# Population genetics of the invasive tick Rhipicephalus microplus in Benin and Burkina Faso (West Africa)

Abel S Biguezoton, Hassane Adakal, Valérie Noël, Sebastien Zoungrana, Christine Chevillon

**Available online at http://www.ifgdg.org**

Int. J. Biol. Chem. Sci. 17(7): 2747-2760, December 2023

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

***Original Paper*** http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

# Population genetics of the invasive tick *Rhipicephalus microplus* in Benin and Burkina Faso (West Africa)

Abel S. BIGUEZOTON[1*], Hassane ADAKAL[2#], Valerie NOEL[3], Souaïbou FAROUGOU[4], Sébastien ZOUNGRANA[1†] et Christine CHEVILLON[3]

*[1] Unité de recherche Maladies à Vecteurs et Biodiversité (UMaVeB), Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), 454 Bobo-Dioulasso 01, Burkina Faso.*
*[2] Département des Sciences et Techniques de l'Elevage (DSTE/FASE), Université Dan Dicko Dan Koulodo, BP 465 Maradi, Niger.*
*[3] MIVEGEC (Maladies Infectieuses et Vecteurs : Ecologie, Génétique, Evolution et Contrôle), Univ. Montpellier-CNRS-IRD, 34090, Montpellier, France.*
*[4] Unité de Recherche sur les Maladies Transmissibles (URMAT), Ecole Polytechnique d'Abomey-Calavi, BP 2420 Abomey-Calavi, République du Bénin.*
*[#]Current address : Centre Régional de Santé Animale (CRSA) Parc de SOTUBA, Bamako, Mali.*
*[†]This co-author died before the manuscript submission.*
*[*] Corresponding author, E-mail: babels005@yahoo.fr*

***ACKNOWLEDGMENTS***

*This research was funded by the Australian AusAid program (AusAID, project WECATiC). AB benefited from a SCAC fellowship from the Embassy of France in Benin and complementary funding from IRD, ARS-USDA (Kerrville, Texas), the International Foundation for Science (http://www.ifs.se) and from the International Laboratory of Vector-borne Diseases in West Africa (http://www.lamivect.net/) that regroups different laboratories working on vectors and vector-borne diseases in West Africa.*

Received: 09-11-2023 Accepted: 25-12-2023 Published: 31-12-2023

## ABSTRACT

*Rhipicephalus microplus*, is an invasive tick species associated with the highest economic losses where it occurs. Invasion of West Africa, where breeding is in 95% of case with low input, started in 2000s and was followed by breeder's complaints of acaricide resistance. Since understanding of population structure could help tick control, this study aimed to investigate processes which influence *R. microplus* invasion in Benin and Burkina Faso. Thus, seven microsatellites (SSRs) markers were applied to analyse 436 ticks from Benin and Burkina Faso. Subsequently, determination of population limits, population size, and investigation on isolation by distance pattern were achieved. Analyses revealed that herd is the relevant level of population limit in Benin and with the whole dataset. Significant differentiation was highlighted between herds and between *R. microplus* population from Benin and Burkina Faso. Migration occurred between herds, as indicated by assignment results and migration rates. Furthermore, any bottleneck was not evidenced within dataset. Results suggested that the origin of *R. microplus* population of Burkina Faso could be Côte d'Ivoire. The limit of population being the herd has obviously to be considered in tick control strategies. Thus, to be efficient, tick control programs should primarily focus on the cattle coming back north after transhumance.


**Keywords**: SSRs markers, genetics of population, limit of population, isolation by distance, tick control.

 
DOI: https://dx.doi.org/10.4314/ijbcs.v17i7.12

## INTRODUCTION

In Benin and Burkina Faso, livestock production represents the second contribution after crops to the gross domestic product, without leading to self-sufficiency in animal protein production (FAO, 2009). In both countries, semi-intensive farming systems and the use of exotic breeds remain exceptional: 95% of the livestock industry relies thus on extensive and low-input systems (CEDEAO and CSAO/OCDE, 2008). In Benin, half of the livestock production is concentrated in the north-east where herd rotation among communal pastures, post-harvested crops, savannahs and woodlands optimizes the use of the rare grazing resources (Djenontin, 2010; Djenontin et al., 2012). In north Benin as in Burkina Faso, the seasonal transhumance of part or whole cattle herds to the south in the dry season (and back to north in rainy season) includes very long-distance movements; i.e. from hundreds to thousands kilometers long. Traditional farming systems in Burkina Faso also include sedentary systems where cattle, sheep and goats forage together on communal pastures. In such low-input systems, breeders cannot afford expensive tick control strategies for limiting the economic costs due to ticks and tick-borne pathogens (Adakal et al., 2013a).

