Molecular characterization of Rickettsia spp., Bartonella spp. and Anaplasma phagocytophilum in hard ticks collected from wild animals in Benin, West Africa

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Abstract

Ticks and tick-borne pathogens constitute a growing veterinary and public health concern around the world. Ticks are considered to be natural reservoirs for tick borne related pathogens and are equally responsible for the spread of infections in animals as well as in humans. In this study, the presence of *Rickettsia* and *Bartonella* species and *Anaplasma phagocytophilum* was investigated in hard ticks collected from reptiles, birds and wild mammalian animals. The samples collection of this study was carried out between December 2020 and September 2021. Adult ticks (male and female) were collected from wild animals in six districts of southern Benin. Molecular analysis was used to verify the presence of pathogens in all the samples (ticks) collected from wild animals. A total of 504 ticks were collected and grouped into 115 different tick pools. The PCR analysis detected 19 out of 115 tick pools positive for *Rickettsia* spp. and 9/115 tick pools positive for *Bartonella* spp., while *Anaplasma phagocytophilum* DNA was not detected in any tick. Several of the tick species collected from our studied reptiles/wild mammalian animals could be potential sources of zoonotic pathogens when subjected to further investigation. Therefore, stringent attention should be paid to tick infestation of reptiles/wild mammalian animals in order to put in place proper control and prevention measures for tick-borne diseases in the wild, which could serve as reservoirs in the infestation of domestic animals/humans in the event of any possible contact.

Introduction

Ticks collected from wild animals are excellent vectors for a variety of agents of emerging zoonotic pathogens (Mitchell et al., 2016) and are important vectors of pathogens of veterinary importance, thus representing a major veterinary and public health problem in the world. Among the tick-borne pathogens, *Rickettsia* spp., *Bartonella* spp. and *Anaplasma phagocytophilum* are commonly identified on ticks collected from wild animals (Ebani et al., 2015). Wild animals play a key role in the ecology of tick-borne diseases and therefore pose a threat to both domestic animals and humans' health (Ogden, 2013; Negi et al., 2021). *Rickettsia* spp. is an obligate intracellular Gram-negative bacterium, belonging to the spotted fever group. This bacterial pathogen has been detected in a variety of tick species belonging to the genus *Rhipicephalus, Ixodes, Dermacentor* and *Hyalomma* parasitizing a range of hosts including domestic and wild animals (Nieri-Bastos et al., 2014; Cicculli et al., 2019). Ticks play an important role in the ecology of most infectious diseases and are at the human, wildlife and domestic animal interface. Ticks in addition to the vectorial role they play, they also serve as reservoirs for *Rickettsia* spp. (Parola et al., 2013; Chisu et al., 2017). The DNA of *Rickettsia* spp. has been detected in the tissue samples of humans, domestic and wild animals (Parola et al., 2013; Chisu et al., 2017; Ribeiro et al., 2021). Bacterial pathogens transmitted by ticks has been found to cause significant diseases in humans and animals (Negi et al., 2021). The epidemiology of rickettsiae involves a wild and a domestic cycle concurrently, and both are interconnected by ticks and thus representing a global threat (Londoño et al., 2017). Bartonellosis is a zoonotic vector-borne disease associated with several *Bartonella* species and are considered potential pathogens for humans, wild and domestic animals (Breitschwerdt, 2014). The vectors confirmed for this pathogen are mainly sandflies, louse and flea (Mosbacher et al., 2011). However the DNA of *Bartonella* spp. has been identified in ticks (Reis et al., 2011) in particular in *Ixodes vespertilionis, Ixodes scapularis* and *Ixodes ricinus* collected in domestic and wild animals (Ebani et al., 2015; Leulmi et al., 2016). But it should be noted that the role of ticks in the transmission of *Bartonella* spp. is unknown (Telford III and Wormser, 2010). Bartonellosis was reported in domestic animals, especially cats, but also in wild animals (Breitschwerdt et al., 2010). *A. phagocytophilum*, responsible for the human granulocytic ehrlichiosis is characterized by acute febrile illness and can cause granulocytic anaplasmosis which is a life-threatening disease in humans (Jin et al. 2012) and is transmitted mainly by ticks which infect the wild and domestic animals (Matei et al., 2017). It is a pathogen of medical and veterinary importance with a wide distribution reported worldwide (Wang et al 2020). While considered as important causes of zoonotic diseases (Noaman, 2019), these organisms are not thoroughly studied in ticks in the Sub-Saharan Africa. Rickettsiosis, bartonellosis and human granulocytic anaplasmosis are vector-borne diseases that inflict severe infection on their hosts and have a wide geographical distribution (Mayne, 2015). However, the climate and biotopes of the West African region are favorable for the introduction and establishment of new tick species (Madder et al., 2012). Climate change is a potential factor that influences the distribution and adaptation of ticks and tick-borne diseases (Léger et al. 2013). As for the wild animal, it serves as suitable host in the life

