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Cattle tick-borne pathogens sharing the same vectors in Benin and Burkina Faso: variations in prevalence and coinfection patterns

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ABSTRACT

Understanding interactions between pathogens at the level of the individual host and the population in West Africa, may have noteworthy implications for predictions of diseases emergence and disease control programmes. Hence, the current study was aimed at investigating the interactions between *Anaplasma marginale*, *Babesia bigemina* and *B. bovis* in West African cattle. Twelve sentinel steers in each of the eight selected herds were randomly chosen to perform a one-year-long survey designed to monthly collect blood sample and to diagnose possible infections of the steers. This allowed identifying *A. marginale* as the most prevalent pathogen across the three surveyed regions (range: 0.60-1), followed by *B. bigemina* (0.24-0.85) and *B. bovis* (0.10-0.64); the same ranking order of the tick-borne pathogens was observed when considering the apparent duration of cattle infection. Regarding interaction patterns, mainly avoidance was revealed between *A. marginale* and *B. bigemina* and between *B. bigemina* and *B. bovis.* Such negative interaction seemed to be beneficial to the West

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African cattle surveyed, helping them to escape from babesiosis caused by *B. bovis*. Altogether, studied *Boophilus* tick-borne pathogens negatively interact within cattle in Benin and Burkina Faso. This pattern raises new questions regarding the underlying mechanisms and potential consequences.

Keywords: Interactions, Tick-borne pathogens, *Anaplasma marginale*, *Babesia bovis*, *Babesia bigemina*, *Rhipicephalus microplus*.

INTRODUCTION

Hosts individuals of pathogens or parasites (e.g. wild and domestic ungulates, rodents, birds, plants, human, etc.) are often infected with more than one pathogenic organism (Ginsberg, 2008; Telfer et al., 2010). Therefore, interactions between different pathogens genotypes or species frequently occur. These interactions may be synergistic (positive interaction) or antagonistic (negative interaction), with potential fitness implications for both the host (morbidity and/or mortality) and parasite (transmission potential). Understanding interactions at the level of the individual host and the population may have noteworthy implications for predictions of disease emergence, disease control programmes and bio-control initiatives (Telfer et al., 2008). Severity of symptoms is often found associated with co-infection by different pathogens (Belongia, 2002; Le Hesran et al., 2004) whereas intolerance between pathogens may appear beneficial to the host. Nevertheless, pathogens mutual intolerance may severely affect the outcome of prophylactic or vaccination campaigns. For instance, when competitive interactions occur, the most sensitive pathogens to a treatment could be the disadvantaged pathogens for the competitions. This may lead to the opening of the widest gate to the most pathogenic species. Therefore, it is essential to better understand which interactions exist between co-occurring pathogens to contribute to better treatment of related diseases. As with diseases affecting humans, such investigations would be essential points of research concerning tick-borne diseases which represent a real threat to livestock on a global scale, particularly in developing countries where they hamper economic improvement (e.g. heartwater, babesioses, theilerioses and anaplasmoses).

Anaplasma marginale is an obligate intracellular bacterium that imposes high morbidity and mortality on cattle by infecting erythrocytes (Kocan et al., 2009). Tick-borne transmission is the most efficient even if alternative routes were documented such as from cow to veal across the placenta, via fomite in presence of dry blood content or via mechanical transmission caused by blood sucking flies (Telfer et al., 2010). In West Africa, three of the four co-occurring tick-species of the *Boophilus* subgenus (*Rhipicephalus annulatus*, *R. decoloratus* and *R. microplus*) are efficient vectors (Aubry and Geale, 2011) within which transmission is transstadial but not transovarial. *Babesia bigemina* and *B. bovis* are tick-borne protozoan parasites (*Apicomplexa*) infecting cattle and buffalo erythrocytes and circulating in locations where at least one tick-species of the *Boophilus* subgenus is present; i.e. throughout Africa, Asia, Australia, Southern Europe, Central and South America (Bock et al., 2004). In West Africa, *B. bigemina* counts four tick-vectors (*R. annulatus*, *R. decoloratus*, *R. geigyi* and *R. microplus*) against three for *B. bovis* (*R. annulatus*, *R. geigyi* and *R. microplus*) (Bock et al. 2004). Within these ticks, *B. bovi*s is the only one that is transmitted by transovarial means.

A 2012-2013 study analyzed the community structure of cattle ticks within 12 herds from Benin and Burkina Faso (Biguezoton et al., 2016). Authors demonstrated that invasive success of *R. microplus* there, did not translate into a reduction of the burden achieve by any native tick species. Moreover, the adults of all native and invasive tick-species tended to aggregate on the same steers all year around (Biguezoton et al., 2016).

Was such aggregation also the rule for the pathogens vectored by *R. microplus*, *R. annulatus*, *R. decoloratus* and *R. geygyi*? To answer, we focused on the eight herds where these vectors were encountered and analyzed, for the same monitored steers and along the same timeframe, the variation in prevalence of single and multiple infections caused by *A. marginale*, *B. bigemina* and *B. bovis*. Hence,

the present study was aimed at investigating the interactions between these three tick-borne pathogens that, in West Africa, share the same vertebrate host, cattle, and the same tick-vectors.

MATERIALS AND METHODS

Sampling

Figure 1 describes the sampling locations. Eight herds nested in three climatic areas experienced 12 consecutive monthly visits. In South Benin (herds 1A, 1K and 1O), the Guinean climate is characterized by a mean annual rainfall of 1 400 mm and the alternation of four seasons; namely, a long rainy season (April to July), a short dry season (August), a short rainy season (September to November) and a long dry season (December to March). In North Benin (herds 2O and 2G), the mean annual rainfall is 1 300 mm and a long rainy season (May to October) alternates with a long dry season (November to April). In South-West Burkina Faso (herds 3F, 3K and 3O), the mean annual rainfall is 1 200 mm and a short rainy season (June to September) alternates with a long dry season (October to May). The survey lasted 12 months in each site, including monthly records on rainfall and mean temperature. Monthly collection of tick was done and blood samples taken from ear and jugular veins from each of 12 randomly chosen sentinel steers per herd (see data and analysis on tick community structure in Biguezoton et al., 2016). Molecular diagnosis was carried out using the blood-sample collected in the jugular vein, maintained for no more than 2 hours in an EDTA-filled tube and then deposited on filter paper (Whatman, n°1 Qualitative, Schleicher & Schuell).

