Vol. 18(9), pp. 704-718, September, 2022
DOI: 10.5897/AJAR2022.15988
Article Number: CC045F469639
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Anaplasma phagocytophilum in cattle parasitism in Benin: An emerging pathogen transmitted by ticks

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Received 17 February, 2022; Accepted 17 August, 2022

***Anaplasma phagocytophilum*, a bacterium transmitted by ticks, is responsible for granulocytic anaplasmosis, an emerging zoonosis that has never been reported nor identified previously in Benin and in the West African sub-region. This study is designed not only to investigate the prevalence of the disease and evaluate mortalities recorded at Kpinnou farm after importing Girolando cattle from Brazil in 2014 but also to assess the prevalence of the emerging disease in Benin in the year 2014 period. A total number of 1427 ticks were collected, including 5 ml of blood from each one hundred cattle. Microscopic diagnosis reveals the presence of *Rhipicephalus* (*Boophilus*) spp., *Rhipicephalus microplus*, *Amblyomma variegatum*, *Rhipicephalus* spp and *Hyalomma* spp. Blood analysis results reveal the occurrence of 55% positive cases for *A. phagocytophilum*. Considering the effeiciency of the methods, 100% of *A. phagocytophilum* reported as positive by microscopy appear to be also positive with molecular analysis. PCR has greater sensitivity and specificity even with microscopy showing appreciable specificity. This study concludes that *Anaplasma phagocytophilum* is the primary suspect responsible for the massive deaths observed in cattle in the study area.**

Key words: *Anaplasma phagocytophilum*, ticks, zoonosis, cattle, Benin.

INTRODUCTION

Ticks transmit various pathogens (protozoa, bacteria and viruses), which cause a wide range of animal and human diseases. Vertebrate animals play an essential role in the life cycle of tick species, while humans are accessory

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hosts (Yessinou et al., 2022).

Bacteria of the Anaplasmataceae family are Gram-negative bacteria (Hauck et al., 2020) and are distributed worldwide (Ouedraogo et al., 2020). As a result of increasing climatic, environmental changes including an increase in world trade, more and more vector-borne diseases tend to be listed as emerging multi-host diseases (Biguezoton et al., 2016). These changes have resulted in metamorphosis and altered distribution of vector and host species (Yessinou et al., 2022).

Granulocytic anaplasmosis, caused by *A. phagocytophilum* is a vector-borne, non-contagious bacterial infection that infects domestic and wild animals and primarily targets neutrophil granulocytes (Schäfer and Kohn, 2020). The disease is transmitted by various vectors such as ticks (which are the main ones), biting insects (Stomoxys and Tabanidae). It is also transmitted biologically or even iatrogenic (needle, blood transfusion, etc.) and mechanically (Fourie et al., 2019).

Granulocytic anaplasmosis, also known as pasture fever, *A. phagocytophilum* ehrlichiosis or large bluegrass disease was first described in ruminants in 1932 by MacLeod in sheep. The latter demonstrated in Scottish sheep a feverish syndrome transmitted by ticks and caused by bacteria. The latter was then named *Rickettsia phagocytophila* then *Cytoecetes phagocytophila* and finally *Ehrlichia phagocytophila* until 2001 (Renard, 2018).

Ticks are blood-sucking arthropods that parasitize almost all vertebrates around the world and can occasionally bite humans. They transmit many disease agents some of which are common to humans and animals. Ticks and mosquitoes are the main vectors of diseases of humans and animals (Zannou et al., 2021). Ticks have been a major concern of medical and veterinary research for the past 150 years (Madder et al., 2014). This is because they have direct effects on their hosts, such as injury to their sites attachment, loss of blood by spoliation and paralysis caused by toxins present in their saliva. They are also efficient vectors of a wide variety of microorganisms (viruses, bacteria, protozoa and helminths).

Bovine anaplasmosis caused by *A. phagocytophilum* is largely unknown, underestimated and never diagnosed in Benin. It is also an emerging zoonosis whose ignorance in human medicine is comparable to that of the veterinary community.

The present study is carried out to study the prevalence of *A. phagocytophilum* in cattle reared on different farms in different regions of Benin. It attempts to identify the main possible risk factors or germs associated with the infection.

MATERIALS AND METHODS

Study areas

This study was carried out from July to November 2014 over the entire national territory of Benin in cattle farms. It aims to check for the presence of *A. phagocytophilum and* other possible hemoparasites in blood samples as well as for the presence of ticks on cattle body. Ticks were therefore taken from one hundred cattle distributed randomly in twenty herds located in five rural townships (Athiémé, Kétou, Tchaourou, Bassila and Gogounou). The selected townships are included in three agro-ecological areas of Benin (Areas II, V and VIII) giving a rate of three selected townships out of eight. They are recognized for holding large cattle herds with heavy tick infestation in livestock (Adehan et al., 2016). Kpinnou Livestock State Farm (FEK) and Okpara Livestock State Farm (FEO) were then included in the sample sites.

In each herd, five cattle aged four to five years visibly infested with ticks, including one male and four females were selected. The sex, presence of ticks and the breeding system practiced were noted. The geographic coordinates of the sites were also taken using a Garmin 3.6 brand GPS.

Benin is a country with three different climatic zones. The south is characterized by four seasons: A main rainy season (April to July), a main dry season (January to March), a shorter dry season (November to December) and a shorter rainy season (August to October). The northern part has two seasons: A rainy season from June to October and a dry season from November to May. It should be noted that during the dry season, a dry wind called harmattan blows from the interior of the continent towards the ocean. Concerning the vegetation, there is woody to grassy trend from south to north, corresponding to a variation from anthropic dry forest relic to anthropic savannah. Wildlife is diversified: there are small and big herbivorous and also carnivorous mammals which are all found in the two national parks. Due to transhumance, herds go through the country seasonally contributing to the microbial and parasitic spread.

Ticks collection and blood sampling

On the body of each selected animal, all the visible ticks were collected and stored in 100 ml tubes containing 70% alcohol. Blood was drawn from the jugular vein in Venoject tubes containing Ethylene-Diamine-Tetra-Acetic (EDTA) and blood smears were taken (Desquesnes, 2012). Next, identified Wattman No. 3 filter papers were soaked with whole blood from each animal and dried away from flies and other insects. Then, they were separated by No. 4 filter papers. These filter paper samples were taken from the four herds at Kpinnou Farm and then sent to the Prince Léopold Institute of Tropical Medicine (IMT), Antwerp, Belgium for molecular identification.

Ticks identification

After collection, ticks were sent to the communicable Diseases Research Unit (URMAT) and microscopically identified using the identification key (Walker, 2003). A first step enabled us to identify the ticks down to genus level using a ×60 magnification stereoscope. The second step was only focused on the ticks of the genus *Rhipicephalus (Boophilus)* to determine its different species under an electric microscope (Olympus) at ×100 magnification.

Hemoparasites identification

The smears produced were fixed with absolute methanol and then stained with a 10% May-Grunwald Giemsa (MGG) solution. They were then observed under an Olympus optical microscope at objective ×100 to identify the various present hemoparasites.

1. Trypanosomes were identified according to morphological criteria which indicate the size and position of the kinetoplast, the shape of

the posterior end, the presence or not of a free flagellum, the presence or not of an undulating membrane and the size (Desquesnes, 2012).

2. *Babesia bigemina* (Smith and Kilborne, 1893) is identified by its intraerythrocytic forms which generally appear as pears measuring 4 - 5 × 2 µm. We sometimes observe round shapes, 2 - 3 µm in diameter, and also irregular shapes. Pear shapes come in pairs (twin shapes) forming an acute angle to each other.

3. *Babesia bovis* (Schäfer and Kohn, 2020) is small in size. Intra-erythrocytic forms are pear-shaped, ring-shaped, or irregularly shaped. The vacuolated annular forms (signet-ring) are frequent. It measures 2.4 × 1.5 µm and has a central location. Twin forms an obtuse angle.

4. *Theileria mutans* (Bettencourt et al., 1907) is widespread in Africa. It causes benign theileriosis, "Turning sickness". The parasitological diagnosis is based on the demonstration of schizonts ("Koch's blue bodies") in lymph node punctures and of piroplasms in red blood cells. These piroplasms appear as multiform parasites represented by numerous bacillary elements, comma, and a few rare oval shapes with masses of purplish-red chromatin. Their dimensions vary between 1.25 and 1.50 µm long axis on 0.5 and 0.75 µm minor axis.

5. *Anaplasma phagocytophilum* (Theiler, 1910) is located inside leukocytes, mainly neutrophils and possibly monocytes. In these white blood cells, the bacteria can either be found as an elementary body isolated in the cytoplasm or as a cytoplasmic cluster of rickettsiae, also called a "morula". Because of the very low parasitaemia, it requires a long and detailed analysis of the smear under microscope.

