (Dantas-Torres, Chomel, and Otranto 2012).

In sub-Saharan Africa, *Rickettsia* infections have been identified in animals and humans, with ticks playing the role of principal vectors (Parola et al., 2013). Ticks are natural reservoir hosts of *Rickettsia* (Piotrowski and Rymaszewska 2020) with sparse knowledge of animals as potential reservoirs. In Spain, researchers detected *Rickettsia* infection in a goat blood sample, suggesting the risk of transmission to humans (Ortuño et al. 2012). Likewise, *Rickettsia* was found in Sika deer in Japan (Inokuma et al. 2008). In a study from China, domestic animals, including cattle and goats, were found infected with *Rickettsia* (Liang et al. 2012). Findings from this study indicate *Rickettsia* DNA in all the types of livestock sampled. Even though animals are not considered reservoir hosts of *Rickettsia*, they could amplify the pathogen and infect feeding ticks and humans.

An estimated 2 billion cattle are at risk of babesiosis exposure despite the efforts in research and preventive measures (Gohil et al. 2013; Bock et al. 2004). In Africa, *B. bovis* and *B. bigemina* transmitted by *Rhipicephalus* ticks cause bovine babesiosis (Bock et al. 2004). Although bovine infections are predominantly caused by *B. bigemina*, *B. bovis* infections are fatal due to neurological