Molecular diagnosis

Six confetti (diameter: 6mm) of filter paper were used per blood sample to extract DNA as described by Ouedraogo et al. (2021) except that the boiled confetti were washed in 100 µl of Chelex-100 solution (concentration 10%) for 10 min at 95°C instead of using a kit. Among the existing molecular methods (Lew and Jorgensen, 2005), the PCR and quantitative PCR protocols described in Buling et al. (2007) and Hornok et al. (2008) were used to diagnose the presence of *A. marginale, B. bigemina* or *B. bovis* DNA.

Prevalence and infection duration

Yearly averages (associated standard errors) in pathogen prevalence were computed per monitored herd. For each of the three tick-borne pathogens, the apparent durations of infection were computed per sentinel steer by counting the number of positive diagnostics between the dates marking the acquisition(s) and loss(es) of the pathogen.

Statistical analyses

The R software *version 3.0.2* was used to perform statistical analyses. The Pearson's product-moment tests were applied to observe if the variations through time in the prevalence of each tick-borne pathogen correlate with those observed for another pathogen, climatic parameters (monthly rainfall and temperature mean), vector abundance or vector incidence (Crawley, 2007). Among herd variations in the assemblage of the surveyed tick-borne pathogens were assessed via correspondence analysis using the R-package ‘ade4’ (Dray and Dufour, 2007).

The impact of co-infection patterns onto the variations in the prevalence of a given pathogen X was tested with generalized linear mixed models that controlled as much as possible the pseudo-replication arising from repeated measures on sentinel-steers and/or sampling geography by declaring the sampling date (DATE) and the monitored herd (SITE) as random factors (Crawley 2007). Such analyses used the glmer function implemented in the ‘lme4’ R-package. The analyses started with the maximal model glmer (X~ Y + Z + (1|DATE) + (1|SITE), family =binomial, nAGQ=1) where X, Y and Z refer to the presence/absence of the three surveyed pathogens. Model simplification was achieved by removing the co-infecting term(s) Y and/or Z when not significant ($P > 0.05$). The possibility of overdispersion was checked *a posteriori* by computing the ratio of residual deviance onto the residual freedom degrees (Crawley, 2007). As an alternative way to avoid any bias due to pseudo-replication, the analyses on restricted datasets focusing on each climatic area and on one of four remarkable sampling dates corresponding to the local extremes in either tick-vectors incidence (Imin or Imax) or vector abundance (Vmin or Vmax) characterized in Biguezoton et al. (2016) was performed. Accordingly, the new maximal model was glmer (X~ Y + Z + (1|SITE), family

=binomial, nAGQ=1). In South Benin, Vmin and Vmax corresponded to March and September 2012 respectively while Imin and Imax corresponded to November and April 2016 respectively (Biguezoton et al., 2016). In North Benin, Vmin and Vmax corresponded to February and November 2012 respectively while Imin and Imax corresponded to February and August 2012 respectively (Biguezoton et al., 2016). In Burkina Faso, Vmin and Vmax corresponded to April and November 2012 respectively while Imin and Imax corresponded to January 2013 and June 2012 respectively (Biguezoton et al., 2016).

Exact Fisher tests were performed on 2 x 2 contingency tables defined on the overall number of steers considered with rows referring the presence/absence of one pathogen and colons referring to the presence/absence of another pathogen. The degree of departure from random associations was quantified with the index I_c presented by Ginsberg (2008), so that I_c <0 referred to cases where coinfections were less frequently observed than expected by chance (Ginsberg, 2008).

Ethics statement

Herders received full information on the study objectives and procedures before signing written informed consent. Sampling was systematically coupled with veterinary inspection of the herd; in the case of infection, animals received free treatment. All study procedures were reviewed and approved by the CSIRO Social Science Human Research Committee under approval number Ref 038/12.