Statistical analyses

The data were encoded in an Excel spreadsheet of WINDOWS version 2007. The statistical processing was mainly carried out in R 3.3.1 software (Team, 2016). The analyses take into account, among other things, the achievement of:

1. Simple correspondence factor analysis to describe associations between breeds or genetic types and agro-ecological zones (or livestock systems) at the sample level;
2. Simple correspondence factor analysis to describe associations between different parasites and agro-ecological zones (or farming systems) at the sample level;
3. Calculus of prevalence rate of the parasite using Bernouilli probability estimation approach
4. A modeling aiming to assess the explanatory power of the prevalence rates of *A. phagocytophilum* from the potential explanatory variables that are “agro-ecological zones (or breeding systems)”, “cattle breeds”, “sex”, “prevalence of *Babesia*”, “prevalence of *Theileria*”, and“ prevalence of *Trypanosoma* ”.
5. An analysis of proportion data according to generalized linear models was then carried out using the GLM function (binomial family), starting from the saturated model. The model was simplified using the step, update and ANOVA (for deviance analysis) functions of R. The model to be retained is the one whose estimated parameters are significant and has the lowest values of the AIC.

RESULTS

The relative importance of the numbers of different sexes in the sample

The distribution of the sexes in the sample is directly linked to the sampling method. The sampling sex ratio is1 male to 4 females. The analysis and interpretation of the influence and relative importance of the sex variable consider the link that resulted from the sex ratio. It was done to limit the biases likely to be generated by making inference on the cattle populations in the five sampled townships (Figure 1).

The overall mapping of the breeds in the sample

Five breeds of cattle or genetic types were identified in the sample: Borgou Girolando, Goudali, White Fulani breeds and the crossbreed genetic type considering the subjects from the crosses Girolando x Azawack, Gir x Azawack, Girolando x Borgou and Gir x Zebu peulh. The importance of each breed in the sample reveals heterogeneity of the distribution of the different sampled sites (Table 1).

The Athiémé and Tchaourou sites have the particularity of each presenting an exclusive breed (or genetic type), as they are state farms engaged in genetic improvement programs based on specific breeds.

From the simple correspondence factor analysis of the sample data, it appears that the first, second and third axes group together almost all of the information they contain. That is 41% of the information for the first axis, 41% also for the second axis and 17.81% for the third axis. The projection of the variables in the factorial plane confirms a particular association between the sampled sites and the cattle breeds (Figure 2).

Goudali and Borgou breeds are respectively associated with Bassila and Gogounou districts while the Girolando race and crossbreeds are respectively associated with Athiémé and Tchaourou districts.

Prevalence rates of hemoparasites in Benin

Overall mapping of parasite prevalence in sampled cattle

All the sampled sites show cattle infected by the four different parasites (Table 2). Simple correspondence factor analysis yielded 80.29%, respectively; 16.29 and 3.42% of the information for the first three factor axes. The projection of the variables in the factorial plane highlights a specific distribution of the parasites according to the agro-ecological zones (Figure 3).

Athiémé District appears to be a hotbed of *Anaplasma* while those of Gogounou and Kétou are to some extent associated with *Theileria* and *Trypanosoma.* Tchaourou is to some extent associated with *Babesia*. The agro-ecological sampled sites are therefore differently infected by the four genera of parasites: *Anaplasma*, *Theileria*, *Babesia* and *Trypanosoma*.

Prevalence of A. phagocytophilum

The prevalence rate of *A. phagocytophilum* observed in

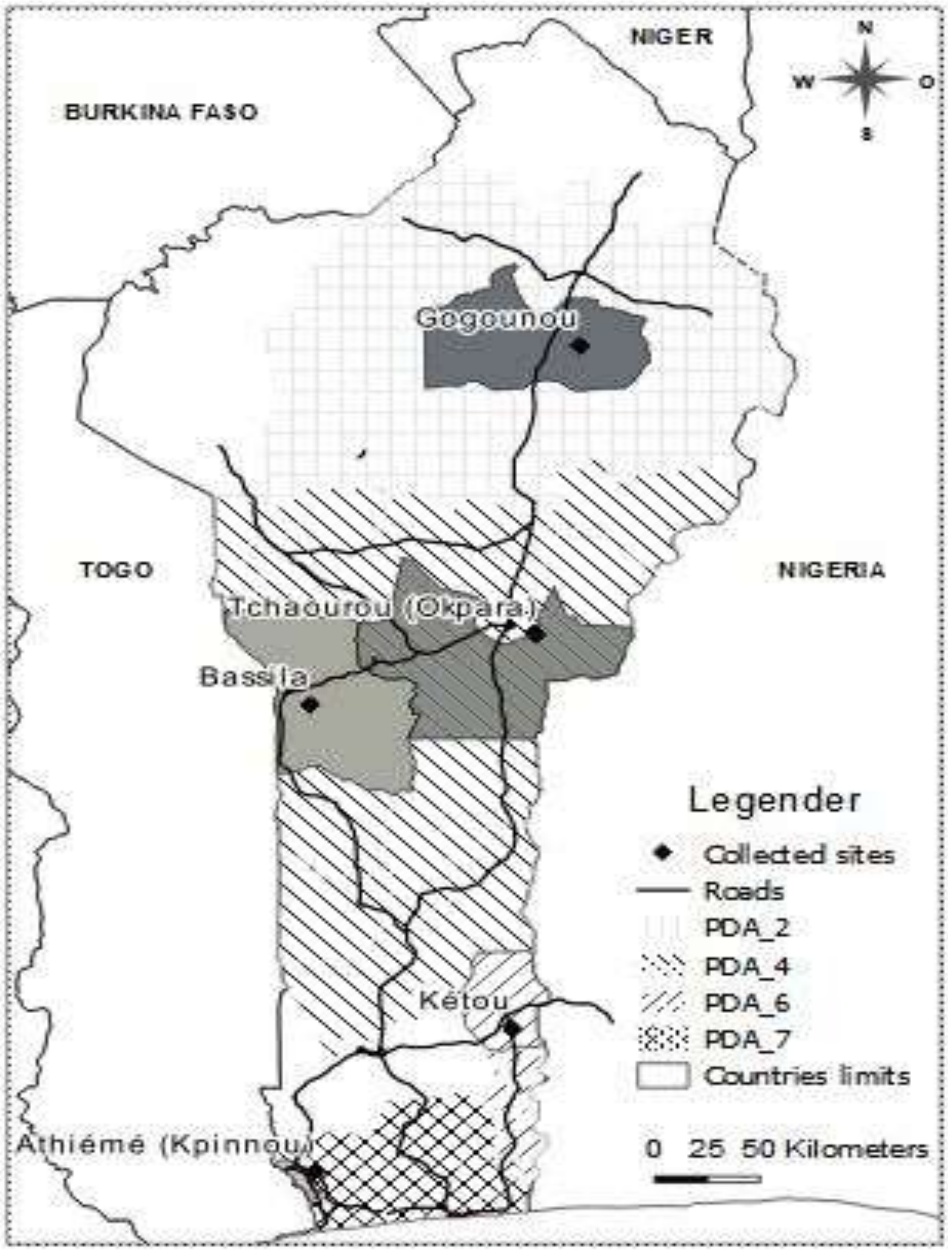

Figure 1. Map representing the study sites.
Source: Authors

Table 1. Number of cattle sampled by site according to their breed or genetic type.

Site	Race				
	Borgou	Girolando	Goudali	White Fulani	Métis
Athiémé	0	20	0	0	0
Bassila	0	0	6	14	0
Gogounou	15	0	0	05	0
Kétou	11	0	2	7	0
Tchaourou	0	0	0	0	20

Source: Authors' Computation.