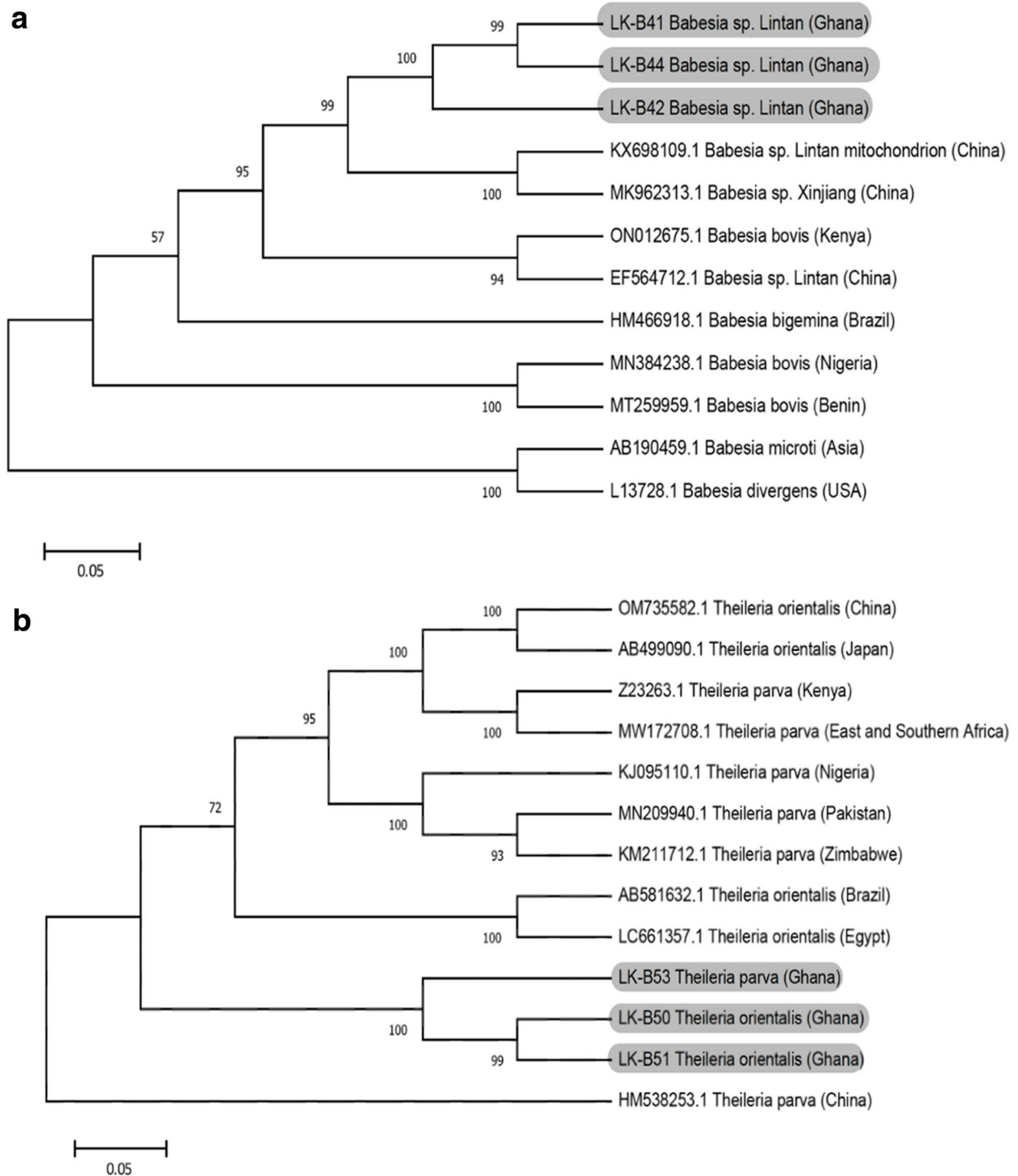


Fig. 1 Phylogenetic analysis of *Babesia* and *Theileria* in the livestock based on the rRNA gene fragments (lsu5-lsu4) of mitochondrial genome. **a** *Babesia*. The sequences obtained in this study are indicated as LK-B41, LK-B42 and LK-B44. **b** *Theileria*. The sequences obtained in this study are indicated as LK-B50, LK-B51 and LK-B53

