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Ruminants

Molecular Detection of *Ehrlichia ruminantium* in Cattle From Different Agro-Ecological Zones of Cameroon: Implication for the Understanding of the Heartwater Epidemiology

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Abstract

Although *Amblyomma variegatum* and *Ehrlichia ruminantium* infections have been reported in cattle from some agro-ecological zones (AEZs) of Cameroon, the transmission patterns of this bacterium seem to vary according to endemic areas and its prevalence as well as that of Heartwater remains not well understood in most sub-Saharan African countries. This study was designed to detect *E. ruminantium* infections in cattle of four AEZs of Cameroon and to identify areas presenting enzootic stability and those with potentially high risk for Heartwater. Blood samples were collected from cattle in four AEZs of Cameroon. DNA was extracted from blood and semi-nested PCR targeting the *16S rRNA* gene of *E. ruminantium* was used to search for this bacterium. From 569 cattle analysed, an *E. ruminantium* DNA fragment was detected in 197 of them, giving an overall prevalence of 34.6%. The highest prevalence of *E. ruminantium* of 48.0% was recorded in cattle from AEZ IV and the lowest (26.0%) in those from AEZ III. Among the AEZs, significant differences ($X^2 = 14.85$, $p = 0.002$) were recorded in terms of the prevalence of *E. ruminantium* infections. Villages of the westerly areas are at higher risk for *E. ruminantium* infections. This study revealed a high prevalence and a wide distribution of *E. ruminantium* infections in AEZs of Cameroon. It enabled the identification of areas showing an enzootic stability for *E. ruminantium* transmission as well as those where the transmission of this bacterium is low and where livestock are at higher risk of developing Heartwater.

1 | Introduction

Heartwater, also called cowdriosis, is a tick-borne disease affecting domestic and wild ruminants. Endemic in tropical and subtropical areas, this infectious disease constitutes a serious threat for livestock breeding and appears as the second most important tick-borne disease for livestock in Africa after East Coast fever (Allsopp 2015). It is caused by *Ehrlichia ruminantium*, which is an intracellular rickettsia transmitted by ticks of the genus *Amblyomma* (Esemu, Ndip, and Ndip 2011; Biguezoton et al. 2016). In all vector tick populations, including adult ticks, the overall infection rates of *E. ruminatium* vary considerably between 11.2% and 40.9% (Allsopp 2015). In areas where *Amblyomma variegatum* is endemic, the infection rates of this bacterium

are always fairly high and can overpass 20% in adult ticks (Allsopp 2015). This variability could lead to some differences in the transmission patterns of *E. ruminatium* and Heartwater in various epidemiological settings. In areas where the transmission of *E. ruminatium* is high, most animals at the early stage of their lives are infested by *A. variegatum*, including those carrying *E. ruminatium* infections. In addition to these early contacts, these animals are regularly exposed to infected *A. variegatum*. Through these early and regular expositions to infected ticks, most animals acquire some degree of immunity against *E. ruminantium* infections. In such context, the important circulation of *E. ruminantium* infections could induce the phenomenon of an enzootic stability of the transmission of this bacterium. This phenomenon occurs when there is a high circulation of a pathogen in such a way that the animals become quickly infected not only after birth but also regularly throughout their lives. Such regular infections maintain their immunity and induce a very low mortality because these pathogens have become less virulent to their hosts. In other settings where the transmission is low, the *E. ruminantium* infections could lead to Heartwater.

In endemic countries, about 150 million animals are at risk of Heartwater, and the mortality rate related to this disease varies from 15% to 82% in adult cattle and also between cattle breeds, agro-ecological zones (AEZs), socioeconomic conditions, the cattle production systems and the level of cattle exposition to ticks and tick-borne pathogens (Jonsson 2006; Swai et al. 2008). Understanding the circulation of *E. ruminantium* infections and the enzootic stability of its transmission, as well as areas where Heartwater may have impacts on animal health, requires generating data on *E. ruminantium* infections in cattle and tick vectors.

Although the monitoring of herds infested by *A. variegatum* and searching for *E. ruminatium* infections in the cortex of dead animals appears as the best approach to generate epidemiological data on Heartwater, such an approach is difficult to implement in rural areas. Moreover, the diagnosis of Heartwater is difficult to perform in live animals because the typical lesions and clinical signs characterising this infectious disease are lacking (Allsopp et al. 1999). As the techniques commonly used to diagnose *E. ruminantium* infections in animals, including indirect immunofluorescence, the isolation and culture of bacteria, are not sensitive enough, Heartwater has been most often misdiagnosed in several endemic areas (Peter et al. 2019). Nevertheless, identifying *E. ruminantium* infections either in animals and/or tick vectors could provide indications on the presence and the circulation of this bacterium. With the development of highly sensitive and specific molecular tools detecting *E. ruminantium* infections in mammals and tick vectors, the identification of this bacterium has been improved (Peter et al. 1995; Bekker et al. 2002; Steyn et al. 2003; Gediyon and Teshale 2014). These reliable tools have yielded considerable data that enabled us to understand the transmission of this bacterium (Gediyon and Teshale 2014; Simuunza et al. 2011).