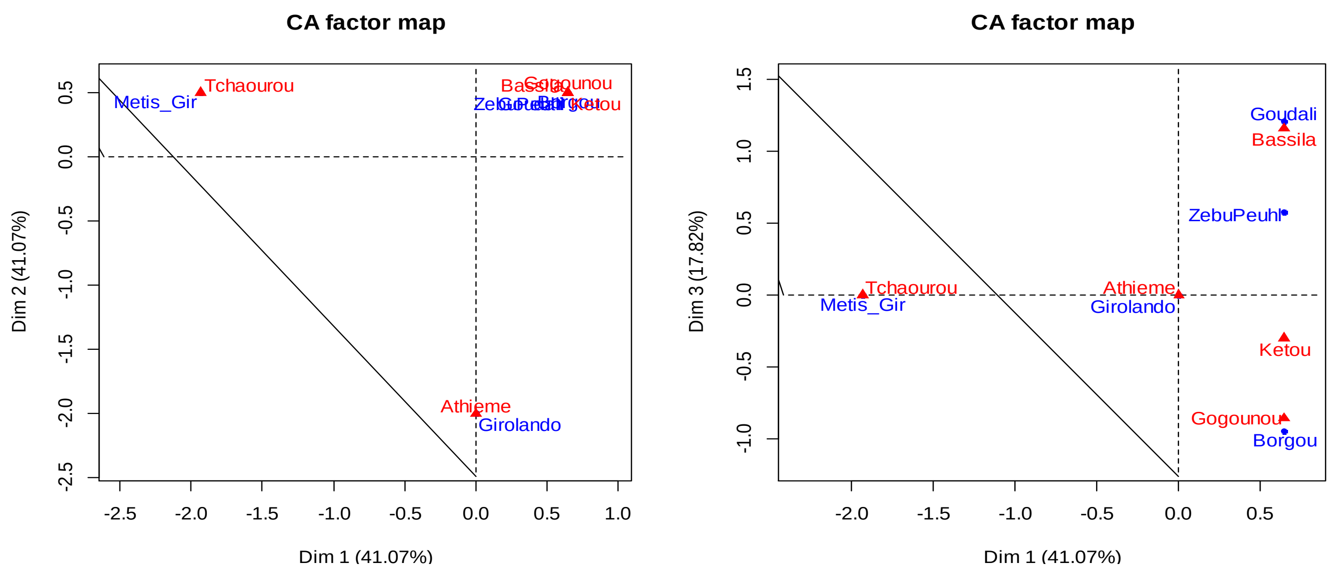

Figure 2. Projection of breeds or genetic types of cattle and agroecological areas in a factorial plane.
Source: Authors.

Table 2. Number of infested cattle in the different sites sampled (Number of heads for 20 cattle/site).

Parasite	Site				
	Athiémé	Bassila	Gogounou	Kétou	Tchaourou
Anaplasma	20	8	7	7	13
Babesia	15	8	8	10	13
Theileria	8	5	8	7	9
Trypanosoma	15	12	11	13	13

Source: Authors' Computation.

the entire sample is 55%. Nevertheless, variations in the prevalence rate seem to appear between the cattle sampled according to the site (Figure 4).

The prevalence rate of *A. phagocytophilum* in the sample from Kpinnou Farm is 100%. This prevalence rate is significantly higher than that of all the other samples. This is followed by the sample from the Okpara State Farm with a prevalence rate of 65%, then Bassila District with an intermediate level of 40% followed and finally those of Gogounou and Kétou districts with identical prevalence rate of 35%.

Deviance analysis leads to an estimation of the prevalence rates of Anaplasmosis at the levels of each herd and agro-ecological area.

There is a link between the prevalence of *A.phagocytophilum* and the type of herd or the agro-ecological area considered (p-value = 1.187×10^{-6}). The herds at Kpinnou State Farm are by far the most infected with *A. phagocytophilum*, followed by those from Okpara State Farm, those of agro-ecological areas II and V, which are Gogounou District and Bassila and Kétou districts. These towns have the lowest levels of *A. phagocytophilum* infestation.

In general, for all the concerned agro-ecological areas and substantially for the whole country, the prevalence rates or the risks of having *A. phagocytophilum* infection are between 54.875 and 74.375% at an uncertainty rate of 0.00125. The estimate of the prevalence rates or risks of infestation by agro-ecological areas or by the herd is given in Table 3.

Furthermore, from the analysis of the variable "Sex", the prevalence rates of *A. phagocytophilum* in the samples are 65% for male cattle and 52.5% for female cattle.

We can deduce that from the herds of Kpinnou State Farms or agro-ecological areas II and V (Gogounou District and Bassila and Kétou Districts), the rate ofprevalence of *Anaplasma phagocytophilum* does not depend on the sex of the bovine (X-squared = 0.56818, df = 1, p-value = 0.451).

Prevalence of theileriosis in the different agro-ecological areas

The prevalence rate of *Theileria mutans* observed in the

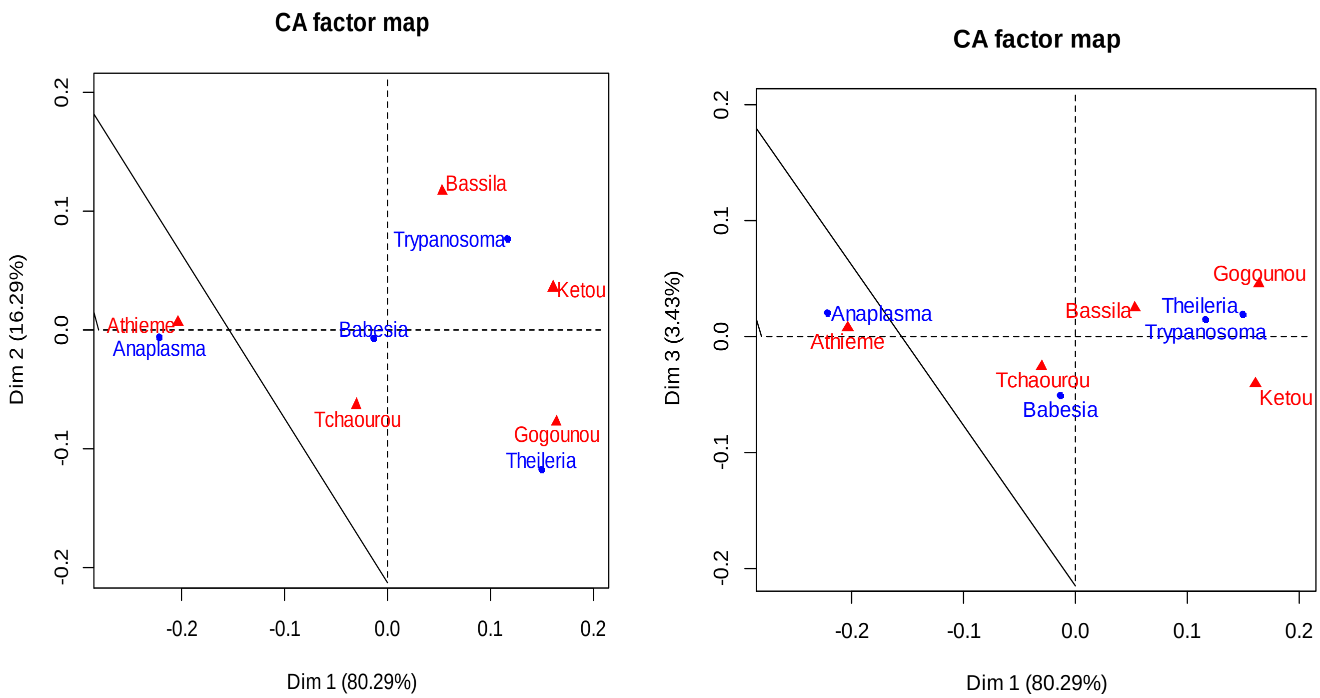

Figure 3. Projection of the different kinds of parasites and agro-ecological areas in a factorial plane.
Source: Authors.

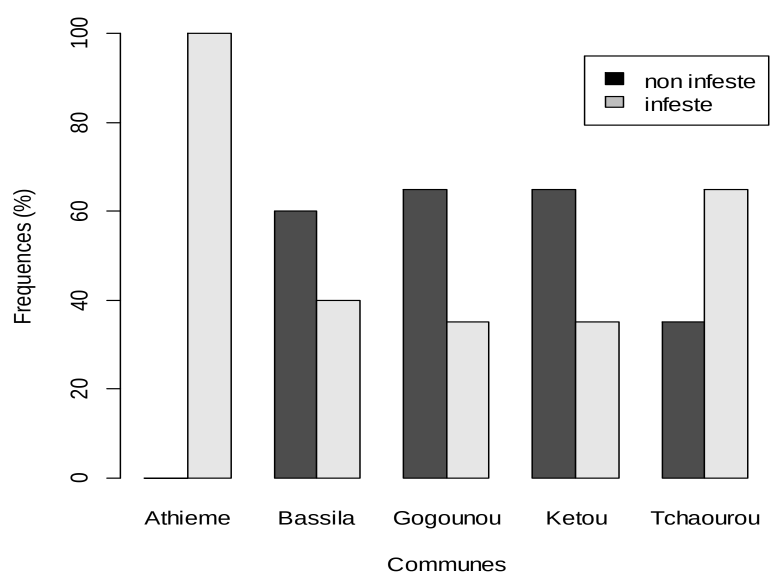

Figure 4. Prevalence of *Anaplasma phagocytophilum* according to agro-ecological areas.
Source: Authors' Computation.