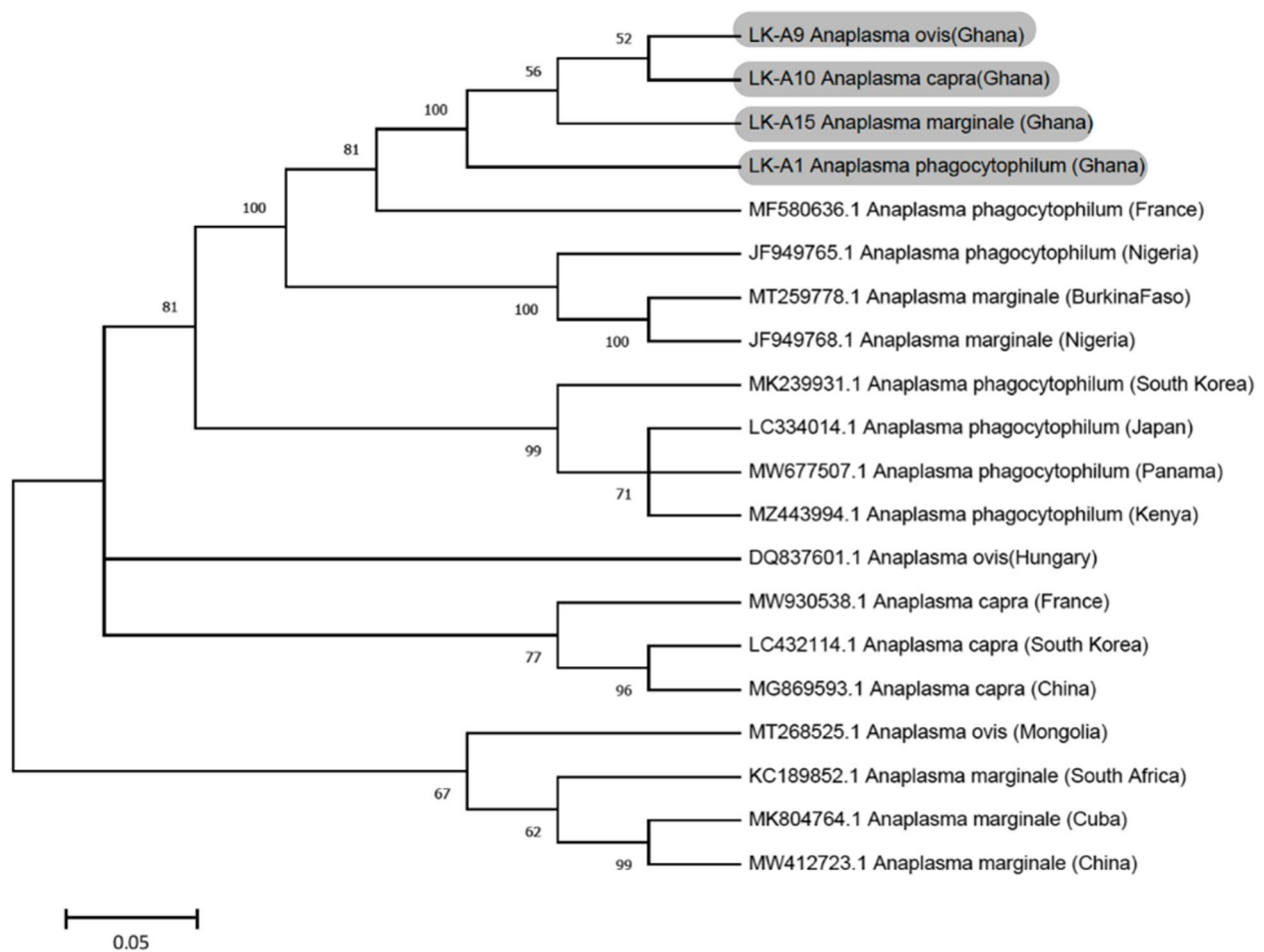


Fig. 2 Phylogenetic analysis of *Anaplasma* in the livestock based on the 16S rRNA gene. The sequences obtained in this study are indicated as LK-A1, LK-A9, LK-A10 and LK-A15

Table 3 Bacterial and parasitic pathogens in association with livestock characteristics

	Total livestock (%)	Bacterial			Parasitic		Bacterial/Parasitic		
		Single infection	Coinfection	P-value	Single infection	P-value	Single bacteria or parasite	Bacteria/Parasite coinfection	P-value
Age									
<=1 year	164 (59.42)	56 (34.15)	2 (1.22)	0.227	9 (5.49)	<i>p</i> <0.001	53 (32.32)	7 (4.27)	0.145
2–5 years	68 (24.64)	17 (25.00)	3 (4.41)		18 (26.47)		20 (29.41)	9 (13.24)	
= >6 years	44 (15.94)	12 (27.27)	0		3 (6.82)		11 (25.00)	2 (4.55)	
Sex									
Female	194 (70.29)	57 (29.38)	4 (2.06)	0.676	14 (7.22)	0.005	59 (30.41)	8 (4.12)	0.051
Male	82 (29.71)	28 (34.15)	1 (1.22)		16 (19.51)		25 (30.49)	10 (12.20)	
Animal host									
Cattle	100 (36.23)	26 (26.00)	2 (2.00)	<i>p</i> <0.001	21 (21.00)	<i>p</i> <0.001	29 (29.00)	10 (10.00)	<i>p</i> <0.001
Goat	81 (29.35)	10 (12.35)	0		0		10 (12.35)	0	
Sheep	95 (34.42)	49 (51.58)	3 (3.16)		9 (9.47)		45 (47.37)	8 (8.42)	

Table 4 Multivariable multinomial logistic regression model for tick-borne bacterial and parasitic pathogens