In 2002 and 2004, *Rhipicephalus microplus*- the tick responsible of the highest economic losses in cattle production worldwide - was incidentally introduced in Benin and Ivory Coast (Madder et al., 2011, 2012). Within a decade, *R. microplus* had invaded West Africa, from Ivory Coast to Togo along the Atlantic Coast, up to Burkina Faso, Mali and the northern border separating Nigeria and Cameroon (Adakal et al., 2013b; Musa et al., 2014). In late 2011, *R. microplus* outnumbered the three native species belonging to the same *Boophilus* sub-genus in the southern half of Benin (De Clercq et al., 2012). A year later, *R. microplus* predominated the entire cattle tick communities in Ivory Coast (Toure et al., 2014) as well as in Benin and south-west Burkina Faso (Biguezoton et al., 2016). In these areas, *R. microplus* adults were present all year around on cattle (Toure et al., 2014; Biguezoton et al., 2016) expressing resistance to acaricides used to control ticks (Achi et al., 2022). Furthermore, in Burkina Faso adults *R. microplus* are similarly infected by *Ehrlichia ruminantium* as the native tick *Amblyomma variegatum* (Some et al., 2023).

Therefore, the population mechanisms involved in such invasion in West Africa need to be investigated. The present population genetics analysis was aimed at analyzing the variations in *R. microplus* effective population sizes across Benin and south-west Burkina Faso. For this purpose, five new microsatellite markers added to two existing ones were applied. The analysis was completed also by testing whether in 2012 the populations of the invasive tick had already reached mutation/drift equilibrium and migration/drift equilibrium in these recently colonized areas.

## MATERIALS AND METHODS

### *R. microplus* ecology in Benin and Burkina Faso

Cattle hosts primarily consist of local breeds of *Bos indicus*, *B. taurus* and hybrids (e.g. zebu white Fulani, Goudali, M'bororo, Lagune, Borgou, Somba) reared in low input systems. Excepted in south Benin where foraging resources occur all year around, most cattle move south in dry seasons (to Côte d'Ivoire or South-Benin, respectively) to return in rainy seasons (to Burkina Faso and north Benin, respectively).

The herd practices differ in the rare state farms where cattle are enclosed and higher economic resources allow chemical tick-control programs. Some of these state farms are involved in programs aimed at improving cattle production via the use of exotic cattle breeds. This is the case of Kpinnou (south-Benin) where the importation of Gir and Girolando steers from Brazil resulted in *R. microplus* introduction (Madder et al., 2012).

**Sampling**

One cohort of *R. microplus* adults was sampled from February to July 2012 with the objectives to involve three steers per herd and to collect 10 males and 10 females per individual-steer (i.e., per infrapopulation). These objectives were mostly completed in five cattle herds located in south-Benin (Athiémé, Kpinnou, Ouidah) and north-Benin (Gogounou, Okpara), except in Gogounou where sampling can only involve two infrapopulations (steer # 1560 on which 10 females & 7 males were collected; steer #1564 on which 9 females and 6 males were collected). The Beninese set was completed by the addition of three central Beninese samples collected on July 2012 (Figure 1). The first consisted of 17 *R. microplus* adults (12 females and 5 males) collected on one sheep at Dassa; this sample was treated as an 'infrapopulation' in population genetic analyses. The two remaining samples were collected at Glazoué on pastures mostly foraged by sheep; one consists in 10 males and 10 females while the other consisted of 9 females and 6 males. The two later samples were treated as two distinct 'infrapopulations' in population genetic analyses.

Tick sampling also involved two cattle herds from south-west Burkina Faso (Figure 1). The sampling objectives were almost achieved in Kimini 1 where 9 females and 10 males *R. microplus* adults were collected on each of two steers while 8 females and 10 males adults were collected on a third steer. Similarly, two steers from Kimini 2 led to samples of 10 males ticks and 7-9 females ticks while a third one allowed collecting 10 adults of each sex.

**Genotyping**

Prior to genotyping, DNA extraction of ticks was carried out. For this purpose, ticks sampled in the field in tubes containing alcohol (70%) were removed, their sex was determined and they were washed twice in sterile water for five minutes. Female blood-meal content was removed to avoid blood PCR-inhibitory effects. DNA extraction was performed with DNeasy Tissue Kit (QIAGEN). Analyses were performed using five new developed microsatellites markers (L22, L29, L33, L40 and L47) (Biguezoton et al., *In press*) and two existing markers (BmCO7 and BmA05) (Koffi et al., 2006b). Genotyping was processed as previously described (Koffi et al., 2006a,b) except for three newly developed markers which required 5 to 10 additional cycles to ease genotype lectures. PCR products were then engaged in automatic electrophoresis on ABI PRISM 310 sequencer pooling 0.15 µL of size standard (GeneScan-500 LIZ, Applera), Hi-Di formamide (13.5 µL QSP), and 1 µL of the PCR product of each locus. When it was possible, multiplex of loci was done for automatic electrophoresis.