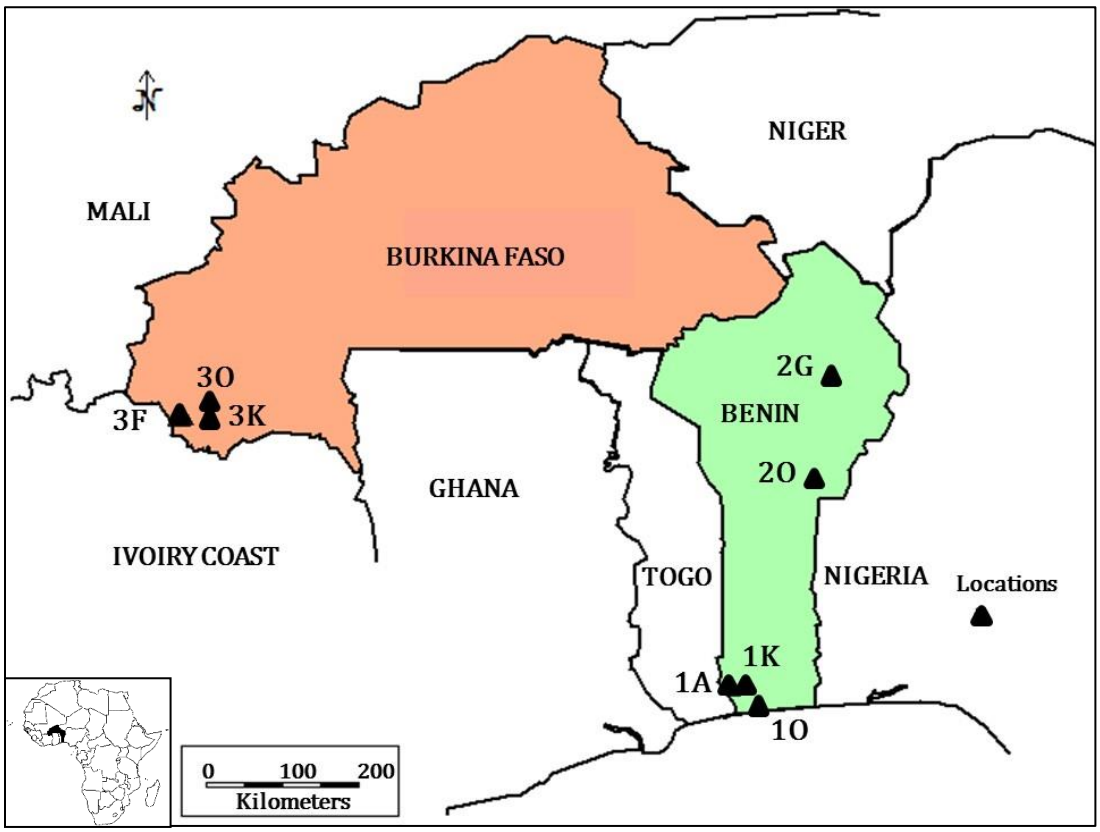

Figure 1: Sampling geography.
Sampling sites (triangles) are identified by an alphanumeric code so that a number refers to the climatic area and a letter to the locality. In South-Benin (area #1), site 1A corresponds to for Athiémé, while 1K and 10 refer to Kpinnou and Ouidah, respectively. In North-Benin (area #2), sites 2O and 2G stand for Okpara and Gogounou, respectively. In south-west Burkina Faso (area #3), sites of Farnifaso, Kimini, and Ouangolodougou are represented by 3F, 3K and 3O, respectively.

RESULTS

Infection duration and tick-borne pathogens prevalence

In all, 1 081 blood-samples were analysed (Table 1) due to presumption of cross-samples contamination of 5.1% (n=58) samples collected.

A. marginale was the most frequently observed pathogen species over the entire study (Figure 2). Its yearly average in prevalence did not differ among herds within climatic areas (P > 0.19) but significantly differed among areas (P = 0.017), ranging from 0.75 to 1 in South-Benin, from 0.78 to 1 in North-Benin and from 0.55 to 0.62 in South-West Burkina Faso. Cattle infections by *A. marginale* usually lasted from two to four months except in two monitored herds (1K and 2O; Figure 2). In 1K and 2O, negative diagnostics were so rare that the estimates of *A. marginale* prevalence and apparent duration of infection were maximal (~100% and ~12 month-long, respectively; Figure 2).

The yearly means in *B. bigemina* prevalence estimates ranged from 0.62 to 0.78 in South-Benin, from 0.41 to 0.85 in North-Benin and from 0.22 to 0.60 in South-West Burkina Faso (Figure 2). No significant differences in the yearly averages in *B. bigemina* prevalence was observed within (P > 0.09) as among areas (P = 0.11). Cattle infection by *B. bigemina* lasted in average from two to three months in South-Benin, from two to four months in North-Benin and from one to two months in South-West Burkina Faso.

If *B. bovis* occurred in all three areas, it was the least frequently observed tick-borne pathogen with its prevalence ranging from 0.18 to 0.58 in South-Benin and from 0.18 to 0.22 elsewhere (Figure 2). Its yearly average in prevalence differed neither within nor between areas (P > 0.11 and P=0.05, respectively). The apparent duration of *B. bovis* infection lasted about a month anywhere but twice as much in 2O (Figure 2).

Importance of environmental and vectors-related parameters

Within herds, neither the variation in rainfall nor that in monthly temperature mean correlated with the variation in the prevalence of *A. marginale*, *B. bigemina* or *B. bovis* (P > 0.05 in all cases).

Significant correlations between pathogen prevalence and either vector incidence or vector abundance appeared in each climatic area (Table 2). In South and North Benin, *A. marginale* prevalence were positively correlated with *R. microplus* abundance (P= 6.10^{-5} and P= 0.0015 respectively) and with *R. microplus* monthly incidence rate (P= 2.10^{-4} and P= 9.10^{-9} respectively) (Table 2). Similarly, in South Benin and Burkina Faso, *B. bovis* prevalence were positively correlated with *R. microplus* abundance (P= 4.4 $10^{-5}$and P= 8.7 10^{-5} respectively) (Table 2). By contrast, *B. bigemina* prevalence in North Benin were significantly negatively correlated with *R. microplus* abundance and with *R. microplus* incidence (P= 8.6 10^{-3} and P= 5.8 10^{-4} respectively) (Table 2).

Coinfections patterns

Multivariate analysis did not allow detecting great differences in the assemblage of *A. marginale*, *B. bovis* and *B. bigemina* aside a tendency for larger variations in four of the eight surveyed herds (1A, 1K, 3K and 3O; Figure 3).

In South Benin, the infections caused by *B. bigemina* and *B. bovis* were significantly and negatively correlated (ρ= - 0.12, P=0.018; Table 3). In North Benin and South-West Burkina Faso, the infections caused by *A. marginale* and *B. bigemina* were also significantly (P=0.0067 and P= 0.0035, respectively) and negatively correlated (ρ= - 0.16 and ρ= - 0.14, respectively; Table 3).

Mixed generalized models also detected significant detrimental impacts of coinfection on the average prevalence at diverse spatiotemporal scales for all three tick-borne pathogens surveyed (Table 3). For instance, the co-occurrence of *B. bigemina* significantly reduced *A. marginale* prevalence from 0.91 ± 0.03 to 0.86± 0.02 over the entire study (P = 0.027) and from 0.64 ± 0.04 to 0.50± 0.04 in Burkina Faso (P = 0.0049). Reciprocally, the co-occurrence of *A. marginale* reduced *B.*

bigemina prevalence from 0.69±0.03 to 0.60±0.02 over the entire study (P =0.018) and from 0.53±0.04 to 0.41±0.04 in Burkina Faso (P =0.028). By contrast, in South Benin, it was the coinfection by *B. bovis* that reduced the *B. bigemina* prevalence from 0.75 ± 0.04 to 0.61± 0.04 (P =0.0041). In turn, coinfection by *B. bigemina* prevalence reduced *B. bovis* prevalence from 0.45±0.05 to 0.28±0.04 in South Benin (P =0.007). Interestingly, *A. marginale* had no significant effect (P > 0.05) on *B. bovis* prevalence at any geographical scale.