Characteristics	Bacterial		Bacteria coinfection	
	Single bacterial infection IRR (95%CI)	P-value	IRR (95%CI)	P-value
Age				
<=1 year vs 2–5 years	0.41 (0.11–1.48)	0.175	2.49 (0.20–31.20)	0.478
<=1 year vs >6 years	0.46 (0.10–2.22)	0.34	0	0.996
Sex				
Female vs Male	1.18 (0.63–2.22)	0.612	0.41 (0.04–4.38)	0.459
Animal host				
Cattle vs Goat	0.19 (0.04–0.90)	0.037	0	0.994
Cattle vs Sheep	1.56 (0.40–6.34)	0.507	2.08 (0.15–29.20)	0.586
Characteristics	Parasite		Parasitic coinfection	
	Single parasite infection IRR (95%CI)	P-value	IRR (95%CI)	P-value
Age				
<=1 year vs 2–5 years	0.48 (0.05–4.96)	0.54	–	–
<=1 year vs >6 years	0.08 (0.01–1.15)	0.063	–	–
Sex				
Female vs Male	1.46 (0.62–3.45)	0.387	–	–
Animal host				
Cattle vs Goat	1	–	–	–
Cattle vs Sheep	0.13 (0.01–1.37)	0.09	–	–
Characteristics	Bacterial and Parasite		Bacteria/Parasite coinfection	
	Single bacteria or parasite infection IRR (95%CI)	P-value	IRR (95%CI)	P-value
Age				
<=1 year vs 2–5 years	0.27 (0.07–1.10)	0.069	0.89 (0.11–7.06)	0.91
<=1 year vs >6 years	0.16 (0.03–0.85)	0.031	0.25 (0.02–3.52)	0.304
Sex				
Female vs Male	0.89 (0.46–1.69)	0.713	2.04 (0.69–6.06)	0.197
Animal host				
Cattle vs Goat	0.06 (0.01–0.34)	0.001	0	0.982
Cattle vs Sheep	0.55 (0.13–2.40)	0.428	0.83 (0.10–2.09)	0.174

symptoms (Uilenberg 2006). In this study, cattle and sheep were infected with *Babesia* sp. Lintan. It causes moderate to severe infections in sheep and goats (Wang et al. 2020; A. H. Liu et al. 2007; Niu et al. 2009). This study reports the first molecular detection of *Babesia* sp. Lintan infections in livestock in Ghana. With no record of infections in cattle, there is a need to determine the epidemiology of *Babesia* sp. Lintan in cattle production. Symptoms of infections such as anemia, hemoglobinuria, fever and jaundice in sheep and goats could be similar to that in cattle since they are ruminants. The detection of *B. bigemina* (Bell-Sakyi et al. 2004) and *B. bovis* (Nagano et al. 2013), coupled with the identification of *Babesia* sp. Lintan suggests multiple *Babesia* species are in circulation within Ghana.

In animals, *Theileria* infections are either asymptomatic or severe, with fever, hemoglobinuria, anemia and death (Zhang et al., 2015). An animal can become infected for life, serving as a reservoir host for tick species (Glass 2001; Ahmed et al. 2008). Most cases of animal infections originate from the tropical and subtropical zones in Asia, Africa, Southern Europe and the Middle East (Sivakumar et al. 2014; Rjeibi et al. 2016; Belotindos et al. 2014; Hussain et al. 2014). In Africa, theileriosis is a significant tick-borne disease that affects domestic ruminants (Clift et al. 2020) with varying mortality and morbidity rates (Mans, Pienaar, and Latif 2015). Many African countries have reported the occurrence of Tropical theileriosis caused by *T. annulata* and East Coast fever (ECF) caused by *T. parva* (Moumouni et al., 2015).

Theileria mutans, *T. velifera* and *T. orientalis* have been reported in Africa (Moumouni et al., 2015; Perveen et al., 2021) to be less pathogenic (Kalume, Losson, and Saegerman 2011). In Ghana, *T. mutans* and *T. velifera* have been identified in domestic ruminants (Bell-Sakyi et al. 2004). This study reports the first molecular identification of *T. parva* and *T. orientalis* in sheep from Ghana. *Theileria parva* causes significant cattle deaths (Nene et al., 2016) and further hinders livestock production in numerous African countries (Mukhebi, Perry, and Kruska 1992; Kivaria 2006; McKeever 2009). *Theileria parva* is thought to kill over a million cattle annually, costing at least \$300,000,000 in lost revenue (Nene et al., 2016). Additionally, livestock production is hampered by sick animals' slower growth, lower productivity, and the high costs of disease control (McKeever 2009; Kivaria 2006). It is thought that *T. parva* evolved from resistant African Cape buffalo to finally infect cattle with East Coast fever (Norval, Perry, and Young 1992). Africa's cattle production has suffered greatly as a result of *T. parva* in nations including Zambia, Uganda, Zimbabwe, Kenya, Rwanda, Burundi, and Sudan (Mukhebi, Perry, and Kruska 1992).