cycle of tick in nature and plays the role of natural host reservoir of tick-borne pathogens (Dantas-Torres et al. 2012, Léger et al. 2013). While considered as important cause of emerging diseases in humans and animals (Mccoy et al., 2013), tick-borne pathogens are not thoroughly studied in West Africa. Therefore, the studies of zoonotic pathogens are important in determining the risks involved in human and animal diseases, and can serve as a guide during diagnosis and treatment of diseases. Rickettsiosis, bartonellosis and human granulocytic anaplasmosis are diseases associated with various symptoms such as febrile illness, fever, malaise, and headache (Faruque et al., 2017), which may be misdiagnosed or confused with malaria due to the poor availability of diagnostic tools leading to lack of thorough examination and subsequently lack of information on the manifestations of the diseases. Despite its public health importance, information on these zoonotic diseases is lacking in West Africa. Therefore this study aimed at identifying the presence of *Rickettsia* spp., *Bartonella* spp., *A. phagocytophilum* in hard ticks collected on reptiles and wild mammalian animals in southern Benin.

Materials And Methods

Collection of ticks

Between December 2020 and September 2021, adult ticks were collected in several areas of Benin from snakes and four-toed hedgehogs in captivity in the Zinvié natural park during the regular veterinary care activity, and from dead animals resulting from hunting in Ayou, Avakpa, Dèkanmè, Hinvi and Tori-Cada (Table 1). All the wild animals sampled were systematically examined to remove all ticks found on them during the regular routine visits, and the animals were handled humanely during the period of the sampling. After collection, ticks were preserved in 70% ethanol and transported to the University of Abomey-Calavi (UAC), Communicable Diseases Research Unit (URMAT) for storage prior to the commencement of the experiment. Subsequently, the ticks were then morphologically identified using a binocular stereo-microscope (Olympus® SZ51) with the key developed by (Hoogstraal and States, 1956; Walker, 2000, 2003).

Table 1
Number of sampled hosts and collected ticks in the different localities

Site of collection	Sampled host	Scientific name	N sampled hosts	N collected tick
Zinvié	Ball python	*Python regius*	4	17
	Black-necked spitting cobra	*Naja nigricollis*	2	29
	Rufous beaked snake	*Rhamphiophis oxyrhynchus*	3	12
	Forest cobra	*Naja melanoleuca*	2	39
	Jameson's mamba	*Dendroaspis jamesoni*	5	31
	Olive grass racer	*Psammophis phillipsii*	3	22
	Puff adder	*Bitis arietans*	2	16
	Four-toed hedgehog	*Atelerix albiventris*	6	5
Ayou	Grasscutters	*Thryonomys swinderianus*	7	71
	Gambian pouched rat	*Cricetomys gambianus*	3	9
	Double-spurred Francolins	*Pternistis bicalcaratus*	3	2
	Squirrel	*Xerus erythropus*	4	10
Avakpa	Grasscutters	*Thryonomys swinderianus*	9	63
	Gambian pouched rat	*Cricetomys gambianus*	3	5
	Double-spurred Francolins	*Pternistis bicalcaratus*	5	8
	Squirrel	*Xerus erythropus*	2	8
Dèkanmè	Grasscutters	*Thryonomys swinderianus*	5	71
	Gambian pouched rat	*Cricetomys gambianus*	2	8
	Squirrel	*Xerus erythropus*	2	4
Hinvi	Grasscutters	*Thryonomys swinderianus*	5	46
	Gambian pouched rat	*Cricetomys gambianus*	2	2
	Squirrel	*Xerus erythropus*	1	5
Tori-Cada	Grasscutters	*Thryonomys swinderianus*	3	17
	Gambian pouched rat	*Cricetomys gambianus*	2	4