Table 3. Confidence interval of prevalence rates (risk of infection) of Anaplasmosis.

	Kpinnou farm	Okpara farm	Area II (Gogounou)	Area V (Bassila-Kétou)
Extreme values of the risk of infestation (%)	[83.125;100[	[40.875; 84.625]	[15.575; 59.125]	[19.125; 63.875]

Source: Authors' Computation.

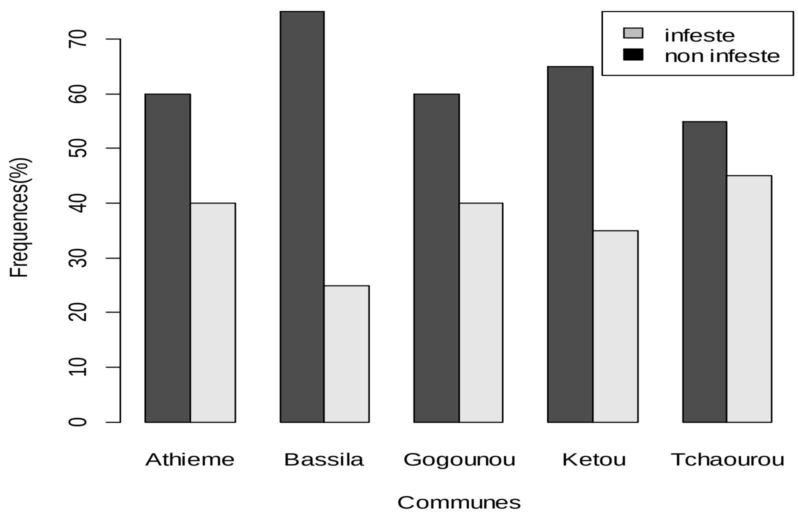

Figure 5. Prevalence of *Theileria mutans* according to agro-ecological areas.
Source: Authors' Computation.

entire sample is 37%. But variations in the prevalence rate seem to appear between the sampled cattle herds (Figure 5).

Sampled cattle in Tchaourou District have the highest prevalence rate of theileriosis (45%). Athiémé, Gogounou and Kétou have intermediate level of prevalence rates (40, 40 and 35%) and finally Bassila District has a lower prevalence rate (25%).

There is therefore no evidence of dependence between the prevalence of theileriosis and the different herds or agro-ecological zones (P-value = 0.7296). Thus, the prevalence rates of *T. mutans* in the herds of the two-state farms or in those of each of the agro-ecological areas II and V (Gogounou district and Bassila and Kétou districts) cannot be considered as different (p-value = 0.7296). The prevalence rate of *T. mutans* in the agro-ecological zones including each of the sampled sites, as well as the average prevalence rate of theileriosis in Benin, should therefore be estimated at 37%. Therefore, the risks of infestation (or prevalence rates) for bovine theileriosis in Benin are between 27.625 and 47.125% with an uncertainty level of 0.00125.

The analysis according to the variable "Sex" gives the prevalence rates of *T. mutans* in the samples as 20% for males and 41.25% for females. We can deduce that from the herds of Kpinnou and Okpara State Farms or the agro-ecological areas II and V (Gogounou Town and Bassila and Kétou districts), the prevalence rate of *Theileria mutans* does not depend on the sex of the bovine (χ^2- = 2.2549, df = 1, p-value = 0.1332).

Prevalence of parasites of genus Babesia in the different agro-ecological areas

The prevalence rate of parasites of genus *Babesia* in all samples is 54%. Babesiosis infestation levels in sampled cattle vary depending on the agro-ecological areas (Figure 6).

Kpinnou farm has the highest prevalence rate of samples (75%) while Bassila and Gogounou have the lowest prevalence rates (40%). Those of Tchaourou and Kétou show intermediate prevalence rates of 65 and 50%, respectively. The prevalence rates of these parasites in the different herds or agro-ecological areas are almost equal (p-value = 0.09). At the traditional 5% threshold, there is no evidence of a statistical difference between the prevalence rates in herds at state farms level or between those in agro-ecological areas II and V; but if we consider a 10% risk of error (1 in 10 chances of being wrong), the prevalence rates in herds at state farms level or in agro-ecological areas could be considered to be different. We deduce that the average prevalence rate of babesiosis in cattle herds in Benin is 54% for a 5% risk error.

Two species of *Babesia* were identified to be infested animals. These are *B. bigemina* and *B. bovis*, represented by respective prevalence of 21 and 33% at the whole sample level (all agro-ecological areas combined). Thus, within infested animals, there is statistically no evidence of a statistical difference between the prevalence rates of the two different *Babesia* species (χ^2- = 2.6667, df = 1, p-value = 0.1025). They are therefore fairly represented in the population of infested cattle; there is just as much chance that an animal infected by a parasite of the genus *Babesia* is infected with both *B. bigemina* and *B. bovis*.

On the whole, the average prevalence rate of babesiosis in agro-ecological areas including each of the sampled sites in Benin is estimated at 54% with a confidence interval between 43.625 and 64.125% at 0.00125 level of uncertainty. The risk of infection with

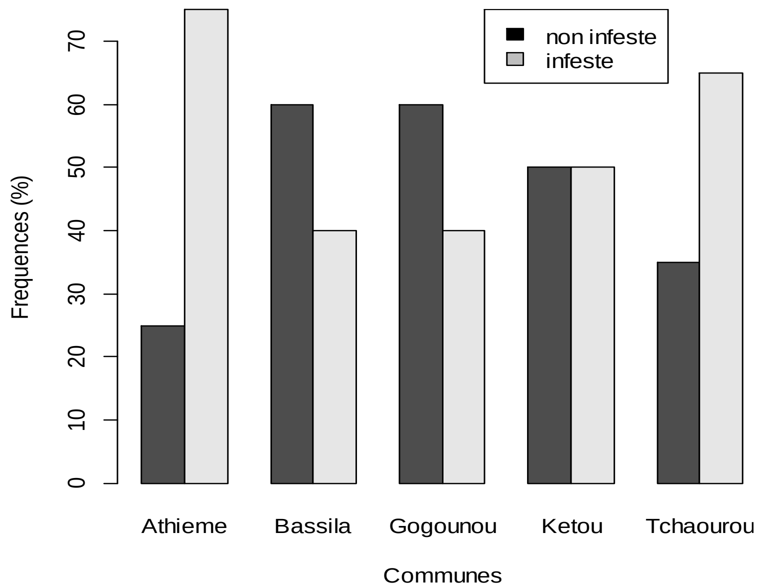

Figure 6. Prevalence of parasites of the genus *Babesia* according to agro-ecological areas.
Source: Authors' Computation.

babesiosis is therefore relatively high in Benin.

Analysis based on "Sex" gives prevalence rates of Babesia in the samples as 65% for male cattle and 51.25% for female cattle. We can deduce that from the herds in Kpinnou and Okpara State Farms or agro-ecological areas II and V (Gogounou District and Bassila and Kétou districts), the prevalence parasite rate of the genus *Babesia* does not depend on the sex of bovine (X-squared = 0.72715, df = 1, p-value = 0.3938).

Prevalence of parasites of the genus *Trypanosoma* in the different agro-ecological areas

The prevalence rate of genus *Trypanosoma* in the entire sample is 64%. The levels of infection in the sampled cattle with trypanosomosis depend on the agro-ecological areas (Figure 7). The cattle sampled from Bassila and Gogounou townships are the least infested (60 and 55% respectively); those from agro-ecological areas including Kétou township and those from Okpara State Farm have intermediate prevalence rates (65% for each of these sites). Kpinnou State Farm has the highest prevalence rates of all the sampled sites (75%). However, there is no evidence of a dependence between the prevalence rates of trypanosomoses in the different herds and agro-ecological areas (p-value = 0.745). Thus, the prevalence of animal trypanosomoses does not depend on the state farm or the agro-ecological area considered. We deduce that the prevalence rate of animal trypanosomoses in Benin is 64%.

Concerning the risk levels specific to each species, *T. congolense* has a prevalence rate of 21%, while *T. vivax* and *T. theileri* have respective prevalence rates of 27 and 16%. Based on infection of the affected animals, there is no statistical difference between the prevalence rates of the three different *Trypanosoma* species (χ^2- = 2.8438, df = 2, p-value = 0.2413). There is just as much chance that an animal infected with a parasite of genus *Trypanosoma* will also be infected with *T. vivax*, *T. congolense* or *T. theileri*.

On the whole, the average prevalence rate of trypanosomosis in agro-ecological areas including each of the sampled sites or substantially in Benin is estimated at 64% with a confidence interval between 53.875 and 73.375% at 0.00125 of uncertainty. The risks of trypanosome infestation are therefore relatively high in Benin.