Avoiding any risk of temporal correlation by focusing on more local scales and on particular dates corresponding either to the sampling dates for the local extremes in vector abundance (Vmax and Vmin) or the local extremes in infection frequency (Imax and Imin) analysis revealed some pathogens associations which were significantly not randomly frequent (Table 4). Regarding climatic areas scale, significant less association than by chance between *B. bigemina* and *B. bovis* (P= 0.04; *lc* = - 10.1) was evidenced at Vmin (D1) within South Benin (Table 4). Likewise, in South-West Burkina Faso significant less association than by chance was observed between *A. marginale* and *B. bigemina* (P= 0.01; *lc* = - 13.8) at Vmin (D2) (Table 4).

Table 1: Sampling actually used in (molecular) diagnostic methods.

	Sampling dates													
Site	**D0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**
1A	12	12	12	11	4	12	0	12	6	6	11	12		
1K	11	12	12	12	12	12	12	12	12	11	11	11		
1O	12	10	12	12	12	11	12	12	12	12	12	11		
2G	10	12	12	12	12	12	11	12	0	11	11	12		
2O	12	12	12	12	12	12	12	12	12	12	12	12		
3F			10	10	12	12	12	12	12	12	11	11	12	12
3K			10	12	12	12	11	12	12	12	12	12	12	12
3O			10	12	12	12	12	12	12	12	11	12	12	12

Sites are coded as described in Figure 1. The first sampling date (D0) and last one (D13) correspond to February 2012 and March 2013 respectively. For each sampling date, the sample size is indicated.

Table 2: Correlations between pathogens prevalence and, either the composition of the vector community or the presence of co-infecting pathogens.

Areas		**Tick abundance**					**Tick incidence**					**Competitors**		
		Ra	Rd	Rg	Rm	ALL	Ra	Rd	Rg	Rm	ALL	Am	Bbg	Bbv
1-South Benin	Am				+***	+***				+***				
	Bbg													–*
	Bbv				+***	+***				+**			–*	
2-North Benin	Am				+**	+**				+***	+**		–**	
	Bbg				–**					–***		–**		
	Bbv													
3-South-West Burkina Faso	Am				–**	–**							–**	
	Bbg				+*	+*						–**		
	Bbv				+***	+***	+*							

Signs + and – refers to significantly positive and negative correlations, respectively. Significance levels are as follows: * means P-value <0.05, ** means P-value <0.01 while *** means P-value <0.001. Am, Bbg, Bbv, Ra, Rd, Rg, Rm and ALL refer to *A. marginale*, *B. bigemina*, *B. bovis*, *R. annulatus*, *R. decoloratus*, *R. geigyi*, *R. microplus* and to all vector-species taken together, respectively.