DNA extraction from ticks

To proceed with the DNA extraction, the collected ticks were pooled into groups of 1–5 ticks per pool, keeping always separate the individuals collected from different host species, either belonging to different species and sex. For each pool, all ticks were individually submerged in distilled water for 10 min, removed from the distilled water and dried-up on sterile filter paper, then cut and crushed into smaller pieces with a sterile scalpel blade in a petri-dish to express the bacterial DNA to facilitate efficient extraction. Separate sterile instruments were used for the expression of DNA on each of the ticks. DNA was then individually extracted using the ISOLATE II Genomic DNA kit (Bioline Meridian Bioscience, Luckenwalde Germany), according to the manufacturer's instructions. DNA extraction and the quality assessment was carried out on agarose gel electrophoresis. The extracted DNA amplicons were analyzed for the presence of *Bartonella* spp. and *Rickettsia* spp. through

the use of a conventional PCR, thereby targeting the citrate synthase (gltA) gene, and for the presence of *A. phagocytophilum* through the nested PCR targeting the 16S rRNA gene of *A. phagocytophilum* (Table 2).

Molecular analyses

The reaction was made up of a final volume of 25 µl containing 12.5 µL µl of Green PCR Mastermix (Rovalab GmBH, Teltow, Germany), 1 µl of 25µM of each primer (Table 1), 9.5 µL of ultra-pure water and 1 µl of genomic DNA. A negative control using ultra-pure water and a DNA positive control was included in each of the PCR analyses. The amplification condition for *Rickettsia* spp. consisted of an initial denaturation step of 2 min at 95°C, 35 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 58°C, and extension of 30 s at 72°C, and a final extension of 2 min at 72°C. The PCR program for *Bartonella* spp. included an initial denaturation step of 5 min at 95°C, 35 cycles of denaturation for 30 s at 95°C, annealing of 30 s at 52°C, and extension of 30 s at 72°C, and a final extension of 5 min at 72°C. For the nested PCR targeting the 16S rRNA gene of *A. phagocytophilum*, a second reaction was performed in 25 µl reaction volume, containing 12.5µL Green PCR Mastermix (Rovalab GmBH, Teltow, Germany), 9.5 µl of ultra-pure water, 1 µl (10 pmol/µl) of each of the two primers (Table 1), and 1 µl aliquot of the first PCR reaction. Reaction conditions for first round were 40 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 1 min and extension at 72°C for 5 min. Then for the second round, the thermocycling condition included: 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min and a final extension at 72°C for 5 min. Negative and positive controls were included in each amplification assay. The PCR products were separated electrophoretically in 1.5% agarose gel under standard conditions. DNA bands were observed under UV302 light using a gel documentation system (Axygen).

Table 2
Primers used for detection of pathogens in ticks

Target species	Gene (~amplicon length)	Forward and reverse primers (5' – 3') (Reference)	References
Bartonella spp.	*glt*A (380–400 bp)	**bart781** : GGG GAC CAG CTC ATG GTG G **bart1137** : AAT GCA AAA AGA ACA GTA AAC A	(Norman et al., 1995)
Rickettsia spp.	*glt*A (381 bp)	**Rsfg877**: GGG GGC CTG CTC ACG GCG G **Rsfg1258**: ATT GCA AAA AGT ACA GTG AAC A	(Regnery et al., 1991)
A. phagocytophilum (nested PCR)	16S rRNA (945 bp)	**Ge3a**: CAC ATG CAA GTC GAA CGG ATT ATT C **Ge10r**: TTC CGT TAA GAA GGA TCT AAT CTC C	(Massung et al., 1998)
	16S rRNA (570 bp)	**Ge9f**: AAC GGA TTA TTC TTT ATA GCT TGC T **Ge2**: GGC AGT ATT AAA AGC AGC TCC AGG	(Massung et al., 1998)

Data analysis

The data collected during the survey were imputed in Excel spreadsheets (Microsoft Corp., Redmond, Washington, USA). The Mean intensity of each species of ticks in each host species was calculated according to Bush et al. (1997).