Previous studies on Heartwater in Cameroon reported *E. ruminantium* infections in livestock of many subdivisions in the northern regions (Awa 1997; Abanda et al. 2019). As these studies were performed in the Sudano-Sahelian zone characterised by dry savannah and steppes, the results of such studies cannot be extrapolated to other regions of Cameroon due to the diversity of the bio-climatic environment within and between AEZs. Considered as Africa in miniature with five AEZs, the bio-climatic diversity observed in these AEZs affects not only the abundance and the distribution of tick species but also the transmission and the prevalence of tick-borne pathogens (Jongejan and Uilenberg 1994).

Amblyomma variegatum, the main tick vector of *E. ruminantium* in Sub-Saharan Africa, has been reported as one of the most abundant tick species in all AEZs of Cameroon (Silatsa et al. 2019). Its presence indicates a possible transmission of *E. ruminantium* infections in these AEZs. However, the significant differences reported in the abundance of *A. variegatum* (varying from 34.8% to 96.5%) in different AEZs suggest some variations in the prevalence and the transmission of pathogens transmitted by this tick species (Silatsa et al. 2019). Confirming this hypothesis requires investigations aiming to search for *E. ruminantium* infections in livestock and/or ticks from different AEZs of Cameroon. Such investigations will give a general overview of the transmission of *E. ruminantium* and also to localise areas presenting an enzootic stability of this bacterial infection as well as areas where such infections could induce Heartwater.

This study was designed to detect *E. ruminantium* infections in cattle of four AEZs of Cameroon and identify areas showing enzootic stability of *E. ruminantium* transmission as well as those presenting a high risk for Heartwater.

2 | Methodology

2.1 | Study Site

This cross-sectional study was performed from April to August 2016. The sampling was performed during the wet season in 39 sites of 17 subdivisions of four AEZs of Cameroon including AEZs II, III, IV and V (Figure 1).

AEZ II, also known as the High Guinea Savannah zone, is characterised by a savannah and degraded forest. It is located at 500–1500 m above sea level. In this AEZ, the wet season of 7 months extends from April to October and the annual precipitations range from 1500 to 1800 mm with an annual average temperature of 22.1°C. Its bio-climatic conditions are favourable for cattle rearing, explaining why this AEZ represents the main cattle production area in Cameroon with a population of about 1.25 million heads of cattle (Motta et al. 2017). This AEZ is the main destination of transhumant herders from neighbouring countries (Motta et al. 2018). In this AEZ, the sampling was performed in April at the beginning of the wet season, where adult ticks infest cattle with a high probability of transmitting tick-borne pathogens.

AEZ III, also known as the Western Highlands zone, is located in the mid- and high-altitude zone of Cameroon. Its vegetation is characterised by a savannah and degraded forest. It has a wet season of 8 months extending from March to October with an annual average temperature of 20.6°C. Its annual precipitations range from 1300 to 3000 mm and its bio-climatic conditions are favourable for cattle rearing. This zone is among the most