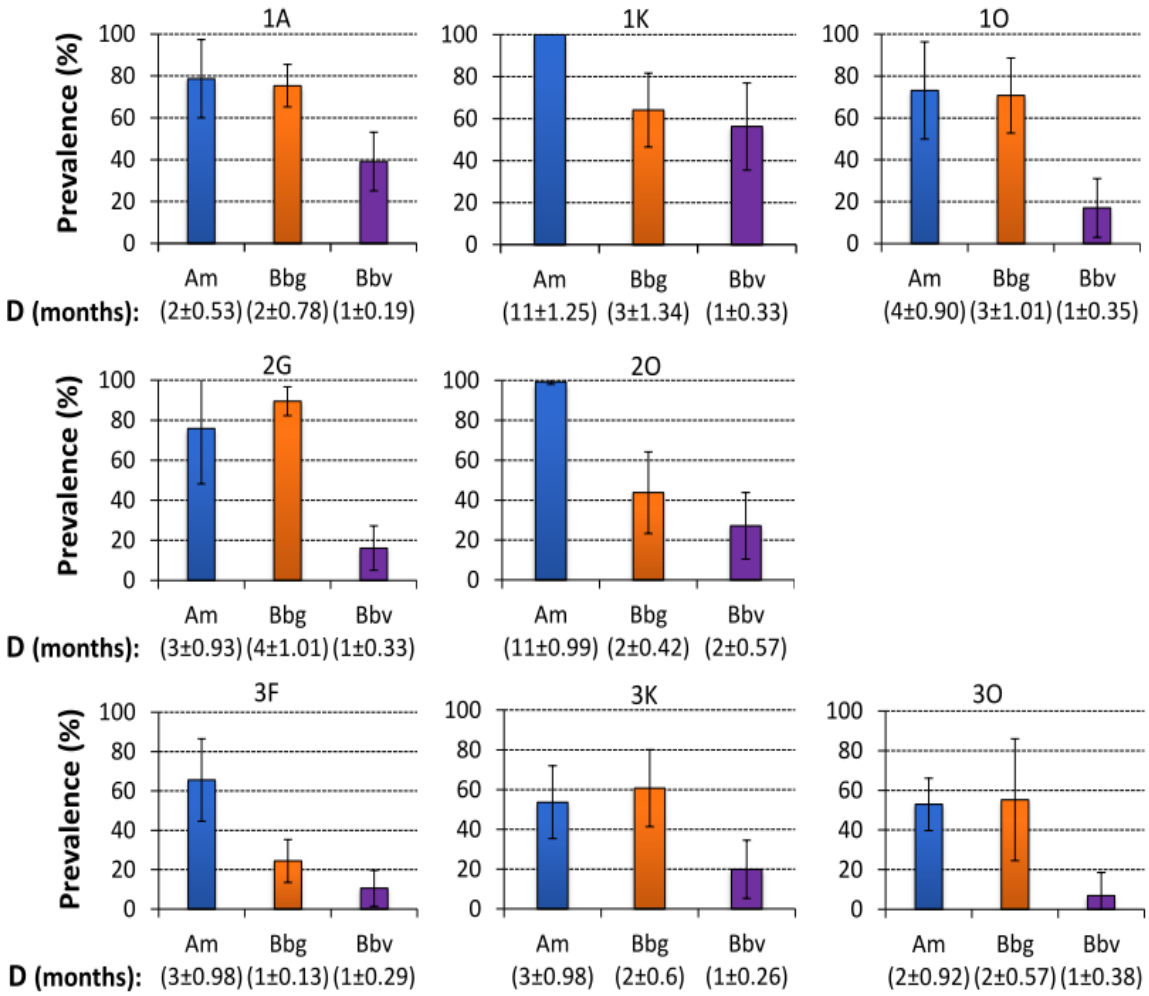

Figure 2: Infection duration and prevalence.
Histograms display the yearly averages in prevalence for *A. marginale* (Am), *B. bigemina* (Bbg) and *B. bovis* (Bbv). The mean apparent durations of infection (D in months) are indicated below histograms.

Table 3: Impacts of coinfection patterns in some prevalence estimates.

Climatic areas	Dates/Periods	Am	Bbg	Bbv
Whole dataset	Twelve months' survey	~ Bbg↓ + (1 | Date) + (1 | Site)	~ Am↓ + (1 | Date) + (1 | Site)	~(1 | Date) + (1 | Site)
1-South Benin	Twelve months' survey	~ (1 | Date) + (1 | Site)	~ Bbv↓ + (1 | Date) + (1 | Site)	~ Bbg↓+ (1 | Date) + (1 | Site)
	Vmax	NA	~(1 | Site)	NA
	Vmin	NA	~ Bbv↓ + (1 | Site)	~ Bbg↓ + (1 | Site)
	Imax	NA	~(1 | Site)	~ (1 | Site)
	Imin	NA	~(1 | Site)	~ (1 | Site)

2-North Benin	Twelve months' survey	~(1 | Date) + (1 | Site)	NA	~(1 | Date) + (1 | Site)
	Vmax	NA	~ (1 | Site)	~ (1 | Site)
	Vmin	NA	NA	~ (1 | Site)
	Imax	NA	~(1 | Site)	NA
	Imin	NA	NA	~ (1 | Site)
3-Burkina Faso	Twelve months' survey	~ Bbg↓ + (1 | Date) + (1 | Site)	~ Am↓ + (1 | Date) + (1 | Site)	~(1 | Date) + (1 | Site)
	Vmax	~ (1 | Site)	~ Am↓ + (1 | Site)	~ (1 | Site)
	Vmin	~ Bbg↓ + (1 | Site)	NA	~ (1 | Site)
	Imax	NA	NA	~ (1 | Site)
	Imin	NA	~(1 | Site)	NA

The minimal models (based on Generalized linear mixed model fit by maximum likelihood, glmer) are detailed in R language. Am, Bbg and Bbv refer respectively to *A. marginale*, *B. bigemina* and *B. bovis*. Vmax and Vmin respectively correspond to the sampling dates for local maximum and minimum in vector abundance. Imax and Imin correspond to the local maximum and minimum in infection cases, respectively. Arrows represent the down effects of the significant co-infection cases.

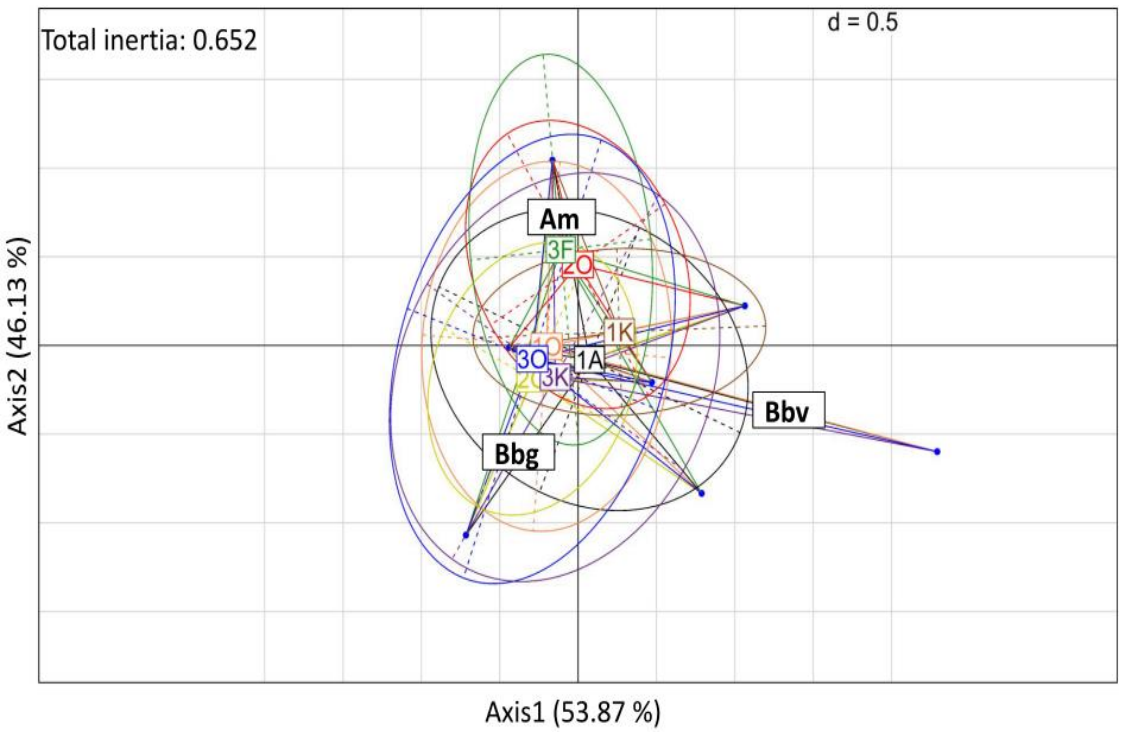

Figure 3: Assemblages in tick-borne pathogens across herds.
Am, Bbg and Bbv refer respectively to *Anaplasma marginale*, *Babesia bigemina* and *Babesia bovis*.