Results

Tick collection and host animals

Overall, 85 wild animals were examined for the ticks sampling, and a total of 504 ticks were collected, 161 males and 344 females. Overall six tick species, belonging to three genera, were identified, namely *Ixodes aulacodi, Rhipicephalus simpsoni, Rhipicephalus sanguineus, Hyalomma (marginatum) rufipes, Amblyomma variegatum* and *Amblyomma latum* (Table 3 and

Fig. 1). *A. latum* was mostly found in snakes, which were sampled in the natural park of Zinvié, with average intensity rates ranging from 4.0 in the Rufous beaked snake to 19.5 in the forest cobra. *I. aulacodi* and *A. variegatum* were found mostly in rodents, and the highest intensity was recorded for the *I. aulacodi* in Grasscutter (I = 7.2), but few individuals were retrieved also from the Double-spurred Francolins. *R. sanguineus* and *R. sampsoni* were infesting only small mammals, but their number was always very limited and generally below the average intensity of 2 ticks for each host. Finally, *H. (m) rufipes* was reported nearly exclusively in the Double-spurred Francolins, with a single sporadic finding in the squirrel (Table 3).

Detection of tick-borne pathogens

The 504 ticks were grouped in 115 tick pools and each pool was amplified by PCR, using specific primers to identify the genus of *Rickettsia* spp., *Bartonella* spp. and *A. phagocytophilum*. The PCR analyses indicated that, out of the 115 tick pools, 19 were positive for *Rickettsia* spp. and 9 for *Bartonella* spp. (Fig. 2, Fig. 3 and Table 3). *Rickettsia* spp was detected in *A. latum* collected on Ball python (4/4 pools; 100%), on Jameson's mamba (2/5; 40%), and on Olive grass racer (1/4; 20%), with positivity findings in both males and females tick pools. Ticks of *I. aulacodi* (Grasscutters) and *R. sanguineus* (Gambian pouched rat, Grasscutters) were also PCR-positive for *Rickettsia* spp. in both males and females ticks. The *Bartonella* gltA genes were detected in *A. latum* (Ball python, Jameson's mamba, Forest cobra, Olive grass racer) and *I. aulacodi* (Grasscutters) (Table 3). The primers Ge3a, Ge10r and Ge9f, Ge2 did not amplify any fragment when tested with the 115 tick pools and accordingly, no bands were produced on the agarose gel despite several attempt. Therefore, *A. phagocytophilum* DNA was not detected in ticks. The investigated pathogens were not detected in *R. simpsoni, A. variegatum* and *H. (m) rufipes* in the study areas.

Table 3 Number and average intensity of ticks collected from wild animals, division in males and females, number of tested pools and relative positivity rate for *Rickettsia* spp. and *Bartonella* spp.

Host species (N sampled individuals)	Tick species	N collected ticks			N tested pools	N pools pos to *Rickettsia*	Positivity rate (%)	N pools pos to *Bartonella*	Positivity rate (%)
		Total	Mean intensity	Males					
				Females					
Ball python (4)	*A. latum*	17	4.2	12	3	3	100	0	0
				5	1	1	100	1	100
Black-necked spitting cobra (2)	*A. latum*	29	14.5	8	2	0	0	0	0
				21	5	0	0	2	40
Rufous beaked snake (3)	*A. latum*	12	4.0	3	1	0	0	0	0
				9	2	0	0	0	0
Forest cobra (2)	*A. latum*	39	19.5	11	2	0	0	0	0
				28	6	0	0	1	17
Jameson's mamba (5)	*A. latum*	31	6.2	9	2	0	0	0	0
				22	5	2	40	0	0
Olive grass racer (3)	*A. latum*	22	7.3	5	1	0	0	1	100
				17	4	1	25	0	0
Puff adder (2)	*A. latum*	16	8.0	7	2	0	0	0	0
				9	2	0	0	0	0
Four-toed hedgehog (6)	*A. variegatum*	5	0.8	2	1	0	0	0	0
				3	1	0	0	0	0
Gambian pouched rat (12)	*I. aulacodi*	3	0.2	3	1	0	0	0	0
				0	0	0	0	0	0
	R. simpsoni	12	1.0	5	1	0	0	0	0
				7	2	0	0	0	0
	R. sanguineus	6	0.5	2	1	1	100	0	0
				4	1	0	0	0	0
	A. variegatum	7	0.6	4	1	0	0	0	0
				3	1	0	0	0	0
Grasscutters (29)	*I. aulacodi*	209	7.2	56	12	3	25	1	8
				153	32	7	22	3	9
	R. simpsoni	41	1.4	14	3	0	0	0	0
				27	4	0	0	0	0
	R. sanguineus	13	0.4	4	1	0	0	0	0
				9	2	1	50	0	0
Double-spurred	*A. variegatum*	2	0.2	0	0	0	0	0	0