Although *T. orientalis* infections are often mild (Aktas, Altay, and Dumanli 2006), reports link the pathogen to sporadic outbreaks that cause clinical signs and notable losses (Kamau et al. 2011; Perera et al. 2014). Mild infections produce symptoms such as anemia and hypoxia, while severe conditions result in weakness, pyrexia, and occasionally abortion (Lawrence et al. 2018; Swilks et al. 2017). Furthermore, hosts' compromised immune system and stress are associated with most clinical cases of *T. orientalis* infection (Watts, Playford, and Hickey 2016). From interactions with livestock owners in the Kassena-Nankana Districts, it was observed that Ivermectin is typically used to treat their cattle. According to a study, Ivermectin protects against *T. orientalis* infection in cattle (Park et al., 2019). The use of Ivermectin may explain why the studied cattle did not have any *T. orientalis* infections. However, infectious diseases are likely to be exchanged because of the frequent interaction between the animals in the Kassena-Nankana Districts.

In this study, *A. phagocytophilum*, *A. capra*, *A. ovis* and *A. marginale* were identified in the sampled livestock. This finding supports studies that indicate these *Anaplasma* pathogens infect animals (Niaz et al. 2021; Renneker et al. 2013). However, due to *A. marginale* and *A. ovis*, the livestock industry experiences huge financial losses on a global scale (Z. Liu et al. 2012; Kocan et al. 2003). While *A. ovis* is only moderately detrimental to goats and sheep (Seong et al. 2015), *A. marginale* can result in lethal acute anaplasmosis (Abdullah et al. 2020).

According to reports from Ghana (Futse et al. 2019), South Africa (Hove et al. 2018), Madagascar, Uganda (Byaruhanga et al. 2018; Muhanguzi et al. 2010), Thailand (Jirapattharasate et al. 2017) and China (Yang et al. 2017), *A. marginale* has been found in cattle. *Anaplasma marginale* has been detected in sheep (Yousefi et al. 2017) and goats (Barbosa et al. 2021; Da Silva et al. 2018), as seen in this study, despite having a substantial impact on cattle production globally (Kocan et al. 2010). Animals with infections produce less milk, have abortions, and face death (Kumar et al., 2015). There is a higher likelihood of disease transmission among the animals in the study areas because ruminants frequently interact with one another. Additionally, animals that recover from severe *A. marginale* infections function as reservoir hosts and spread the disease to attached ticks (Eriks, Stiller, and Palmer 1993; Kieser, Eriks, and Palmer 1990).

Cattle and sheep were discovered to be infected with *A. ovis* in this study. There have been reports of *A. ovis* infections in sheep and goats across Africa, Europe, Asia and North America (Yin and Luo 2007; Han et al. 2017). Finding *A. ovis* in sheep can be compared to studies in Sudan (Lee et al. 2018), Tunisia (Said et al. 2015), Senegal (Dahmani et al. 2019), West Iran (Mohammadian, Noaman, and Emami 2021), and Uganda (Kasozzi et al. 2021) which recorded a higher prevalence of infections. The variations in *A. ovis* prevalence may be caused by stress factors such as coinfection and a dry and hot climate (Renneker et al. 2013). Given the zoonotic nature of the disease as described in Iran, the aforementioned reports indicate that *A. ovis* has a major impact on the production of small ruminants and poses a risk to those who handle animals (Hosseini-Vasoukolaei et al. 2014). The experimental infection of various wild ruminants with *A. ovis* illustrates the pathogen's diverse host range (la Fuente et al., 2006; Zaugg, 1987, 1988; Zaugg et al., 1996). This, coupled with the continuous interactions amongst the ruminants in the Kassena-Nankana Districts, may be the cause of finding cattle with *A. ovis* infections.

This study reports the first detection of *A. phagocytophilum* in cattle from Ghana. A zoonotic pathogen with a broad host range, *A. phagocytophilum* can infect both domestic and wild animals and humans (Fuente et al., 2005; Dumler et al., 2001; Zhan et al., 2010). This pathogen causes infections that induce respiratory symptoms, fever, infertility, and decreased milk production in afflicted cattle (Noaman and Shayan 2009).