Table 4: Within-areas pairwise coinfection patterns at particular dates.

Climatic area	Dates	Pathogens		P	*Ic*
1-South Benin	Vmax	*A. marginale*	*B. bigemina*	1.00	0.0
		A. marginale	*B. bovis*	1.00	0.0
		B. bigemina	*B. bovis*	0.26	-5.7
	Vmin	*A. marginale*	*B. bigemina*	0.48	-4.3
		A. marginale	*B. bovis*	0.70	4.4
		B. bigemina	*B. bovis*	0.04*	-10.1
	Imax	*A. marginale*	*B. bigemina*	1.00	0.7
		A. marginale	*B. bovis*	0.05	7.2
		B. bigemina	*B. bovis*	0.55	0.9
	Imin	*A. marginale*	*B. bigemina*	0.64	3.2
		A. marginale	*B. bovis*	1.00	-0.2
		B. bigemina	*B. bovis*	0.57	-3.5
2-North Benin	Vmax	*A. marginale*	*B. bigemina*	1.00	0.0
		A. marginale	*B. bovis*	1.00	0.0
		B. bigemina	*B. bovis*	0.27	-5.0
	Vmin	*A. marginale*	*B. bigemina*	1.00	2.4
		A. marginale	*B. bovis*	1.00	2.6
		B. bigemina	*B. bovis*	1.00	2.1
	Imax	*A. marginale*	*B. bigemina*	1.00	0.0
		A. marginale	*B. bovis*	1.00	0.0
		B. bigemina	*B. bovis*	0.52	-1.8
	Imin	*A. marginale*	*B. bigemina*	1.00	2.4
		A. marginale	*B. bovis*	1.00	2.6
		B. bigemina	*B. bovis*	1.00	2.1
3-Burkina Faso	Vmax	*A. marginale*	*B. bigemina*	1.00	0.0
		A. marginale	*B. bovis*	1.00	-2.8
		B. bigemina	*B. bovis*	1.00	-2.4
	Vmin	*A. marginale*	*B. bigemina*	0.01**	-13.8
		A. marginale	*B. bovis*	1.00	1.9
		B. bigemina	*B. bovis*	0.47	3.8
	Imax	*A. marginale*	*B. bigemina*	0.71	-1.7
		A. marginale	*B. bovis*	0.56	3.2
		B. bigemina	*B. bovis*	1.00	-1.0
	Imin	*A. marginale*	*B. bigemina*	0.68	4.2
		A. marginale	*B. bovis*	0.70	4.4
		B. bigemina	*B. bovis*	1.00	1.4

Significance levels are as follows: * means P-value <0.05 while ** means P-value≤0.01. The four particular dates were defined as the sampling dates corresponding to the local maximum or minimum in vector abundance (Vmax and Vmin, respectively) or as the local maximum or minimum in the number of infection cases (Imax and Imin, respectively). P and *Ic* correspond to the Fisher exact test P-values and the index of coinfection, respectively.

DISCUSSION

Variation in *Boophilus* tick-borne pathogens assemblage and host susceptibility

Tick-borne pathogens are not homogeneously distributed in the eight herds (Figure 3). Herds 1A and 1K presented singular pattern due to most frequent *B. bovis* infection (Figures 2 and 3). Likewise, singular pattern of herds 3K and 3O, is supported by the fact that they are the only herds where prevalence of *B. bigemina* exceeded those of *A. marginale* with moderate estimate (~60%) (Figures 2 and 3). Such variability in these blood-associated pathogens assemblage could be deriven from host characteristics and not interspecific interactions (Cohen et al., 2015). The diversity of cattle race involving in the study, unfortunately uncontrolled here, could reinforce this hypothesis. Furthermore, as the monitored steers were not all from the same breed, and given that West Africa holds a great bovine genetic diversity (Hanotte et al., 2002), surveyed steers' immune reaction could be different. For instance, *Bos indicus* (zebu) breeds that are as that sampled in South-West of Burkina Faso, is known to be less susceptible (i.e., presenting milder symptoms when infected) to babesiosis than *Bos taurus* (taurine) breeds taurine cattle and to display less sever clinical symptoms whenever babesiosis occurs (Chartier et al., 2000). Indeed, less animal were infected by *B. bovis* in herds within South-West of Burkina Faso than elsewhere (Figure 2). Such variation of *Bos taurus* and *Bos indicus* susceptibility in regard to *A. marginale, B. bigemina* and *B. bovis* was investigated; *Bos indicus* being less sensitive than *Bos Taurus*. Moreover, variation of susceptibility between *Bos taurus* and *Bos indicus* to tick infestation was shown to lay on differentiation of genes involved in innate inflammatory processes (Piper et al., 2008). It is noteworthy that parasites coinfection affects host susceptibility even within a subpopulation (Cattadori et al., 2007). Herein, parasites coinfection variation among hosts could influence hosts susceptibility variation. In other words, steers from the different areas may be coinfected with variable degrees or with variable prevalence as observed among areas in the present study (Tables 3 and 4).

Relationship between the local prevalence and the apparent duration of infection

In the present study, *A. marginale* was the most prevalent, followed by *B. bigemina* and then *B. bovis* (Figure 2). The same ranking order of the three tick-borne pathogens was noticed for infection duration calculated (Figure 2). This pattern could result from differences in incubation periods (prepatent period) between pathogens. Regarding *A. marginale*, this period lasts in average 28 days varying from 7 to 60 days according to the infective dose. For *Babesia* species, the prepatent period is shorter, lasting generally 12–18 days after tick attachment for *B. bigemina* and 6–12 days for *B. bovis*. However, *B. bigemina* prepatent period could be shortened (6-12 days) when vectored by male *R. microplus*.