Francolins (8)				2	1	0	0	0	0
	H. (m) rufipes	8	1.0	8	2	0	0	0	0
				0	0	0	0	0	0
Squirrel (9)	*I. aulacodi*	7	0.7	0	0	0	0	0	0
				7	2	0	0	0	0
	R. simpsoni	12	1.3	3	1	0	0	0	0
				9	2	0	0	0	0
	R. sanguineus	3	0.3	0	0	0	0	0	0
				3	1	0	0	0	0
	A. variegatum	9	1.0	2	1	0	0	0	0
				7	2	0	0	0	0
	H. (m) rufipes	1	0.1	0	0	0	0	0	0
				1	1	0	0	0	0
	Total	504	-	504	115	19	16.5	9	7.8

Discussion

Among the 504 ticks collected, six species were identified, and their host preference was in line with the literature. *A. latum* was limited to reptile hosts, whereas other tick species were found mainly on mammals. *I. aulacodi*, *R. simpsoni* and *R. sanguineus* were infesting mostly the wild rodents, whereas *H. (m) rufipes* preferred the wild bird, and *A. variegatum* showed the highest adaptability, being found on most of the investigated host species, excluding reptiles.

I. aulacodi has been identified as the most abundant in our study and this could be related to the high number of grasscutters which are the most favorite host of this tick species in southern Benin. The grasscutter is found to be widely distributed in West Africa in the wild especially in Ghana, Ivory Coast, Burkina Faso, Togo, Benin, Nigeria (Andoh et al., 2015; Zouh Bi et al., 2015). In these study areas, the grasscutters are found in abundance in the Panicum plantations where the preferred fodder of the grasscutters is also found in abundance. Indeed, Ntiamoa-Baidu et al. (2005) reported that *I. aulacodi* and *R. simpsoni* are species that are very specific to grasscutters in different vegetation zones (savanna Saharan or rain-forest tropical zones) in West Africa. Zouh Bi et al. (2015) reported a high prevalence of *I. aulacodi* in grasscutters in southern Ivory-Coast. In Ghana, *I. aulacodi* have been predominance among ticks collected from wildlife (Adenyo et al., 2020). *I. aulacodi* and *R. simpsoni* are mainly collected on the grasscutters but other types of ticks such as *Amblyomma* spp., *Haemaphysalis* spp., *Hyalomma* spp. and *Rhipicephalus* spp. has also been found on the grasscutters (Ntiamoa-Baidu et al., 2004, 2005; Adenyo et al., 2020). In addition to grasscutters, *I. aulacodi* has been reported on other mammals as reported by Chiţimia-Dobler et al. (2016). On the other hand, some study carried out in Benin did not reveal the presence of *I. aulacodi* and *R. simpsoni* on grasscutters, but rather *R. sanguineus* has been reported in Benin in such studies (Adinci et al., 2018). Indeed, the presence of *R. sanguineus* could probably be due to the use of dogs which are the main host of *R. sanguineus* as hunting animals which could eventually serve as a source of the contamination of the wildlife overtime (Smith et al., 2011; Dantas-Torres and Otranto, 2015).

A. latum tick has been found on several snakes' species in the world, confirming the findings of our study, where *A. latum* was the only tick species found on all reptiles. Nowak (2010) and Turner et al. (2017) showed that snakes are primarily infested by *A. latum* unlike other tick species such as *R. sanguineus, R. microplus* and *A. variegatum*. However, Burridge, 2001 reported that *A. latum* is an African tick, which feeds mainly on snakes, but can also occasionally feed on lizards and other types of hosts including humans. Report on the presence of *A. latum* on reptiles and some wild mammalian animals has

been documented in Ghana, Madagascar, Togo as well as Florida, Argentina, Chile and USA (González-Acuña et al., 2005; Andoh et al., 2015). It was also reported on imported reptiles from Europe and Japon (Andoh et al., 2015; Mihalca, 2015). *A. latum* has a high potential to establish well beyond its natural habitats (Burridge and Simmons, 2003).