The analysis based on variable "Sex" gives the prevalence rates of *Trypanosoma* in the samples as 60% for male cattle and 65% for female cattle. We can deduce that from the herd of Kpinnou and Okpara State Farms or the agro-ecological areas II and V (Gogounou Township and Bassila and Kétou districts), the prevalence rate of genus *Trypanosoma* does not depend on the sex of the bovine (X-squared = 0.024414, df = 1, p-value = 0.8758).

Factors influencing the prevalence rate of *A. phagocytophilum*

The saturated model based on the agro-ecological areas, breeds, sex of the animals as well as the presence or

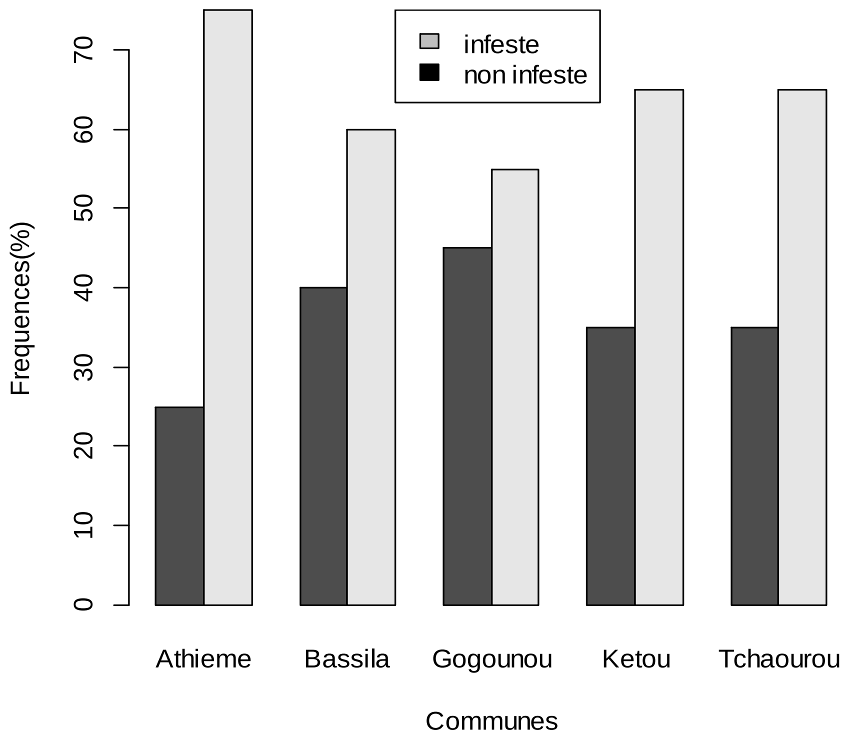

Figure 7. Prevalence of parasites of the genus *Trypanosoma* according to agro-ecological areas.
Source: Authors' Computation.

Table 4. Estimated parameters of the explanatory model of the prevalence rate of *Anaplasma* and associated probabilities.

Coefficient	Estimator	Standard deviation	z value	Pr(>|z|)
Intercept	0.09247	0.49804	0.186	0.85271
FACthelthel	0.49809	0.62698	0.794	0.42695
FACbabesNbabes	1.18968	0.83867	1.419	0.15604
FACtryptryp	1.41735	0.61497	2.305	**0.02118***
FACthelthel:FACbabesNbabes	2.5101	0.93414	2.687	**0.00721****
FACbabesNbabes:FACtryptryp	1.48385	0.94716	1.567	0.11720

Intercept, FACthelthel, FACbabesNbabes, FACtryptryp, FACthelthel:FACbabesNbabes FACbabesN babes:FACtryptryp,- Finally, the minimum explanatory model retained is: z ~ FACtryp + FACthel: FACbabes where z represents the prevalence of *Anaplasma.*
Source: Authors' Computation.

absence of the parasites of genus *Theileria*, *Babesia* and *Trypanosoma* does not make it possible to identify factors likely to explain the prevalence of *Anaplasma* within the cattle throughout the study area. It is therefore not statistically possible to link the prevalence of *Anaplasma* to agro-ecological factors (or the production system), neither race nor sex. However, the model explaining the prevalence of Anaplasma by the presence or absence of parasites from the genus *Theileria*, *Babesia* and *Trypanosoma* is significant (Table 4).

It therefore appears that the prevalence of *Anaplasma* is as high as the bovine infected with trypanosomes on one hand; while on the other hand, this prevalence is as high as the bovine infested with *Theileria* but not infested with *Babesia*. Taken together, these results reveal that *Anaplasma* parasitism is strongly supported by a community of associated parasites. It underlines the danger of the situation since trypanosomiasis is endemic in most agro-ecological areas in Benin.

Agreement and inconsistency of results according to the diagnostic method

The results of parasitic diagnosis by microscopy and PCR reveal a perfect agreement between the diagnostic

Table 5. Estimates of the minimum chances of accurately identifying the species of parasites by microscopy in the event of a positive test.

	Anaplasmosis	Theileriosis	Babesiosis	Trypanosomiasis
Minimum chances (%) at an uncertainty level of 0.00125	83.16	63.06	78.18	78.18

Source: Authors' Computation.

Table 6. Number of coherent versus opposite microscopy and PCR test's results (Number of cases out of 20).

Result	*Anaplasma*	*Theileria*	*Babesia*	*Trypanosome*
Positive concordant	20	8	15	15
Negative concordant	0	12	5	5

Source: Authors' Computation.

methods for Anaplasmosis (Table 5). Regarding Theileriosis, Babesiosis and Trypanosomiasis, discrepancies are observed (Table 5).

The proportion of discordant and concordant results for the diagnosis of parasites by microscopy and PCR as well as the estimates of the minimum chances of accurately identifying the species of the parasite by microscopy in the event of a positive test are summarized in Tables 5 and 6.

DISCUSSION

Various bacterial diseases are caused by bacteria and transmitted by vectors. The latter are hematophagous arthropods that ensure active, and biological transmission of an infectious agent from one vertebrate to another, which in our study are mainly ticks. These diseases can exclusively affect humans or animals, but it is not uncommon for them to be zoonoses or anthropozoonoses.

In many countries, including Benin, the livestock sector faces a major constraint related to animal health. Bacteria of the genus *Anaplasma* are among the pathogens that are of great public health interest. Their phylogenic affiliations have evolved a lot and are still evolving. They parasitize the blood cells of many animal species including humans. Their ability to cross-species barrier complicates their study and often delays their identification. *A. phagocytophilum* (*Ehrlichia phagocytophila*) is one of the family members with unidentified members. The disease cycle is trixene, made up of its definitive host, reservoir and vector, all of which can be the source of the infecting parasite ((Fourie et al., 2019). In addition, the epidemiology of vector-borne diseases depends on: Reservoirs, susceptible populations, vectors, infectious agents, but also on human activities, the environment and climatic conditions. Because this bacterium is transmitted by ticks, it is all in one biotope disease and seasonal.

Today, we see an expansion of vector-borne diseases resulting from the intensification and globalization of trade, climate change, and changes in interactions between humans and their environment. These diseases are of real significance, both through the zoonotic role of some and through their economic impact on animal health (Zehnter, 2014). Ticks are considered to transmit the greatest number of pathogens compared to other arthropod vectors (Beugnet and Marié, 2009; Heyman et *al*., 2010; Baneth, 2014). Several of these pathogens are of both veterinary and medical significance causing various diseases, including anaplasmosis, babesiosis, Lyme borreliosis, ehrlichiosis and rickettsiosis (Heyman et al., 2010; Baneth, 2014). Animal movements due to international trade associated with climate change and many other factors are causing changes in the distribution of tick species and the disease agents they transmit (Aydin and Bakirci, 2007). Regular study of tick species and associated infectious agents is therefore essential for epidemiological control. From numerous studies already carried out, *A. phagocytophilum* is mainly transmitted by ticks of genus *Ixodes (*not identified among those found during our study*).* However other genera of *Anaplasma* vector ticks have also been described. These are *Dermacentor*, *Rhipicephalus* and *Amblyomma* (Rymaszewska and Grenda, 2008). Thus, *I. ricinus*, *I. persulcatus*, *I. scapularis*, *Rh. Sanguineus*, *Rh. purse*, *Rh. turanicus*, *A. americanum*, *D. variabilis*, *D. anderson*, *D. auratus*, *D. silvanum*, *Ha. lagrangei*, *Ha. leporispalustris*, *Ha. longicomis*, *Ha. concinna*, *Ha. punctata*, *Ha. megaspinosa*, *Hy. marginatum*, *Hy. asiaticum* and *Hy. detritum* have been confirmed as vectors for *Anaplasma* in various studies (Parola and Raoult, 2001; Goethert and Telford, 2003; Kim et al., 2003, 2006; Parola et al., 2003; De La Fuente et al., 2004; Kawahara et al., 2006; Shpynov et al., 2006; Stafford III, 2007; Rymaszewska and Grenda, 2008; Jiang et al., 2011; Meng et al., 2012; Bonnet et al., 2013; Palomar et al., 2015).