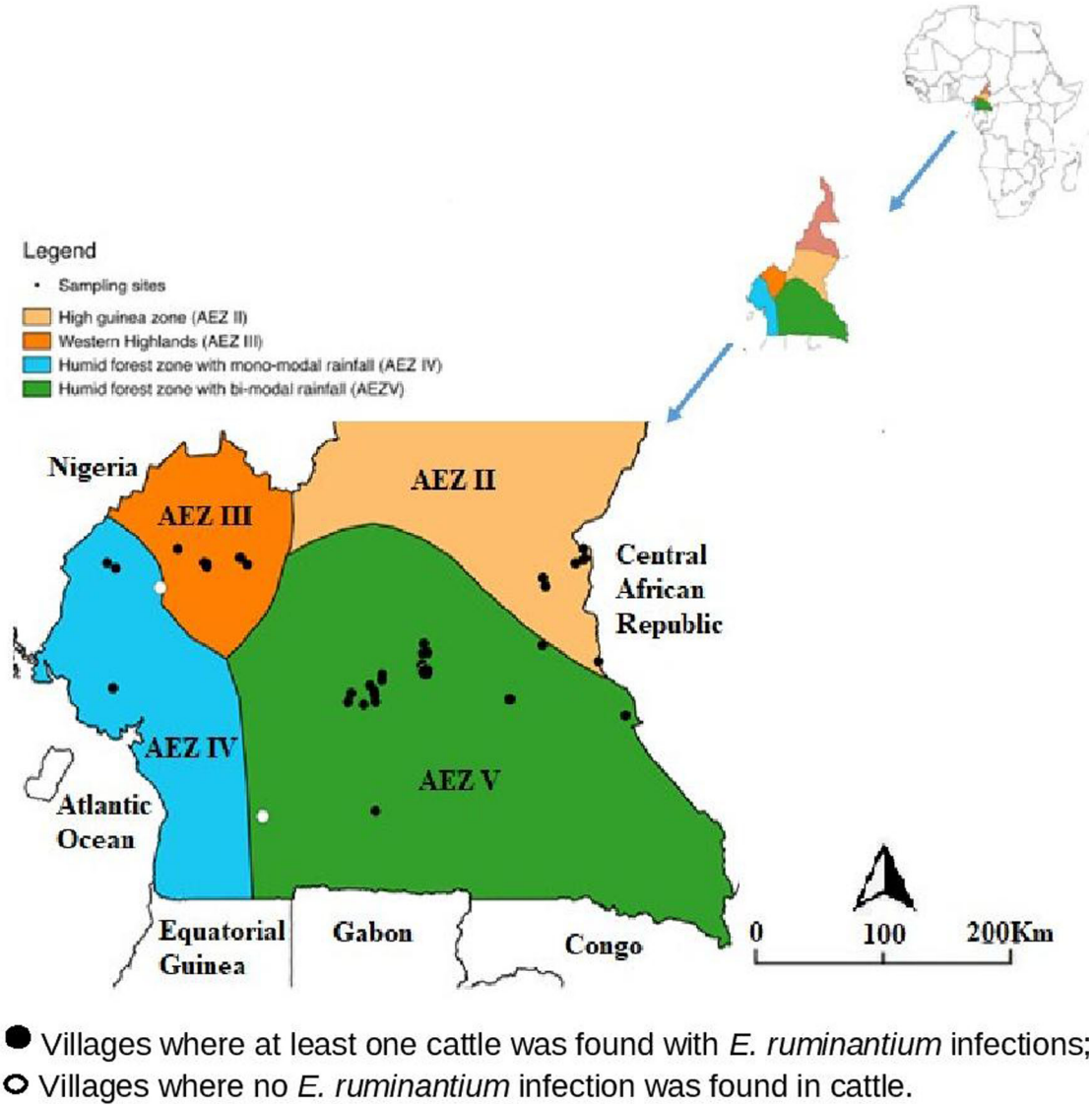

FIGURE 1 | Map showing AEZs and sampling sites of the Southern Cameroon where *Ehrlichia ruminantium* infections were detected in cattle. ● Villages where at least one cattle was found with *E. ruminantium*. ○ Villages where no *E. ruminantium* infection was found in cattle.

important cattle production areas of Cameroon, with a population estimated at 610,000 heads of cattle (Motta et al. 2017). In this AEZ, blood samples were collected at the end of April and the beginning of May. This sampling period corresponds to the beginning of the wet season.

AEZ IV, also known as the humid forest zone, has an evergreen forest. It is located in the west of the South Cameroon plateau, with an altitude varying between 0 and 2500 m above sea level. This AEZ has a wet season of about 8 months (from March to October) and its annual rainfall ranges from 3000 to 4000 mm with a temperature varying between 23°C and 28°C. Its cattle population was estimated at 1472 heads. This AEZ is less favourable for cattle rearing. In this AEZ, the sampling was performed between June and July.

AEZ V, also known as the humid forest zone with bimodal rainfall, has an altitude varying from 400 to 1000 m above sea level. This zone is characterised by a humid forest and a mosaic savannah. It has a wet season of 8 months extending from March to October and its average temperature is at around 26°C with an annual average rainfall of 2457 mm. The cattle population of this AEZ was estimated to 276,855 heads. Many farmers of this AEZ are refugees from conflict zones of Central African Republic. In this AEZ, the sampling was performed in August.

In the four AEZs, the majority of inhabitants were traditional smallholder farmers practicing small-scale animal husbandry. Cattle were generally reared together with sheep and goats. In most AEZs, the grazing system is essentially free grazing and, to a lesser extent, a combination between free grazing and stall-feeding. Few ranches were sampled in each AEZ and in areas where farms were not accessible, cattle were sampled in markets and in slaughterhouses. Except for a minority of farms where a combination of stall feeding and free grazing is practiced, most cattle were reared under an open grazing system. Moreover, *A. variegatum*, which is the main tick vector of *E. ruminantium*, has been reported in cattle of all four AEZs with significant differences in its abundance (Silatsa et al. 2019). Cattle of AEZ IV had the lowest *A. variegatum* burden of 34.8% compared to 96.5%

in cattle of AEZ II, 43.1% in those of AEZ III and 36.3% in those of AEZ V (Silatsa et al. 2019).

2.2 | Collection of Blood Samples From Cattle

Blood samples were collected from April to August 2016 in cattle of local breeds (*Bos indicus*) and also in a few exotic cattle breeds (*Bos taurus*). In each AEZ, the number of sampling sites was determined by the livestock density, the willingness of farmers to participate in the study, and to some extent, the security prevailing in each site. From each animal, 5 mL of blood was collected from jugular vein in EDTA-coated tubes. The collected blood samples were kept in a cool box that was transferred to the laboratory where the samples were stored at 4°C for less than 1 week before DNA extraction. The age of each animal was estimated using the tooth method as described by Ron et al. (2003). Thereafter, the sex, the breed, the grazing system and the name of the subdivision as well as the AEZ were recorded for each animal in which a blood sample was collected. In addition to that, geographical coordinates of each sampling site were also recorded using a global position system device (eTrex, Garmin International, Olathe, KS, USA).

2.3 | DNA Extractions

Genomic DNA was extracted from cattle blood using the DNeasy Blood and Tissue Kit (Qiagen; Hilden, Germany) as recommended by the manufacturer. DNA extracts were aliquoted and stored at −20°C for molecular analyses.