There is very limited information on tick species found in the other species of wild mammals investigated in our study, and also concerning the double-spurred francolin. Investigation on ecto-parasites infesting wildlife reported the presence of *H. aegyptium, Hyalomma* spp., *Haemaphysalis* spp., *H. aegyptium, I. ricinus* and *I. hexagonus* on the hedgehog (Kar et al., 2011). Khattak et al (2012) and Hosseini-Chegeni et al., (2019) has noted that birds are infested by *Hae. leachi, Argas hermanni, Alveonasus canestrinii, H. marginatum, Hae. leporispalustris, I. pacificus* and *A. maculatum.* Despite the parasitic specificity of certain tick species, ecological constraints and host availability could give rise to accidents during the search for the host/ blood meal (Laamari et al., 2012; Mccoy et al., 2013).

Concerning the molecular investigation, this is the first report on the presence of DNA from *Rickettsia* spp. and *Bartonella* spp. in ticks collected from wild animals in Benin. In general, tick and tick-borne diseases are some of the primary cause of morbidity and mortality in some domestic animals and this condition could constitute a brake on animal production and can also expose humans to infections from zoonotic related tick-borne pathogens (Dantas-Torres et al., 2012). Most tick-borne diseases in humans and domestic animals originated from the wildlife reservoirs (Stenos et al., 2003; Dantas-Torres et al., 2012). The number of cases of pathogens identified in ticks collected from wild animals are considered to be quite high looking at the recent global emergence of zoonotic diseases, great attention must be paid to vectors, hosts and communicable diseases (Halsey and Miller, 2018; Kulkarni et al., 2018). In recent decades, research on tick-borne diseases has been very active around the world, but little scientific work has been done on tick-borne pathogens in sub-Saharan Africa. Ticks were reported to be reservoirs of *Rickettsia* and *Bartonella* species and also play a role in the transmission of zoonotic related pathogens (Legendre and Macaluso, 2017; Saengsawang et al., 2021) and they can potential serve as vectors for animal and human diseases.

In our study three ticks species (*A. latum, I. aulacodi* and *R. sanguineus*) were found to be infected with *Rickettsia* spp. and two species (*A. latum* and *I. aulacodi*) with *Bartonella* spp., which is in line with previous studies that implicated these tick species as potential hosts for tick-borne diseases. Kenny et al. (2004) reported tick-borne pathogen on *A. latum* collected on *Bitis gabonica, Python regius* and *Rhamphiophis oxyrhynchus* transported from Ghana to UK. Pathogens identified on reptile ticks are thought to be capable of infecting humans, suggesting a precautionary approach to a potential public health risks (Václav et al., 2011). Despite the invasion of reptiles ticks into natural environments in Europe, it was reported that the risk that these parasitic arthropods cause by their invasion is reasonably low (Mihalca, 2015). On the other hand, in Africa the environmental, ecological and climatic conditions are suitable for the distribution and abundance of reptile suitable hosts, most especially in the wild. Thus, further studies to clarify the role and the vectorial competence of different tick species in the transmission of pathogenic agents are needed (Stenos et al., 2003; Nowak-Chmura, 2012). In our study area *A. latum* was the only tick found in reptiles, but it was found infected with both *Rickettsia* spp. and *Bartonella* spp., suggesting a potential vectorial capacity. A tick as *A. latum* usually thrives very much in areas of high humidity and shows little particular preference for habitat (Nowak-Chmura, 2012).

Concerning the ticks collected from mammals, the majority of the infected ones were *I. aulacodi* found on grasscutters, and this is an important finding, since there has been no previous report on the presence of tick-borne pathogens on the ticks of reptiles and grasscutters in Benin. The limited information available are retrievable from neighboring countries, since *A. phagocytophilum* and *Rickettsia* spp. were detected and reported in ticks attached to reptiles exported from Ghana (Nowak et al., 2010; Andoh et al., 2015). The widespread presence of *Rickettsia* spp. (10 positive/44 tested pools; 23%) and the more sporadic presence of *Bartonella* spp. (4/44; 9%) in grasscutter *I. aulacodi* ticks should alert public health managers, since these tick-borne pathogens could pose a major threat to people whose livelihoods depend on animal production and in particular grasscutter farms, which have been set up to meet the high demand for its meat in the local market (Adenyo et al., 2020). However, studies must be carried out to identify the species of these pathogens incriminated.

In conclusion, this study has confirmed the molecular evidence of the presence of *Rickettsia* and *Bartonella* species in some common tick species parasitizing the wildlife in Benin, West Africa, where very little information exists on these tick-borne diseases. However, ticks play an important role as vectors in the transmission of *Rickettsia* and *Bartonella*. Our findings therefore could be of substantial help for physicians and veterinarians during the diagnosis of tick related ailment. The role of ticks in the maintenance and distribution of pathogens should furthermore not be underestimated, especially in wild animals. This will allow adequate measures to be taken for the surveillance and control of zoonotic pathogens.