In addition, infections caused by *A. phagocytophilum* can be mild to severe, leading to multiple organ failures and death (Battilani et al., 2017; Li et al., 2015). Ticks

from Kenya (Mwamuye et al. 2017), Tunisia (Sarin et al. 2005), livestock in Uganda (Muhanguzi et al. 2010), and ticks and cattle in Ethiopia (Teshale et al. 2018; Teshale et al., 2015) have all been found to carry this zoonotic pathogen. Infection with *A. phagocytophilum* in cattle from this investigation was lower than that reported in Iran (Mohammadian, Noaman, and Emami 2021), Uganda (Muhanguzi et al. 2010), Ethiopia (Teshale et al., 2018), and China (Zhou et al. 2019). However, it was higher than a study from Tunisia that found a prevalence rate of 0.6% in cattle for *A. phagocytophilum* (M'Ghirbi et al., 2016). Even though several tick-borne pathogens cause diseases in Africa (Kocan, Blouin, and Barbet 2000), there is little evidence to support the distribution of *A. phagocytophilum*. This is most likely because no reliable diagnostic methods are available (Stuen, Granquist, and Silaghi 2013). People who work closely with animals or reside in rural areas with tick-friendly habitats are more likely to contract the disease (Thomas, Dumler, and Carlyon 2009).

Additionally, this study also reports the first identification of *A. capra* in cattle, sheep and goats in the Kassena-Nankana District. This zoonotic pathogen infects humans, ruminants, and wild animals (Amer et al., 2019; Jouglin et al., 2019; Li et al., 2015; Peng et al., 2018). After *A. capra* was first discovered in asymptomatic goats, a case of human infection was later reported from China in 2015 (Li et al., 2015). *Anaplasma capra* was more prevalent in this investigation than in studies from China (Peng et al. 2018) and Turkey (Altay, Erol, and Sahin 2022).

Infections with *A. capra* in sheep and goats can range from mild to severe, with symptoms such as fever, weight loss, decreased milk production, miscarriage and death (Said et al., 2018; Yasini et al., 2012). Numerous hard tick species can carry and spread *A. capra* to zoonotic and domestic hosts (Guo et al., 2019; Segura et al., 2020; Seo et al., 2018; Yang et al., 2016). This pathogen thus poses a serious risk to public health, necessitating the development of efficient preventative and control methods. *Anaplasma* prevalence may be impacted by husbandry practices, according to some observations (Fuente et al., 2005). Suitable husbandry methods and tick control measures will help stop the spread of the *Anaplasma* pathogens and lessen the risk to animal and human health.

Conclusion

This study demonstrates the use of DBS in the surveillance of tick-borne pathogens in livestock. Pathogens of zoonotic and veterinary importance were identified primarily in the abattoir. Among these pathogens were *Anaplasma capra* and *Anaplasma phagocytophilum* which

can cause infections in animals and humans. Poor tick prevention increases the risk of infections among the district's Abattoir workers and animal handlers. It is evident that the trade of livestock across borders has an influence on the distribution and spread of tick-borne pathogens. As such, there is a need for effective measures to control tick populations that will prevent the spread of tick-borne pathogens to humans and animals. Furthermore, there is a need to educate the abattoir workers on hygienic practices and the use of personal protective equipment to reduce the risk of infections.

Methods

Study area and ethical approval

The study was conducted in seven locations within the Kassena-Nankana districts of Ghana. Sampling sites included the abattoir, where livestock from within the community and beyond are slaughtered daily. Other locations were based on livestock availability and the animal owners' willingness to allow sample collections. Tick species including *Amblyomma variegatum*, *Rhipicephalus sanguineus*, *Hyalomma truncatum*, *Hyalomma rufipes*, *Rhipicephalus evertsi* and *Rhipicephalus (Boophilus) sp.* are prevalent in the Kassena-Nankana districts (Paintsil et al. 2022).