Examination of ticks collected in the present study allowed us to identify tick species such as *Amblyomma variegatum*, *Rhipicephalus microplus*, *Rhipicephalus* spp., *Hyalomma* spp., *R. decoloratus* and *R. annulatus*. *Ixodes ricinus.* The most implicated vector in Europe and America in the transmission of *A. phagocytophilum* (Satta et al., 2011), was not identified in any of the five sites of interest in this study.

Ouedraogo and Coll (2020) found the presence of granulocytic anaplasmosis caused by *A. phagocytophilum* in areas where the *I. ricinus* tick has not been found. This suggests the existence of other arthropod vectors. This thesis corroborates our results. In addition, other biting arthropods such as stomoxys and tabanids have been encountered.

Prevalence of different hemoparasites

Out of the 100 blood samples analyzed, 55 tested positive for *A. phagocytophilum*, that is, a prevalence rate of 55%. Other parasites such as *Theileria mutans*, *Babesia bigemina*, *B. bovis*, *Trypanosoma vivax*, *T. congolense* and *T. theileri* were also encountered during the analysis of blood samples with rates respectively 39, 22, 33, 27, 21 and 16%. A large proportion of animals collected during the study show good health trend, but some of them had arthritis and edema of the limbs. These findings corroborate those of many researchers' results (Guyot et al., 2011; Belkahia et al., 2013; Stuen et al., 2013) who reported that clinical signs associated with tick-borne fever and pasture fever included a sudden onset of high fever, anorexia, dullness, arthritis, edema of the limbs and weight loss. Also, infection with *A. phagocytophilum* can induce an immune deficiency in ruminants leading to secondary opportunistic infections such as those with *Staphylococcus aureus*, *Pasteurella* spp. and *Listeria monocytogenes* (Carrade et al., 2009; Guyot et al., 2011; Ben Said et al., 2014). This would explain the cases of co-infections observed in Kpinnou State Farm animals. The farms of Kpinnou and Okpara have the particularity of each sheltering only one breed (or genetic type) of cattle. This is as a result of the farms being involved in genetics selection programs based on specific breeds.

The prevalence rate due to *A. phagocytophilum* in the FEK sample is 100% and is significantly higher than that which is seen in all other samples. It is followed by that of Okpara Breeding Farm (FEO) which is 65%, then Bassila sample which is 40% and finally those of Gogounou samples and Kétou which are identical and equivalent to 35%. These results testify to the susceptibility of this animal species to infection by *A. phagocytophilum*, although no particular clinical sign has been demonstrated. The lower prevalence observed in both Gogounou and Kétou could be due to several factors such as bioclimatic conditions, low intensity of tick infestation, infesting tick species and tick control practices. Moreover, since these areas are cotton production areas, the use of large quantities of insecticides by cotton growers could lead to reduced tick populations.

A first serological study on *A. phagocytophilum* prevalence in dromedaries (*Camelus dromedarius*) in Tunisia was carried out (Ben Said et al., 2014). The results obtained showed prevalence rates of 29.2% in 226 dromedaries tested or 66 positive subjects. This is close to the rates of 35% obtained with the samples from Gogounou and Kétou cattle herds (Ben Said et al., 2014).

The prevalence of infection with *A. phagocytophilum* is 36.7% in traditional farms versus a minimum of 82.5% in productive farms with high health monitoring and control system. This difference in prevalence between the two types of farming shows that the type of activity seems not to influence the rate of infection and that the health control of productive farms remains insufficient and needs to be strengthened. These results corroborate those of many researchers (Lebland et al., 2005; Hansen et al., 2010) concerning equine granulocytic anaplasmosis, which showed that infection by *A. phagocytophilum* was not correlated with this type of breeding.

All parasite-borne infections associated with Anaplasmosis are of high importance even if the prevalence rates of each show no particular differences according to agro-ecological areas. In the study area, the two species of *Babesia* seen show the same risk of infection just as the three species of Trypanosomes. In the particular case of trypanosomosis, it has been reported that *T. vivax* is transmitted by all species of tse-tse flies and also by mechanical vectors such as Stomoxys and Tabanids (Lekeux, 2006).

The average prevalence rate of *T. mutans* at the level of agro-ecological areas including each of the sampled sites, as well as the average prevalence rate of theileriosis in Benin, calculated from the data is 37%. Therefore, the infection risks (or average prevalence rates) of bovine theileriosis in Benin are calculated from the data between 27.625 and 47.25% with an uncertainty level of 0.00125.

The average prevalence rates for babesiosis in the different herds or the different agro-ecological areas are almost equal (p-value = 0.09). At the traditional 5% threshold, there is no evidence of a statistical difference between the prevalence rates in the herds of state farms or those of agro-ecological areas II and V. But if one fixes a risk of error of 10% (1 chance in 10 of being wrong), the prevalence rates in the herds of state farms or agro-ecological areas could be considered as different (quasi dependence between the prevalence and the herd or the agro-ecological area). We therefore, deduce that the average prevalence rate of babesiosis in cattle herds in Benin is 54% on average.

The studies carried out have made it possible to identify two species of *Babesia* in the infected animals;

these are *B. bigemina* and *B. bovis*, represented by respective prevalences of 21 and 33% at the level of the entire sample (all agro-ecological areas combined). Thus, within infected animals, there is statistically no evidence of a statistical difference between the prevalence rates of the two different species (X - squared = 2.6667, df = 1, p-value = 0.1025); they are therefore fairly represented in the population of infected cattle. There is just as much chance that an animal infected by a parasite of the genus *Babesia* is infected with both *B. bigemina* and *B. bovis*

In total, the average prevalence rate of babesiosis in agro-ecological zones including each of the sampled sites or substantially in Benin is calculated as 54% with a confidence interval between 43.625 and 64.125% at the level of uncertainty of 0.00125. The risks of infestation with babesiosis are therefore relatively high in Benin.

This study revealed the presence of three species of trypanosomes namely *T. congolense*, *T. vivax* and *T. theileri* in the study area with the predominance of *T. vivax* followed by *T. congolense* and finally *T. theileri*. This predominance can be justified by the fact that *T. vivax* is transmitted by all species of tsetse flies and also by mechanical vectors such as stomoxys and tabanids (Lekeux, 2006).

Regarding the risk levels specific to each species, *T. congolense* prevalence rate is calculated as 21%, while *T. vivax* and *T. theileri* have respective prevalence rates of 27 and 16%. Therefore, within the infested animals, there is no evidence of a statistical difference between the prevalence rates of the three different species of trypanosomes (X - squared = 2.8438, df = 2, p-value = 0.2413). There is just as much chance that an animal infected with a parasite of the genus *Trypanosoma* will be infected with *T. vivax*, *T. congolense* or *T. theileri* as well.

In total, the average prevalence rate of trypanosomosis in agro-ecological areas including each of the sampled sites or substantially in Benin is estimated at 64% with a confidence interval between 53.875 and 73.375% at the level of uncertainty of 0.00125. The risks of trypanosomosis infestation are therefore relatively high in the agro-ecological areas concerned in Benin.

Factors influencing prevalence

In contrast to the other herds, all the sampled cattle from Kpinnou State Farm tested positive for *A. phagocytophilum*. Kpinnou State Farm herds being by far the most infested by *A. phagocytophilum*, they are followed by the FEO herds. Finally, the herds of agro-ecological zones II and V (Gogounou Township and Bassila and Kétou townships) have the lowest levels of *A. phagocytophilum* infection. There is a link between the prevalence of *A. phagocytophilum* and the type of herd or the agro-ecological area considered (p-value = $1.187.10^{-6}$). This particular trend should normally be linked with the pasture feeding approach and also the humidity and temperature conditions that are on average 85 to 90% and 27 to 28°C, all corresponding to the optimal conditions for ticks. The case confirms that the transmission of *A. phagocytophilum* is linked to ticks and their presence. However, the pasture feeding approach as temperature and humidity conditions has not been proved to be statistically different from one sampled site to another. Thus, the only factors of importance are the type of pasture (opened or closed) and also the origin of the animal breed (abroad or local). Kpinnou and Okpara state farms use closed pasture. Their herds are relatively separate from neighboring farm herds which is not the case for agro-ecological II and V sampled sites. The latters are then more exposed to herds and infestation sources. Unfortunately, agroecological sampled sites are less infested than state farms. Consequently, animal breed origin remains the most important factor. Animal exchange and in this case Girolando breed importation from Brazil to the state farms could have contributed to particular *Anaplasma* genus introduction in Benin as Aydin and Bakirci (2007) found in their study. Secondly, the closed breeding approach that makes the state farms closed systems with the least anthropogenic agricultural actions such as pesticide use could explain the expansion and the increase of the parasite genus in the state farms making them permanently infested area.