Declarations

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Data availability The data are shown in this manuscript.

Code availability Not applicable.

Ethics approval No approval of research ethics committees was required to accomplish the goals of this study because it was conducted with routine data from farms.

Consent to participate Not applicable.

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Conflict of interest The authors declare no competing interests.

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Figures

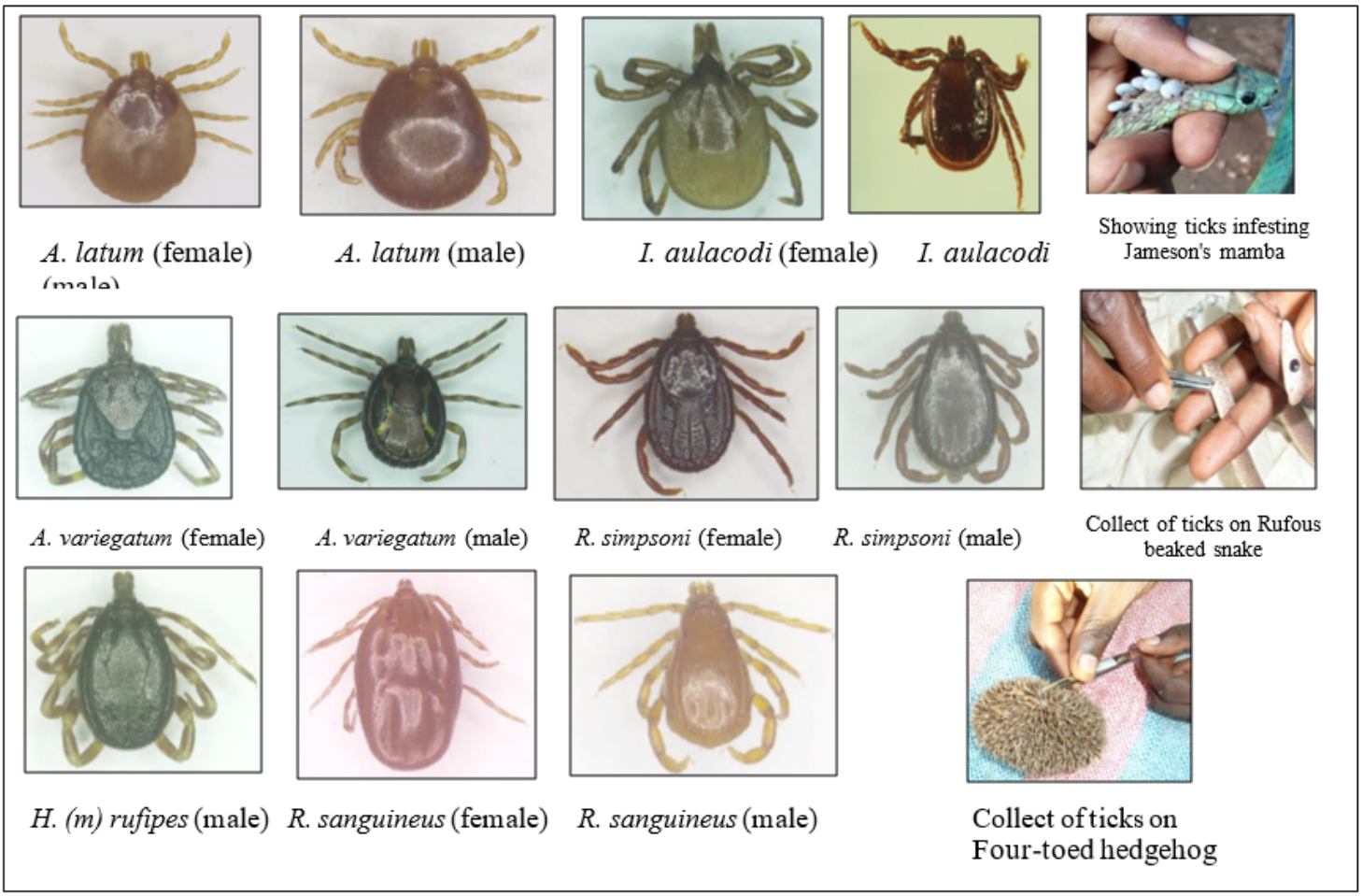
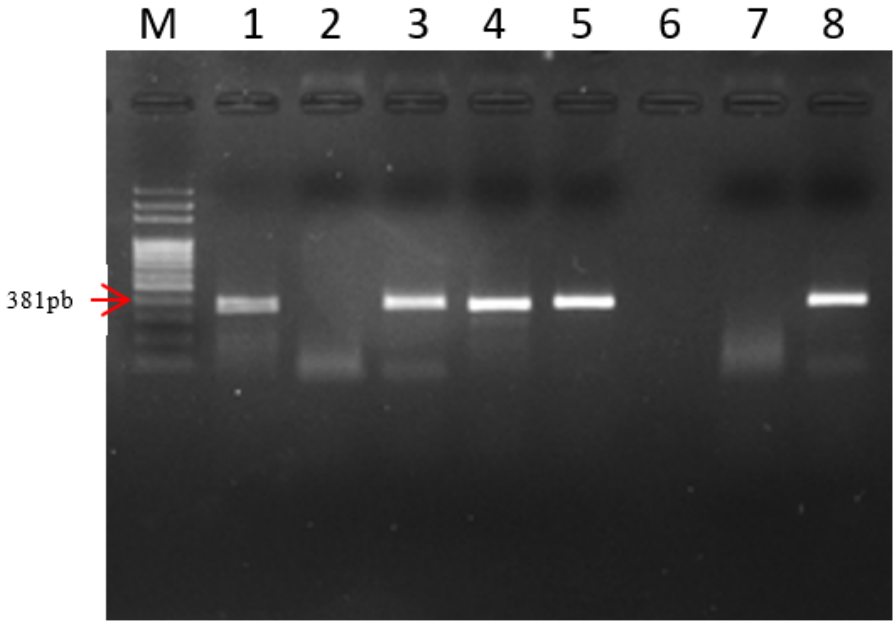