2.4 | Molecular Detection of *E. ruminantium* Infections

This detection was performed using semi-nested PCR as described by Bekker et al. (2002). For this detection, three primers, including Ehr16SF (5′-GGT TTA ATT CGA TGC AAC GCG A-3′), Ehr16SR (5′-CGT ATT CAC CGT GGC ATG -3′) and Ehr16SNR (5′-GAG TGC CCA GCA TTA CCT GT-3′), were used to amplify, in two PCR rounds, DNA fragments of the *16S rRNA* gene of *E. ruminantium*. Ehr16SF primer was used as forward primer for the two PCR rounds, while Ehr16SR was used as external reversed primer for the first PCR round. Ehr16SNR was subsequently used as internal reversed primer for the second PCR. Ehr16SF and Ehr16SR are universal primers that amplify DNA fragments of 430 bp common to *Ehrlichia and Anaplasma*, while Ehr16SF and Ehr16SNR primers amplify a DNA fragment of about 201 bp which is specific to *E. ruminantium*.

The first PCR round was performed in a final volume of 20 µL containing 2 µL of PCR buffer (10 ×), 0.4 µL of dNTPs (10 mM), 2 µL of Ehr16SF primer (10 pmol), 2 µL of Ehr16SR primer, 9.5 µL of sterile water, 0.1 µL (0.5 unit) of Taq DNA polymerase and 4 µL of DNA extract. For this first PCR, amplification was performed as described by Bekker et al. (2002). The amplification program was made up of an initial denaturation step at 95°C for 5 min followed by 25 amplification cycles. Each of these cycles consisted of a denaturation step at 95°C for 30 s, an annealing step at 58°C for 30 s and an extension step at 72°C for 30 s. A final extension was performed at 72°C for 10 min.

For the second PCR, amplicons of the first PCR were diluted 100 times and 2 µL of each dilution was used as DNA template for the second PCR that was also performed in a final volume of 20 µL. The composition of the master mix was similar to that of the first PCR with some slight modifications. The volume of each primer was 0.5 µL, while that of the water was 14.5 µL. The amplification program of the second PCR was identical to that of the first PCR. For each PCR, positive and negative controls were used. In the negative control, nuclease-free water was used instead of a DNA template, while for the positive control, DNA samples previously confirmed to be *E. ruminantium* were used.

Amplicons of the second PCR were resolved by electrophoresis on 1.5% agarose gel that was stained with ethidium bromide. The stained gel was visualised under UV light and photographed using UVItec (Cambridge, UK). Samples for which a DNA fragment of about 201 bp was observed after amplification and electrophoretic separation were considered to carry *E. ruminantium* infections.

2.5 | Mapping of *E. ruminantium* Infections

During the collection of blood samples, the geographical coordinates of each sampling site were recorded using a Global Positioning System device (Garmin eTrex 20). Each location's coordinates were transferred into QGIS version 2.18 software and plotted on satellite maps. Spatial distribution of *E. ruminantium* infections as well as its prevalence was carried out using the gstat package in R. With the geographical coordinates of each subdivision, the disease prevalence was interpolated using inverse distance weighting (IDW), which predicts the prevalence at an unobserved point or unmeasured location based on the assumption that things that are close to one another are more alike than those that are farther apart. As the locations get farther away, the measured prevalence values will have little relationship to the value of the predicted location. IDW therefore assigns more weight to the observed values closer to the unobserved point, and the weight diminishes as the distance of the observed values increases (Bartier and Keller 1996).

2.6 | Data Analysis

The chi-square test was used to compare the prevalence of *E. ruminantium* infections between AEZs and subdivisions. All analyses were performed using the XLSTAT software version 2017. Significance was defined at p value < 0.05.

3 | Results

3.1 | Distribution of Cattle According to Sampling Areas

For this study, 569 cattle, including 151 (26.5%) males and 418 (76.5%) females, were sampled in 39 sites of four AEZs of Cameroon. These 39 sites were located in 18 subdivisions (Table 1).

TABLE 1 | Prevalence of *Ehrlichia ruminantium* infections according to agro-ecological zones and subdivisions.

				***E. ruminantium* infections**	
AEZs	**Regions**	**Subdivisions**	**NCA**	**Number of positive cattle**	**Prevalence (%)**
AEZ II	East	Garoua-Boulai	60	23	38.3
		Betare-Oya	66	23	34.9
		Total AEZ II	126	46	36.5
		X^2	0.04		
		p-value	0.82		
AEZ III	West	Galim	50	12	24.0
		Foumban	50	14	28.0
		Total West	100	26	26
	Northwest	Bamenda II	9	6	66.7
		Total AEZ III	109	32	29.4
		X^2	6.7		
		p-value	0.03		
AEZ IV	Southwest	Mamfe	10	7	70.0
		Fontem	10	0	0.0
		Kumba	30	17	56.7
		Total AEZ IV	50	24	48.0
		X^2	0.13		
		p-value	0.70		
AEZ V	Centre	Nkoteng	14	11	78.6
		Mbandjock	40	18	4.5
		Lembe Ezoum	46	21	45.7
		Minta	33	7	21.2
		Bibey	45	16	35.6
		Nanga	27	9	33.3
		Kentzou	25	3	12.0
		Kette	25	9	36.0
		Total Centre	255	94	36.7
	South	Ebolowa I^{er}	9	0	0.0
		Meyongmessala	20	1	5.0
		Total South	29	1	3.4
		Total AEZ V	284	95	33.5
		X^2	37.62		
		p-value	< 0.0001		
Total			**569**	**197**	**34.6**
***X*^{2}**			**5.66**		
p-value			**0.129**		

AEZ: agro-ecological zone; NCA: Number of cattle analysed.