Regarding the common prevalence rate trend of the other parasites genus, *Babesia*, *Theileria* and *Trypanosoma*, the fair explanation should be that they were present and well established in the local parasite genus system in Benin a long time ago.

With regard to the sex factor, it appears that none of the parasite genus infestation rates shows dependency. Gender therefore does not have a significant effect on the infection rate. There should be an identical susceptibility of both sexes to the parasite genus or its vectors. These results confirm current results (Acapovi-Yao et al., 2010). These researchers demonstrated that the phenotype, sex and age class of the cattle did not influence the prevalence of trypanosome infestations in Korhogo and Odiénné. Moreover, analogous results concerning *A. phagocytophilum* have been found with horses (Lebland et al., 2005; Hansen et al., 2010).

On the contrary, Ingabiré (2009) found a higher prevalence rate in females than in males for *Trypanosoma* genus as Acapovi-Yao et al. (2009) in Koutouba. The only explanation given for the different prevalence rates in sex is the gentle and impetuous behavior respectively for females and males when facing vector attacks. The mitigation result related to sex, influencing or not the prevalence rate makes implemented rigorous experimental design based on sex, age, physiological status, breed and also breeding system factors.

Comparative efficacy of diagnostic methods by microscopy and PCR

The precision of microscopy results only perfectly matched that of the PCR in the case of *A. phagocytophilum* at Kpinnou State Farm (only samples diagnosed by both microscopy and PCR), in the differentiation of the two species of *Babesia* (*B. bigemina* and *B. bovis*) and the three species of Trypanosomes (*T. theileri*, *T. congolense* and *T. vivax*). Thus, several samples diagnosed negative by microscopy for the rest of the parasites (in addition to *A. phagocytophilum*) were found to be positive by PCR. This shows that microscopy displays lower sensitivity than PCR; that is, there were false negatives with the microscopy. However, their specificities are equivalent since there were no false positives detected by PCR. The low level of sensitivity of the microscopic method could be induced by a blood load of parasites low enough to be detected and therefore linked to the diagnostic method. The diagnostic method by PCR appears therefore to be the most reliable.

Anaplasmosis microscopy seems to offer the greatest chance of identification of the parasite species (minimum chance of 83, 16% with an uncertainty of 0.00125) than those directed towards other parasites. It could be due to the single Anaplasma species of interest in the study. Babesiosis and Trypanosomosis microscopies came second (with respective minimum chances of 78.18% each). Regarding Theileriosis microscopy, it offers the lowest chances of species identification (63.06%) although there is only one prevalent species of Theileriosis against three for trypanosomiasis and two for babesiosis in the study.

Concerning trypanosomosis, a perfect agreement is observed in 17 cases (17/20), that is 15 true positives and 2 true negatives, and a disagreement in 3 cases (3/20). The chances of concordance of the results of the tests for the detection of trypanosomosis by microscopy and PCR under the conditions of the present experiment vary between 62.11 and 96.78% with an uncertainty of 0.00125.

In the concordant positive cases, the microscopy allowed a good differentiation or identification of the species. For each of the species, at the level of the positive cases of the study, the diagnostic methods by microcopy and by PCR give identical results about the precise identification of the parasite species. The minimum chances of identifying the precise species of parasite by microscopy vary depending on the parasite (Table 6). These changes could be equated with the chances of identifying true positives (if the PCR result was considered to be 100% reliable). But in the present study all the samples that are positive by microscopy are also positive by PCR. Therefore, they are true positives, which give the two methods an equally important specificity.

Conclusion

A. phagocytophilum is known as a veterinary pathogen causing disease in ruminants for several decades. Its zoonotic potential is not yet well understood. The ability to cause disease in humans with potentially serious consequences, the global distribution and the growing attention to ticks and transmitted diseases have increased the interest and awareness of veterinarians and other health workers about this emerging pathogen.

The advent of significant advances in molecular biology in recent years has enabled us to confirm part of the microscopic analysis of samples by PCR. The study of *A. phagocytophilum* highlights several important elements among which the first effective prevalence was reported in Benin. Its pathogenic role, albeit moderate at the individual level, can be significant at the herd level. Moreover, the lack of interest shown in it by practitioners certainly masks a significant impact.

It emerges that the economic losses caused by *A. phagocytophilum* deserve a better awareness of those involved in breeding. The implementation of laboratory tests that must be simple, economical and reliable is essential. This is ideally the place that PCR should take in the future to systematize the search for and the precise identification of the bacteria. Finally, the zoonotic nature of *A. phagocytophilum* infection gives it additional appeal, although it is difficult to predict its incidence in the coming years.

To better consider the impact of diseases transmitted by ticks to livestock, bovine anaplasmosis requires large-scale epidemiological studies in Benin. This is due to the low number of research activities carried out on this disease which could be considered a major animal or even human health problem. Both for public health and economic reasons, it is advisable to fight against the disease of the pasterns. However, this control is hampered by a lack of knowledge about the epidemiology of *A. phagocytophilum* and the quality and duration of the immunity it confers in domestic ruminants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Acapovi-Yao G, Desquesnes M, Hamadou S, N'Goran E (2010). Prévalence parasitologique et sérologique des trypanosomoses chez trois races bovines en zones à glossines et présumée indemne, Côte d'Ivoire. Agronomie Africaine 21(2).

Adehan SB, Biguezoton A, Adakal H, Assogba MN, Zoungrana S, Gbaguidi AM, Tonouhewa A, Kandé S, Achi L, Kagone H (2016). Acaricide resistance of *Rhipicephalus microplus* ticks in Benin. African Journal of Agricultural Research 11(14):1199-1208.

Aydin L, Bakirci S (2007). Geographical distribution of ticks in Turkey. Parasitology Research 101(S2):163-166.

Baneth G (2014). Tick-borne infections of animals and humans: a common ground. International Journal for Parasitology 44(9):591-596.

Belkahia H, Sayahi L, Aloui M, Jemli MH, Sassi L, Darghouth MA, Djaïem AA, Bayoudh M, Messadi L (2013). First serological study of the prevalence of *Anaplasma phagocytophilum* in dromedary (*Camelus dromedarius*) in Tunisia. Bulletin de la Societe de

Pathologie Exotique 107(1):1-6.

Ben Said M, Belkahia H, Sayahi L, Aloui M, Jemli MH, Hadj Mohamed B, Sassi L, Darghouth MA, Djaïem AA, Bayoudh M, Messadi L (2014). Première étude sérologique de la prévalence d'*Anaplasma phagocytophilum* chez le dromadaire (*Camelus dromedarius*) en Tunisie. Bulletin de la Société de Pathologie Exotique 107(1):1-6.

Bettencourt A, Franca C, Borges J (1907). Un cas de piroplasmose bacilliforme chez le daim. Arquivas do Instituto Bacteriologico Camara Pestana 1:341-349.

Beugnet F, Marié JL (2009). Emerging arthropod-borne diseases of companion animals in Europe. Veterinary Parasitology 163(4):298-305.

Biguezoton A, Adehan S, Adakal H, Zoungrana S, Farougou S, Chevillon C (2016). Community structure, seasonal variations and interactions between native and invasive cattle tick species in Benin and Burkina Faso. Parasites and Vectors 9(1):1-16.

Bonnet S, Fuente J de la, Nicollet P, Liu X, Madani N, Blanchard B, Maingourd C, Alongi A, Torina A, Fernández de Mera IG, Vicente J, George J-C, Vayssier-Taussat M, Joncour G (2013). Prevalence of Tick-Borne Pathogens in Adult *Dermacentor* spp. Ticks from Nine Collection Sites in France. Vector-Borne and Zoonotic Diseases 13(4):226-236.

Carrade DD, Foley JE, Borjesson DL, Sykes JE (2009). Canine granulocytic anaplasmosis: a review. Journal of Veterinary Internal Medicine 23(6):1129-1141.

De La Fuente J, Vicente J, Höfle U, Ruiz-Fons F, De Mera IGF, Van Den Bussche RA, Kocan KM, Gortazar C (2004). Anaplasma infection in free-ranging Iberian red deer in the region of Castilla-La Mancha, Spain. Veterinary microbiology 100(3-4):163-173.