Besides, the maximal duration of infection observed in this study concerned *A. marginale* in herds 1K and 2O (i.e. 11 to 12 months) (state farms). Such results are very likely to result from chronic infection of the steers (Suarez and Noh, 2011) or superinfection. Given the associated high prevalence estimates (i.e. 1) during the twelve months' longitudinal survey and the absence of mortality, it was concluded that the concerned steers reach enzootic stability in *A. marginale*. The high prevalence estimates registered here might arise from the cattle race involving in this study in herds 1K and 2O: Girolando, i.e. hybrid between *Bos indicus* (Gir) and *Bos taurus* (Holstein) breeds given that hybrids are known to be more sensitive to *A. marginale* than pure *Bos indicus* breeds raised in the other monitored herds (e.g local breed of West Africa). It is noteworthy to mention that cattle "Girolando" were imported from Brazil and some of them died in the past, before the present study in herds 1K and 2O due to *A. marginale* infections (Farougou Souaïbou, data not published). Thus, the current enzootic stability is a post event after the elimination of the animals with weak immunity against *A. marginale* strains within the state farms where

the cattle "Girolando" share the same spaces with local cattle races.

Current prevalence compared to other published results in West Africa: has current strains of *B. bovis* been co-introduced with *R. microplus* in Benin?

Globally, in Benin, 86.20% of surveyed steers were infected by *A. marginale* while 67.96% were infected by *B. bigemina* and 30.88% by *B. bovis*. In Burkina Faso, 57.24% of steers were infected by *A. marginale*, 46.87% by *B. bigemina* and 12.52% by *B. bovis*. The higher levels of *A. marginale* infection in both countries could be explained by strains superinfection (Castañeda et al., 2015), host susceptibility or an enzootic stability. Such trends of higher prevalence estimates concerning *A. marginale* were reported in a study covering four West African countries (including Benin and Burkina Faso) and three East African Countries (Heylen et al., 2023). However, opposite trends to the highest dominance of *A. marginale* have also been reported. Furthermore, a recent study carried out in 2016-2017 in North-Benin and East-Burkina Faso, indicated lower prevalence (<30%) of the three tick-borne pathogens in cattle studied (Ouedraogo et al., 2021). Possible decrease of hosts susceptibility or pathogens virulence could be the reasons of such differences, though the number and races of the cattle surveyed in the compared studies might induce these results.

Besides, since the prevalence of *B. bovis* in North Benin in the present study is higher than in past studies and given that *R. microplus* is known to be a better competent vector, therefore, the possibility of recent new introduction of *B. bovis* through *R. microplus* introduction in Benin was checked. Prior to that it is noteworthy to underline that the review of past researches published in West Africa concerning *B. bovis* indicated that first infections to cattle were encountered before the 1990's and its prevalence was even sometimes superior to that of *B. bigemina* (e.g. in Benin 0.31 for *B. bovis* and 0.14 for *B. bigemina*). However, at the start of 2000's most of tick-borne pathogens studies didn't report the presence of this *Babesia* (Farougou et al., 2007) until the introduction of the invasive tick, *R. microplus*. Further to this introduction, some mortalities of bovines due to suspect cases of babesiosis caused by *B. bovis* were recorded in Benin (Maxime Madder, data not published) as when animals get first contact with a virulent pathogen. Thereafter, in 2011 an epidemiologic study determined a prevalence of 0.24 of *B bovis* on 210 bovines at the national scale with a variation of 0.09 (Atacora, North-Benin) to 0.58 (Zou, towards the Centre Benin) (Patsanza, 2012). The same study highlighted a positive correlation between the invasive tick and *B bovis* (Patsanza, 2012) as it is recorded in the current study in South-Benin (area #1) (Table 2). Thus, *R. microplus* would have been introduced into Benin with strains of its vectored pathogens, *B. bovis*, just like it was the case in South Africa (Tønnesen et al., 2006).

Variation of relationships between *Boophilus* tick-borne pathogens and vectors according to *R. microplus* history of invasion and abundance geographic distribution

It is noteworthy to underline the chronology of *R. microplus'* invasion: it was imported in South Benin in 2004 (area #1), colonized North-Benin (area #2) between 2004 and 2011 but was firstly detected in South-West Burkina Faso (area#3) in late 2011, i.e. a few months before the start of the present survey. Regarding the study on *R. microplus* relative abundance checked during the current study within the targeted climatic areas it was as follows: 71% in South Benin; 62% in North Benin and 75% in South-West Burkina Faso.

Positive correlations were noticed between *B. bovis* prevalence and *R. microplus* abundance in area #1 and area#3 (Table 2). Thus, variations in prevalence of *B. bovis* appeared to be driven by the variation in *R. microplus* abundance in South Benin and South-West Burkina Faso. Positive correlation was also previously evidenced between *B. bovis* and *R. microplus* in 2011 in Benin (Patsanza, 2012). *R. microplus* introduction in Benin seemed to have been achieved with *B. bovis* like in South Africa (Tønnesen et al.,

2006) but the positive correlations registered are worrying, since *R. microplus* present high abundance in several herds/areas in Benin and South-West Burkina Faso (Biguezoton et al., 2016). Because *B. bovis* is an acute infection resulting in more severe disease of cattle – though, fortunately, it was not the case with the surveyed steers – and in Benin and Burkina Faso, as in most West African countries, breeding lay in 95% on agro-pastoral system with low input (CORAF/WECARD, 2010).

Interestingly, in areas #2 where the presence of *R. microplus* is more recent than in area #1 no significant correlation was evidenced between *B. bovis* and *R. microplus* abundance. Besides, since the way of transmission is not the same for the three parasites studied, and as we only focused our analyses on adult tick, relationships between ticks' incidence and that of pathogens were also investigated. Negative correlation was revealed between *B. bigemina* and *R. microplus* incidences within area #2 (Table 2). Moreover, positive correlation was observed between *A. marginale* and *R. microplus* incidences within area #1 and area#2 (Table 2). Thus, relationships between vectors and pathogens are variable according to areas. Yet, some correlation computed were not significant but have the same trends that those cited above. The variation observed here may be partly due to variations in susceptibility among breeds, though, climate difference between areas could also lead to such results.