Figure 1

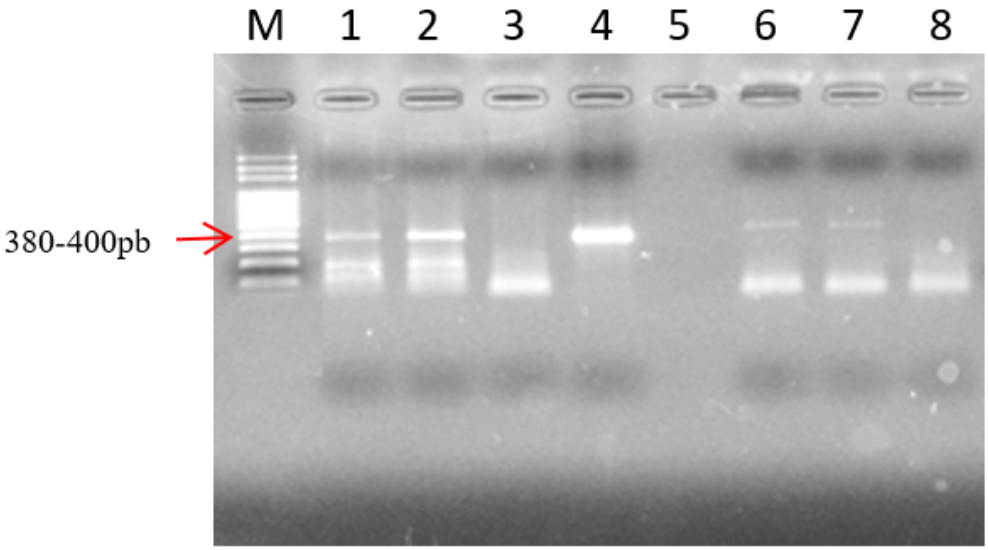
Tick species and infested hosts



Legend: M = Molecular Marker, 5= Positive Control (+), 6 = Negative Control (-), 1, 3, 4, 8= positive and 2, 7=Negative for *Rickettsia spp.*

Figure 2

PCR-gltA product of *Rickettsia spp.* on Agarose gel



Legend: M = Molecular Marker, 4= Positive Control (+), 5 = Negative Control (-), 1, 2, 6, 7 = positive and 3, 8=Negative for *Bartonella spp.*

Figure 3

PCR-gltA product of *Bartonella spp.* on Agarose gel