The highest number of cattle (44.8%; 255/569) was sampled in the Center region (AEZ V) and the lowest number (1.6%; 9/569) in the Northwest region (AEZ III). Regarding the cattle distribution according to subdivisions, the highest number (11.6%; 66/569) was from Betare-Oya followed by Garoua-Boulai (10.5%; 60/569), all in AEZ II. The lowest number of cattle (1.6%; 9/569) was from Ebolowa I^{er} (AEZ V) and Bamenda II (AEZ III). Comparing the numbers of sampled cattle, a significant difference ($X^2 = 164.17$; $p < 0.0001$) was recorded between subdivisions. From 569 sampled cattle, 126 (22.1%), 119 (20.9%), 40 (7.0%) and 284 (50.0%) were

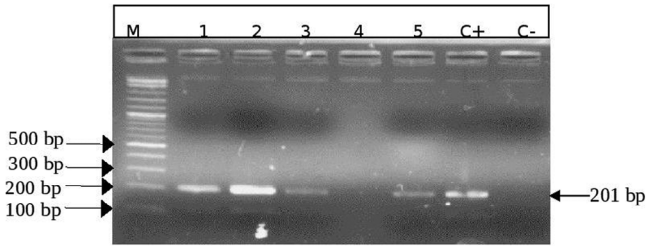

FIGURE 2 | Example of an agarose gel illustrating the electrophoretic resolution of amplified products of *16S rRNA* gene of *E. ruminantium*. Lane M: 100 kb ladder; lanes 1, 2, 3 and 5: samples carrying *E. ruminantium* infections or samples in which DNA fragments of 201pb were amplifying using primers targeting the *16S rRNA* gene; lanes 4 and 5: samples negative for *E. ruminatium* infections or samples in which primers targeting *16S rRNA* gene did not amply specific DNA fragment of *E. ruminantium*; lane C−: negative controls in which distilled water was used instead of DNA; lane C+: positive control in which purified *E. ruminantium* DNA was used as template.

from AEZs II, III, IV and V, respectively. A significant difference ($X^2 = 293.87$; $p < 0.0001$) was also recorded between AEZs. Out of 569 sampled cattle, 540 (94.9%) were local breeds, while 29 (5.1%) were exotic.

3.2 | Prevalence of *E. ruminantium* Infections

From the 569 blood samples collected in cattle, a specific DNA fragment of *E. ruminantium* (Figure 2) was detected in 197 of them, giving an overall prevalence of 34.6%. No *E. ruminantium* infection was detected in cattle from two sampling sites: one site in AEZ IV and the other one in AEZ V (Figure 1).

3.3 | Prevalence of *E. ruminantium* Infections According to AEZs and Subdivisions

In all four AEZs, at least one bovine was found with *E. ruminantium* infections (Table 1). The highest prevalence of *E. ruminantium* of 48.0% was recorded in cattle from AEZ IV, followed by those from AEZ II (36.51%). The lowest prevalence of 29.4% was recorded in cattle from AEZ III. Comparing the overall prevalence of *E. ruminantium* infections, a significant difference ($X^2 = 5.67$; $p = 0.13$) was recorded between the AEZs (Table 1).

The four AEZs in which cattle were sampled belong to eight regions of Cameroon: the West, the Northwest, the Southwest, the Centre, the South, the Littoral, the East and the Adamawa regions. Owing to the small size of nine cattle from the Northwest region, and the fact that no sample was collected in the Littoral and Adamawa regions, only data from the remaining five regions were considered for subsequent analyses (Table 1). The highest prevalence of *E. ruminantium* infections of 48.0% (24/50) was found in cattle from the Southwest region, followed by those from the Centre (36.7%; 94/255) and the East (36.5%; 46/126). The lowest prevalence of 3.4% (1/29) was recorded in cattle from the South region (Table 1). Comparing the prevalence of *E. ruminantium* infections, significant differences ($X^2 = 3.72$; $p < 0.0001$) were recorded between regions (Table 1). No *E. ruminantium* infection was detected in cattle from Ebolowa I[er] and Fontem, respectively, in AEZ V and AEZ IV (Table 1). The highest prevalence of *E. ruminantium* infections was recorded in cattle from Nkoteng and Mamfe, located respectively in the Centre (AEZ V) and the Southwest (AEZ IV) regions. The prevalence of *E. ruminantium* infections varies within and between AEZs (Table 1). Comparing these prevalences of *E. ruminantium* infections, significant differences were recorded between the subdivisions of AEZ III ($X^2 = 6.7$; $p = 0.03$) and AEZ V ($X^2 = 37.62$; $p < 0.0001$). However, no significant difference was recorded in the prevalence of *E. ruminantium* infections between the Subdivisions of AEZ II ($X^2 = 0.04$; $p = 0.82$) and those of AEZ IV ($X^2 = 0.13$; $p = 0.70$) (Table 1).

3.4 | Prevalence of *E. ruminantium* Infections According to Other Factors

The overall prevalence of *E. ruminantium* infections was 33.3% (139/418) in females and 38.4% (58/151) in males. Comparing these prevalences of *E. ruminantium* infections, no significant difference ($X^2 = 1.303$; $p = 0.254$) was recorded between males and females (Table 2).