Avoidance between babesiosis and anaplasmosis-causing pathogens within cattle in Benin and Burkina Faso

The pattern of the interaction between pathogens could influence disease epidemiology or the host survival (Onah et al., 2004) and consequently the disease control strategy. Unfortunately, interactions between cattle blood parasites were scarcely studied, particularly in West Africa. Researches done there focused in majority on single or co-occurred prevalence of parasites (Nwoha et al., 2013). Therefore, the current work is the first in West Africa dealing with *Boophilus* tick-borne pathogens interaction.

Mainly, negative interaction between *Anaplasma marginale* and *Babesia bigemina/ Babesia bigemina* and *Babesia bovis* was highlighted in this study, though at regional scale using the 12 months' dataset, only avoidance between *A. marginale* and *B. bigemina* were revealed (Table 3). Thus, these tick-borne pathogens seemed to avoid each other according to areas/herds where analyses were possible (Table 3). Herein, pathogens studied negatively interact on the contrary to their vectors which were demonstrated to be aggregative (Biguezoton et al., 2016). Past researches focusing on vector-borne pathogens interactions, including sometimes at least one of the *Boophilus* tick-borne pathogens studied here highlighted also negative interaction. For instance, in Algeria, absolute exclusion between *T. annulata* and *B. bovis*, strong avoidance between *T. annulata* and *A. marginale* and a moderate one between *A. marginale* and *T. orientalis* within cattle, were demonstrated (Dib et al., 2008). Negative interactions between pathogens or parasites were also demonstrated elsewhere using others models than cattle. Herein, *Plasmodium* sp. and *Babesia* sp. infections study in a wild primate, *Propithecus verreauxi* in Western Madagascar suggested negative interaction (Springer et al., 2015). Others examples of negatives interactions between pathogens (parasites) are available in the literature (Ginsberg, 2008; Telfer et al., 2010).

Such negative interaction could be due to cross-immunization or resource competition (Dib et al., 2008). Interestingly, it could help animals to more survive than in case of single infection (Onah et al., 2004) or other pattern of interactions. Since any animal mortality was not observed during current study, despite non-zero prevalence of the most virulent *Babesia*, *B. bovis*, it could be assumed that such interaction might be beneficial to the steers. In fact, *B. bovis*, the most virulent *Babesia* would be more virulent for West African cattle. However, neither clinical case nor surveyed steers mortality was encountered till the end of the twelve months' longitudinal survey. Therefore, such results could be one of the consequences of the negative interactions

occurring between pathogens (Onah et al., 2004). In this latter cited work, authors demonstrated that concurrent *Strongyloides ratti* and *Trypanosoma brucei* infection resulted in the prolongation of the life span of host (Onah et al., 2004). Immune responses would be the mainly mechanism of such result.

However, the three tick-borne pathogens targeted in this study might not be the only one which contributed to the extending of the steers' life span. Otherwise, other pathogens transmitted by other tick species (e.g. *Amblyomma variegatum*) could also be in cause. Therefore, it would be useful to include such pathogens in futures studies. Besides, steers could also have been infected by multiple *B. bovis* strains, which competition enhanced animals' survival. Such intraspecific competition enhancing host survival was observed with the causal agent of human African sleeping sickness, *Trypanosoma brucei* (Balmer et al., 2009). In this case, the explanation of the results could base on: resource competition, direct allelopathic interference competition or immune-mediated apparent competition (Balmer et al., 2009). Nevertheless, it is possible that steers immunity simply helped them to escape from babesiosis caused by *B. bovis*.

Otherwise, even if it was marginal, positive interaction between *B. bovis* and *A. marginale* was observed within South Benin when the three studied pathogens co-occurrence's prevalence reached its highest value (Imax=D9; Table 4). Elsewhere, positive interaction was also reported within mice between *Anaplasma phagocytophilum* and *Borrelia burgdorferi* (Holden et al., 2005). Indeed, authors of this work demonstrated that antibody response to *A. phagocytophilum*, but not to *B. burgdorferi*, decreased when coinfection occurred (Holden et al., 2005). Ginsberg (2008) also showed that there was a positive interaction between *Ehrlichia chafeensis* and *Ehrlichia ewingi in A. americanum* ticks. Likewise, positive interaction between HIV and *Plasmodium sp* is known. Cooperation between pathogens where the immunocompromising by one agent opening the way to infection by the other could explain positive association observed here (Dib et al., 2008; Holden et al., 2005). However, since both involved pathogens (i.e. *B. bovis* and *A. marginale*) are vectored by the same *Boophilus* species, identical ecological needs could also lead to such, though marginal, aggregative interaction (Dib et al., 2008). Another explanation of this interaction could be the immunosuppressive effects.

Deep analyses are now needed to more understand the mechanism involving in revealed interactions.

Conclusion

Large prevalence of *A. marginale* are likely related to large apparent period of infection which could be in relation with host susceptibility. This study has shown for the first time that imported "Girolando" in Benin within the state farms (1K & 2O) reach enzootic stability in *A. marginale* infection. It has also been demonstrated that tick-borne pathogens assemblage varies according to herds. Furthermore, results suggest that *R. microplus* was co-introduced in Benin with *B. bovis* like in South Africa. One of the most attractive result of this paper is mainly avoidance evidenced between the *Boophilus* tick-borne pathogens using samples from West Africa. Such negative interaction could influence concerned diseases epidemiology and be used in disease control strategies. This main interaction is opposite to that observed with the vectors and is likely the reason why cattle seemed to subvert to babesiosis caused by *B. bovis* within herds from both Benin and Burkina Faso. Therefore, it would be a mistake considering pathogen species in isolation rather than pathogens communities. Such results open the way to future works focusing on the mechanisms and impacts of these interactions on epidemiology of concerned diseases. Moreover, positive correlation was evidenced between *B. bovis* and *R. microplus*. Given the potential impacts of these two parasites, more attention should be paid in corresponding areas towards breeders and veterinary surgeons.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

ASB, SA, HA, SF, designed the study. HA, ASB, SA, SZ coordinated and participated to field sampling. ASB, HB and MT performed pathogens diagnosis. ASB and CC analysed data and wrote the first draft. All authors read and approved the final version of the manuscript.

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