Desquesnes M (2012). Trypanosomosis (tse-tse-transmitted). https://www.woah.org/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/TRYPANO_TSETSE.pdf

Fourie JJ, Evans A, Labuschagne M, Crafford D, Madder M, Pollmeier M, Schunack B (2019). Transmission of Anaplasma phagocytophilum (Foggie, 1949) by Ixodes ricinus (Linnaeus, 1758) ticks feeding on dogs and artificial membranes. Parasites and Vectors 12(1):1-10.

Goethert HK, Telford SR (2003). Enzootic Transmission of *Anaplasma bovis* in Nantucket Cottontail Rabbits. Journal of Clinical Microbiology 41(8):3744-3747.

Guyot H, Ramery E, O'Grady L, Sandersen C, Rollin F (2011). Emergence of bovine ehrlichiosis in Belgian cattle herds. Ticks and Tick-Borne Diseases 2(2):116-118.

Hansen MG, Christoffersen M, Thuesen LR, Petersen MR, Bojesen AM (2010). Seroprevalence of *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum* in Danish horses. Acta Veterinaria Scandinavica 52(1):1-6.

Hauck D, Jordan D, Springer A, Schunack B, Pachnicke S, Fingerle V, Strube C (2020). Transovarial transmission of Borrelia spp., Rickettsia spp. and Anaplasma phagocytophilum in Ixodes ricinus under field conditions extrapolated from DNA detection in questing larvae. Parasites and Vectors 13(1):1-11.

Heyman P, Cochez C, Hofhuis A, Giessen J van der, Sprong H, Porter SR, Losson B, Saegerman C, Donoso-Mantke O, Niedrig M, Papa A (2010). A clear and present danger: tick-borne diseases in Europe. Expert Review of Anti-infective Therapy 8(1):33-50.

Jiang JF, Jiang BG, Yu JH, Zhang WY, Gao HW, Zhan L, Sun Y, Zhang XA, Zhang PH, Liu W, Wu XM, Xu RM (2011). *Anaplasma phagocytophilum*</i> Infection in Ticks, China–Russia Border. Emerging Infectious Diseases 17(5):932-934.

Kawahara M, Rikihisa Y, Lin Q, Isogai E, Tahara K, Itagaki A, Hiramitsu Y, Tajima T (2006). Novel Genetic Variants of *Anaplasma phagocytophilum* , *Anaplasma bovis* , *Anaplasma centrale* , and a Novel *Ehrlichia* sp. in Wild Deer and Ticks on Two Major Islands in Japan. Applied and Environmental Microbiology 72(2):1102-1109.

Kim CM, Kim MS, Park MS, Park JH, Chae JS (2003). Identification of *Ehrlichia chaffeensis* , *Anaplasma phagocytophilum* , and *A. bovis* in *Haemaphysalis longicornis* and *Ixodes persulcatus* Ticks from Korea. Vector-Borne and Zoonotic Diseases 3(1):17-26.

Kim CM, Yi YH, Yu DH, Lee MJ, Cho MR, Desai AR, Shringi S, Klein TA, Kim HC, Song JW, Baek LJ (2006). Tick-borne rickettsial pathogens in ticks and small mammals in Korea. Applied and Environmental Microbiology 72(9):5766-5776.

Lebland A, Pradiar S, Pitelº PH, Fortier G, Boireau P (2005). Enquête épidémiologique sur l'anaplasmose équine (*Anaplasma phagocytophilum*) dans le sud de la France. Revue scientifique et technique-Office international des épizooties 24(3):899-908.

Lekeux M (2006). La trypanosomose bovine africaine: Généralités et situation au Bénin. Doctoral dissertation, Thèse vétérinaire, Université de Lyon, 94p.

MacLeod J (1932). The bionomics of *Ixodes ricinus L.*, the "sheep tick" of Scotland. Parasitology 24(3):382-400.

Madder M, Horak I, Stoltsz H (2014). Tick identification. Pretoria: Faculty of veterinary Science University of Pretoria 58 p.

Meng QL, Qiao J, Chen CF, Cai XP, Wang GC, Zhang ZC, Zhao CG, Zhang LJ, Cai KJ, Yang LH (2012). A preliminary survey on the ticks carrying *Ehrlichia* and *Anaplasma* in the Southern marginal zone of Gurbantunggut Desert. African Journal of Microbiology Research 6(43):7073-7077.

Ouedraogo A, Luciani L, Zannou O, Biguezoton A, Pezzi L, Thirion L, Belem A, Saegerman C, Charrel R, Lempereur L (2020). Detection of two species of the genus parapoxvirus (Bovine papular stomatitis virus and pseudocowpox virus) in ticks infesting cattle in Burkina Faso. Microorganisms 8(5):644.

Palomar AM, Portillo A, Santibáñez P, Mazuelas D, Roncero L, García-Álvarez L, Santibáñez S, Gutiérrez Ó, Oteo JA (2015). Detection of tick-borne *Anaplasma bovis*, *Anaplasma phagocytophilum* and *Anaplasma centrale* in Spain: Detection of *Anaplasma* spp. in Spain. Medical and Veterinary Entomology 29(3):349-353.

Parola P, Cornet JP, Sanogo YO, Miller RS, Thien HV, Gonzalez JP, Raoult D, Telford SR, Wongsrichanalai C (2003) Detection of *Ehrlichia*</i> spp., *Anaplasma*</i> spp., Rickettsia spp., and Other Eubacteria in Ticks from the Thai-Myanmar Border and Vietnam. Journal of Clinical Microbiology 41(4):1600-1608.

Parola P, Raoult D (2001). Ticks and Tick-borne Bacterial Diseases in Humans: An Emerging Infectious Threat. Clinical Infectious Diseases 32(6):897-928.

Renard T (2018). Etude dynamique de l'infection par *Anaplasma phagocytophilum* dans un élevage de bovins. Doctoral Dissertation, 125 p.

Rymaszewska A, Grenda S (2008). Bacteria of the genus *Anaplasma* – characteristics of *Anaplasma* and their vectors: a review. Veterinarni Medicina 53(11):573-584.

Satta G, Chisu V, Cabras P, Fois F, Masala G (2011). Pathogens and symbionts in ticks: a survey on tick species distribution and presence of tick-transmitted micro-organisms in Sardinia, Italy. Journal of Medical Microbiology 60(1):63-68.

Schäfer I, Kohn B (2020). Anaplasma phagocytophilum infection in cats: A literature review to raise clinical awareness. Journal of Feline Medicine and Surgery *22*(5):428-441.

Shpynov S, Fournier PE, Rudakov N, Tarasevich I, Raoult D (2006). Detection of Members of the Genera *Rickettsia*, *Anaplasma*, and *Ehrlichia* in Ticks Collected in the Asiatic Part of Russia. Annals of the New York Academy of Sciences 1078(1):378-383.

Smith T, Kilborne FL (1893). Investigations into the Nature, Causation, and Prevention of Texas or Southern Cattle Fever. US Department of Agriculture, Bureau of Animal Industry.

Stafford III KC (2007) Tick Management Handbook. The Connecticut Agricultural Experiment Station. Bulletin (1010):79.

Stuen S, Granquist EG, Silaghi C (2013). *Anaplasma phagocytophilum*—a widespread multi-host pathogen with highly adaptive strategies. Frontiers in Cellular and Infection Microbiology 3:31.

Team RC (2016). R: A Language and Environment for Statistical Computing [Manual de software informático]. Vienna, Austria Descargado de https://www R-project org.

Theiler A (1910). *Anaplasma marginale.* Annals of the Transvaal Museum 2(2):53-55.

Walker AR (2003). Ticks of Domestic Animals in Africa: A Guide to Identification of Species. Bioscience Reports Edinburgh

Yessinou RE, Cazan CD, Bonnet SI, Farougou S, Mihalca AD (2022). Geographical distribution of hard ticks (Acari: Ixodidae) and tick-host associations in Benin, Burkina-Faso, Ivory-Coast and Togo. Acta Tropica P 106510

Stuen S, Granquist EG, Silaghi C (2013). Anaplasma phagocytophilum—a widespread multi-host pathogen with highly adaptive strategies. Frontiers in Cellular and Infection Microbiology 3:31.

Zannou OM, Ouedraogo AS Biguezoton AS, Yao KP, Abatih E, Farougou S, Lenaert M, Lempereur L, Saegerman C (2021). First tick and tick damage perception survey among sedentary and transhumant pastoralists in Burkina Faso and Benin. Veterinary Medicine and Science *7*(4):1216-1229.