The five samples from the Mamfe subdivision of AEZ IV were excluded because the grazing system was not clearly defined there. From the 564 remaining samples, 486 (86.2%) were from the opened grazing system and 78 (13.8%) from the combination of the opened and stalled feeding system (Table 2). The prevalence of *E. ruminantium* infections was 35.0% (170/486) and 30.8% (24/78), respectively, in cattle from the opened grazing system and those from the combination of an opened and stalled feeding system. Comparing these prevalences of *E. ruminantium* infections, no significant difference ($X^2 = 0.528$; $p = 0.467$) was recorded between the two grazing systems.

The prevalence of *E. ruminantium* infections was 36.4% (196/539) in cattle of local breed and 3.5% (1/29) in exotic ones. Comparing these prevalences of *E. ruminantium* infections, a significant difference ($X^2 = 13.161$; $p < 0.001$) was observed between cattle breeds (Table 2).

From the 569 cattle analysed in this study, 293 (51.5%) were 1–4 years, 224 (39.4%) were 5–8 years and 52 (9.1%) were at least 9 years. The highest prevalence of *E. ruminantium* infections of 40.6% was recorded in younger cattle and the lowest prevalence of 20.8% in old ones. Comparing these prevalences of *E. ruminantium* infections, significant differences ($X^2 = 11.499$; $p = 0.003$) were recorded between age groups (Table 2).

3.5 | Heat Map of *E. ruminantium* Infections in the Southern Cameroon

The four AEZs where cattle were sampled have shown a gradient risk for *E. ruminantium* infections that varies within and between AEZs (Figure 3). Some Subdivisions had a lower risk for *E. ruminantium* infections while, in others, this risk is higher (Figure 3). Subdivisions of the South, the Southwest and the East regions of Cameroon had a very little risk for *E. ruminantium* infections (yellow zones in Figure 3), while those of the red zones are at higher risk (Figure 3). Subdivisions of the westerly areas are

TABLE 2 | Prevalence of *E. ruminantium* infections in cattle according to sex, grazing system, breeds and age.

Factors		Number of cattle analysed	*E. ruminantium* infections		X^2	*p*-value
			Positive cattle	Prevalence (%)		
Sex	Female	418	139	33.3	1.303	0.254
	Male	151	58	38.4		
Total		569	197	34.6		
Grazing system	Open grazing	486	170	35.0	0.528	0.467
	Combination	78	24	30.8		
Total		564[a]	194[b]	34.4		
Breeds	Local	540	196	36.4	13.161	0.000
	Exotic	29	1	3.45		
Total		569	197	34.6		
Age groups (years)	1–4	293	119	40.6	11.499	0.003
	5–8	224	67	29.9		
	≥ 9	52	11	21.3		
Total		569	197	34.6		

X^2: Chi-square test.

[a]Five animals from the Mamfe Subdivision in AEZ IV were excluded because the grazing system was not clearly defined there.

[b]Three infected animals from the Mamfe Subdivision in AEZ IV were excluded because the grazing system was not clearly defined there.

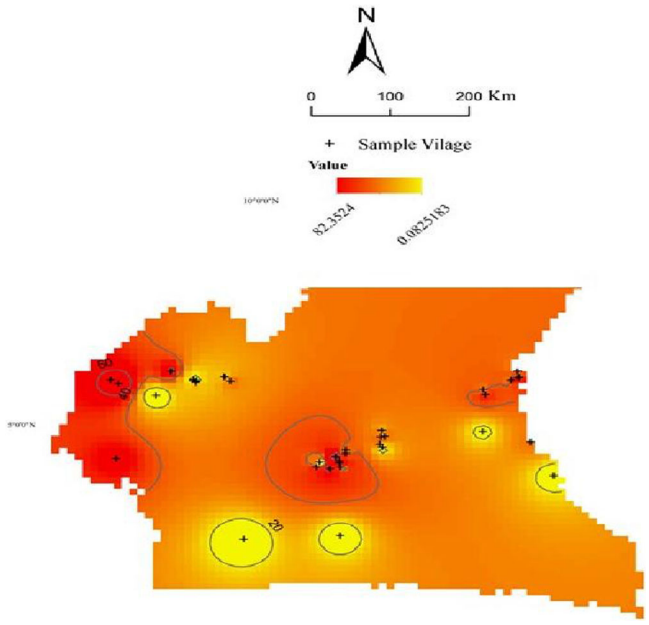

FIGURE 3 | Map showing areas showing a gradient risk for *E. ruminantium* infections; yellow: lower risk for *E. ruminantium* infections; red: high risk for *E. ruminantium* infections.

at higher risk of *E. ruminantium* infections (red zones) than those of easterly zones (yellow).