Before this study, ethical approval was obtained from the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC; UG-IACUC 001/19–20). After verbal consent from the livestock owners, the animals were restrained with the help of the owners, and samples were collected under the instruction of a local veterinarian.

Sample collection

A minimum of 248 livestock was required for this study using Epi Info V. 6. The sample size was calculated based on the following assumptions; a population size of 5000 livestock (local veterinarian estimate), a prevalence rate was 21.6% (Johnson et al. 2019), and a 95% confidence level with a 5% error margin. The breed of livestock was not taken into consideration in this study. The livestock included in this study were physically examined to be healthy by the attending Veterinarian before the blood sample collection.

At the abattoir, blood samples were collected from the slaughtered livestock and spotted onto labelled FTA Gene cards (GE Whatman, Maidstone, Kent, United Kingdom), air-dried overnight, and stored in sample bags containing silica gel. Within the communities, each animal was restrained, and the blood collection area (facial vein) was disinfected. Using animal lancets (Goldenrod™ Animal Lancet, Medipoint, NY, USA) blood was drawn

and spotted on FTA cards. The cards were subsequently dried overnight and stored in sample bags containing silica gel. All the samples were then transported to the laboratory and stored at -80°C pending analysis.

DNA extraction and molecular analysis

DNA was extracted from the livestock dried blood spots using Qiagen DNA Mini Kit (Qiagen Inc. Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was screened for *Coxiella burnetii* using an *IS1111* assay that targets the 295bp fragment of the transposase gene of *C. burnetii* *IS1111a* element (Klee et al. 2006) (see Additional file 1).

Again, *Rickettsia* DNA was detected in the livestock DBS using a quantitative PCR which targets the 115bp fragment of the 17kDa surface protein of *Rickettsia* species (Jiang et al., 2004). Samples that were *Rickettsia* positive were subsequently characterized using primers that target the rOmpA gene (ompA) of *Rickettsia* amplifying at 632bp (Jiang et al. 2005) (see Additional file 2). To identify *Babesia/Theileria* in the samples, a conventional PCR that amplifies the 150bp fragment of rRNA gene fragments (lsu5-lsu4) of the *Babesia* mitochondrial genome (Quorllo et al. 2017) as well as other apicomplexan pathogens was performed (see Additional file 3). Furthermore, *Ehrlichia* and *Anaplasma* DNA were detected in the samples using conventional PCR that amplifies the 345bp fragment of the *Ehrlichia* genus 16SrRNA gene (Nazari et al. 2013). The primers used in the PCR reaction were designed to amplify a wider spectrum of organisms in the *Ehrlichia* and *Anaplasma* genera (see Additional file 4). All positive PCR products were shipped to MacroGen Europe B.V. (Amsterdam, the Netherlands) for Sanger sequencing.

Phylogenetic analysis

The sequences obtained in this study were mapped to similar sequences in the NCBI database, including reference sequences. Sequences were further aligned using the Clustal Omega tool in MEGA X (Kumar et al. 2018). The relationships between the isolates were determined using phylogenetic trees created in MEGA X based on the Neighbour-joining method.

Statistical analysis

Chi-square analysis was used to determine the association between parasite/bacterial infection status with livestock characteristics. Logistic or multinomial regression was fitted to parasite/bacterial infection status with the animal characteristics. The statistical level was set at $p < 0.05$ and analysis was performed using STATA version 13.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s44149-022-00064-6>.

Additional file 1.

Additional file 2.

Additional file 3.

Additional file 4.

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Authors' disclaimer statement

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the US Government. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army.

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Authors' contributions

SOA and REB wrote the main manuscript. SOA, REB, KNY, JA, EB, PO, and SB conducted the laboratory analysis. EB analysed the data. SOA, VA, SM, JAL, PKB, MDW, JWD and SKD designed the study. JAL, PKB, MDW and SKD supervised this study. All authors reviewed and approved the final manuscript.

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Availability of data and materials

All the data supporting this study are included in the article.

Declarations

Ethics approval and consent to participate

Approval was obtained from the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC; UG-IACUC 001/19–20).

Competing interests

The authors declare no conflict of interest.

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