4 | Discussion

Despite the fact that *A. variegatum* has been reported as one of the main tick species in all the AEZs of Cameroon (Silatsa et al. 2019), data on *E. ruminantium* infections and Heartwater remain scarce in most AEZs. Our results revealing *E. ruminantium* infections in cattle from these AEZs are in agreement with previous ones reporting this bacterium in cattle as well as in *A. variegatum* from Cameroon (Awa 1997; Esemu, Ndip, and Ndip 2018; Abanda et al. 2019). The wide distribution of *E. ruminantium* infections in cattle from the four AEZs is also in agreement with data from Silatsa et al. (2019) reporting not only *A. variegatum* in these AEZs, but also with previous observations emphasising that from the knowledge on tick species distribution, it is possible to predict the potential distribution of tick-borne diseases (Awa 1997; Teklu et al. 2017)

The overall high prevalence of *E. rumninatium* infections of 34.6% recorded in cattle of this study is significantly higher than 6.6% and 0.7% reported respectively in the Southwest (AEZ IV) and the Northwest (AEZ III) regions of Cameroon (Esemu, Ndip, and Ndip 2018; Abanda et al. 2019). The discrepancies between these results could be explained by the technical approaches used to identify this bacterium. Although primers targeting the *pCS20* gene were used to detect *E. ruminantium* infections in previous studies, some polymorphisms in the sequence of this gene have been reported among isolates of *E. ruminantium* from different countries (Allsopp et al. 1998; Steyn et al. 2003). Moreover, the sequencing of the *pCS20* gene of *E. ruminantium* showed seven novel *E. ruminantium pCS20* variants that were grouped into two separate clusters with sequences from other parts of Africa and Asia (Byaruhanga et al. 2021). In addition to that, the *pCS20* probe that has been reported to be specific and the most sensitive for the detection of *E. ruminantium* infections revealed some polymorphisms in the amplified target located on the *pCS20* gene (Allsopp et al. 1999; Collins, Allsopp, and Allsopp 2002). It is likely that the primers targeting the *pCS20* gene may not amplify all *E. ruminantium* strains because over 37 different strains have been reported in previous studies (Allsopp et al. 1998). If these primers

were not designed from the conserved sequences of the *pCS20* gene of *E. ruminantium*, some strains could not be amplified. The use of these primers has probably led to an underestimation of the prevalence of *E. ruminantium* infections. In the present study, the primers targeting the *16S rRNA* gene of *E. ruminantium* enabled its detection in samples that could have been probably negative if the primers targeting the *pCS20* gene were used. The 16S rRNA primers have probably improved the detection of this bacterium. These results are strengthened by those emphasising that one probe from the 16S gene of *E. ruminantium* was able to detect five genotypes of this bacterium (Allsopp et al. 1998; Collins, Allsopp, and Allsopp 2002). Compared to previous studies reporting a low prevalence of *E. ruminantium* infections using primers targeting *pCS*20 gene, the high prevalence recorded with primers targeting *16S rRNA* gene suggests the circulation of different *E. ruminantium* strains. Some of these strains may have some genetic polymorphisms at the binding sites of *pCS20* primers. This hypothesis needs to be confirmed by isolating and genetically characterising *E. ruminantium* isolates from animals of various AEZs of Cameroon.

The significant differences observed in the prevalence of *E. ruminantium* infections according to AEZs and regions could be related to the variations of bio-climatic conditions within and between AEZs, the abundance of tick vectors in various sampling sites and the sampling period. These factors may affect the transmission of tick-borne pathogens (Mdladla, Dzomba, and Muchadeyi 2016). In previous studies, Silatsa et al. (2019) reported not only a high abundance (59.4%) of *A. variegatum* compared to other tick species in various AEZs of Cameroon, but also some significant differences in its abundance according to AEZs. The high prevalence of *E. ruminantium* infections in cattle from AEZ II is in agreement with the high abundance (96.5%) of *A. variegatum* infestations in cattle of this AEZ (Silatsa et al. 2019). However, in AEZ IV with the lowest abundance of *A. variegatum* infestations, *E. ruminantium* infections have been recorded with the highest prevalence. In addition to the fact that 50 cattle were sampled in this AEZ compared to 100, 126 and 284 sampled respectively in AEZs II, III and V, the discrepancies between these results could also be explained by some variations in the *E. ruminantium* infection rates in tick vectors as well as the sampling period. In AEZ II for instance, the sampling was performed at the beginning of the wet season, where adult ticks infest cattle and may highly transmit tick-borne pathogens. In AEZs IV and V that have bimodal rainfall compared to monomodal one observed in AEZs II and III, the models of tick infestations could be probably different. Such differences may influence the transmission of tick-borne pathogens and, therefore, their prevalence in infested animals.

Our results showing no significant difference in the prevalence of *E. ruminantium* infections between male and female cattle are in agreement with those of Esemu, Ndip, and Ndip (2018). However, they contrast with the results of Peter et al. (2019), reporting a high prevalence of *E. ruminantium* infections in female compared to male cattle. The discrepancies between these results could be explained by the number of males and females that were sampled in each study. In the present study, males and females were randomly sampled, while in other studies, sampling was performed in dairy farms. Such sampling has probably induced some biases in the interpretation of results because about 90.5% (268/296) of cattle from dairy farms were females (Peter et al. 2019). Moreover, our results report no significant difference in the prevalence of *E. ruminantium* infections according to grazing systems contrast with those of Peter et al. (2019) mentioning the effect of feeding system on the prevalence of such infections. In previous studies, the stall grazing system was reported to have a high risk for *E. ruminantium* infections compared to the free-opened grazing system. The transmission and prevalence of *E. ruminantium* infections seem to be influenced by the grazing systems as well as other factors prevailing in the endemic zones. Such factors may influence the distribution and the density of tick vectors and, subsequently, the transmission of tick-borne pathogens.

The high prevalence of *E. ruminantium* infections reported in cattle of local breeds compared to exotic ones could be explained by the sampling strategies, the sample size and the treatment allocated to cattle breeds in different sampling sites. In the present study, cattle of local breeds were abundant in most villages, while exotic ones were sampled only at Ebolowa in AEZ V. The low *E. ruminantium* infection rate recorded in cattle of exotic breeds could be explained by the treatments provided to these animals. Abundant at Ebolowa and belonging to rich people, exotic breeds were regularly followed up for tick infestations and tick-borne diseases. The use of acaricides to treat cattle during these follow-ups could explain the low prevalence of *E. ruminantium* infections in animals from Ebolowa. However, we cannot rule out the fact that exotic cattle may die as soon as they are infected because they are more susceptible and have never acquired immunity since acaricides have been regularly used on them to fight against tick infestations. In such context, such dead animals cannot be sampled. Cattle of local breeds were significantly more infected because they received less attention and were not regularly treated against tick infestations due to their lower susceptibility to Heartwater compared to exotic ones.

Results of the present study reporting no *E. ruminantium* infections in cattle from Fontem could be explained by the fact that most animals from this locality were old and have probably developed strong immunity against tick-borne diseases. This hypothesis is strengthened by results showing that the prevalence of *E. ruminantium* infections was significantly higher in young cattle compared to old ones. The high prevalence of *E. ruminantium* infections in younger cattle compared to old ones is in agreement with results obtained in Zambia (Simuunza et al. 2011). These results suggest that the young age of cattle can be considered as a risk factor for *E. ruminantium* infections in these epidemiological settings. They point out a large circulation of this bacterium and plaid in favour of the enzootic stability of *E. ruminantium* transmission in these AEZs. In such context, cattle reared in these AEZs have probability been immunised because they have been exposed to *E. ruminantium* infections at the early stage of their lives. In addition, being continuously and regularly exposed to *A. variegatum*, including those infected by *E. ruminantium*, these animals have developed strong immunity against this bacterium. This hypothesis is strengthened by results showing a low prevalence of *E. ruminantium* infections in older cattle compared to young ones. As some investigations reported no significant difference in the prevalence of *E. ruminantium* according to cattle age (Peter et al. 2019), the effect of age on the prevalence of *E. ruminantium* infections seems to vary according to epidemiological settings.

The heat map shows that the risk of having *E. ruminantium* infections varies according to regions or AEZs. This risk is very low in some regions, like the South, the Southwest and the East regions, while in other regions, like the westerly areas, this risk is high. As dead cattle linked to Heartwater have not been regularly reported in different sampling sites, areas showing high risk for *E. ruminantium* infections are likely those presenting an enzootic stability for these infections. In areas where the risk of contracting *E. ruminantium* infections is low, cattle from these areas have been less exposed to this bacterium. Such cattle are more likely to develop Heartwater because they have not been exposed to *E. ruminantium* infections and therefore have not acquired immunity against these infections.

The heat map enabled not only to establish a risk gradient for *E. ruminantium* infections and from which that of Heartwater can be inferred, but also to identify areas presenting an enzootic stability of the *E. ruminantium* transmission. Understanding the transmission of *E. ruminantium* as well as the prevalence of Heartwater requires considering the distribution and the density of tick vectors, the transmission of *E. ruminantium* and the enzootic stability phenomenon. In this phenomenon, a high prevalence or a large circulation of *E. ruminantium* infections should not be necessarily considered a problem because it leads to a rapid and early immunisation of animals so that they become less susceptible to this bacterium. If that occurs, the number of animals developing Heartwater will be reduced, as will its impact on animal health. As *A. variegatum* was reported in all AEZs (Silatsa et al. 2019), the possibility of having Heartwater cannot be ruled out in localities where *E. ruminantium* was not detected in cattle because these animals have not probably been exposed to this bacterium and hence did not develop immunity against *E. ruminantium*. If such cattle become infested with infected ticks, the losses resulting from Heartwater could be significant even among cattle of local breeds that are much less susceptible than other animal species like small ruminants.

5 | Conclusion

This study revealed a high prevalence of *E. ruminantium* infections in cattle from four AEZs of Cameroon. The wide distribution and the high prevalence of this bacterium could probably indicate an enzootic stability in the *E. ruminantium* transmission in the four AEZs of Cameroon. Mapping *E. ruminantium* infections enabled us to localise areas presenting this enzootic stability and those where the transmission of *E. ruminantium* is low. These areas of low transmission can be considered as high risk for Heartwater, and attention must be paid to the development of control measures that will help to improve cattle health and limit economic losses resulting to Heartwater.

Author Contributions

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Ethics Statement

This study was approved by the Ministry of Livestock, Fisheries and Animal Industries with the reference number 026/L/MINEPIA/MSEG. It was also approved by the review board of the Molecular Parasitology and Entomology Unit of the Department of Biochemistry of the Faculty of Science of the University of Dschang. Moreover, signed informed consent forms were obtained from farmers and herders before blood collection from their cattle.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated and/or analysed are included in this article.

Peer Review

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