**Population genetic analyses**

***Genotypic biases***

Absence of stuttering errors and large allele dropout (i.e., short allele dominance) was checked using MICRO-CHECKER software (Van Oosterhout et al., 2004). Short allele dominance could also change allele frequency estimates (De Meeûs et al., 2004).

***Hierarchical genetic structure***

This analysis was performed without the central samples associated to sheep. HierFstat (Goudet, 2005) package in R was used to evaluate the relative importance of herds and infrapopulations in *R. microplus* genetic structure. The tests were realised with 10 000 permutations of tick genotypes among infrapopulations within or among herds. Hierarchical F-statistic was also computed at country and herd levels in order to assess the structure presumably resulting from two independent introduction events (in south Benin and Ivory Coast, respectively).

***F-statistics***

F-statistics analyses were performed using *FSTAT* software *version* 2.9.3.2. Estimates of $F_{is}$ and $F_{st}$ unbiased parameters were computed according to Weir and Cockerham (1984). $F_{is}$ represents the

probability of allele identity between the two alleles born by individuals relatively to that two alleles borne by distinct individuals within populations; it is thus a measure of deviation from random union of gametes within infrapopulation (sample) ($F_{is}$ = 0 under local panmixia). $F_{st}$ represents the probability of allele identity between two alleles borne by distinct individuals within populations relatively to that of individuals sampled in distinct populations; it is thus a measure of genetic differentiation between infrapopulations ($F_{st}$ = 0 under free migration across samples). For per-locus estimates, means and standard errors were computed by jackknifing over populations. The means and standard errors of global $F_{is}$ and $F_{st}$ were computed by jackknifing over loci and confidence intervals by bootstrapping over loci.

Hardy–Weinberg equilibrium was tested by performing 10 000 permutations of alleles among individuals within populations. Differentiation between populations was tested using the log-likelihood ratio G with 10 000 permutations among samples of individuals genotypes.

BAPS4 (Corander and Marttinen, 2006) were used to identify possible clustering within infrapopulations and thus to evaluate the possibilities of Walhund effects and/or sib-clustering within infrapopulations *via* examining the impact of such a clustering on $F_{is}$ estimates (Chevillon et al., 2007).

***Sex-biased dispersal***

The central samples associated to sheep (Dassa and Glazoué) were removed before investigating the possibility of sex biased dispersal. $F_{is}$, $F_{st,}$ AIc (i.e. assignment index) and vAIc (i.e. variance of assignment index) tests were used. Analyses were performed with Fstat software. The sex that disperses the least is expected to be associated with lower $F_{is}$, higher $F_{st}$, higher AIc and lower vAIc. Using bilateral test with 10 000 permutations, statistical analyses were computed according to Goudet et al. (2002).

***Assignment and migration***

Individual assignment likelihood analysis was carried out with GENECLASS2 software *version 2.0* (Piry et al., 2004). This allowed computing the mean individual assignment likelihood of each individual-genotype **i** to each possible source population **s**, and thus the percentages of correct assignments per sample.

Migration rate from one sampling locality to any other was computed using BayesAss software *version 1.3* according to Wilson and Rannala (2003). Evidences of migration were also assessed using maximum likelihood method of MIGRATE software *version 3.6. 11* as described by Beerli and Felsenstein (2001).

***Population effective size of R. microplus***

Tick population effective sizes was evaluated using the linkage disequilibrium model of NeEstimator *version 2.01* and considering 0.01 as critical allele frequency (Do et al., 2014).

***Mutation/drift balance and migration/drift balance (i.e. isolation by distance)***

BOTTLENECK software was used to evaluate whether the sampled *R. microplus* populations were at mutation/drift equilibrium. Given He, the expected heterozygosity of a population, let Heq be the heterozygosity that would be expected for a population at mutation/drift equilibrium with the same sampling size and allele number. As allele number decreases faster than heterozygosity when populations size reduced, bottlenecks are signed by He > Heq in subsequent generations. Heq was determined by assuming infinite allele mutation model, a single-step mutation model and/or a mixed mutation model.

GENEPOP software *version 3.4* was used to investigate the occurrence of a signal isolation by distance between cattle herds. Mantel tests based on 10 000 permutations were carried out to test whether the regression slopes between $[F_{ST}/(1-F_{ST})]$ and the logarithm of geographic distances were positive or null.

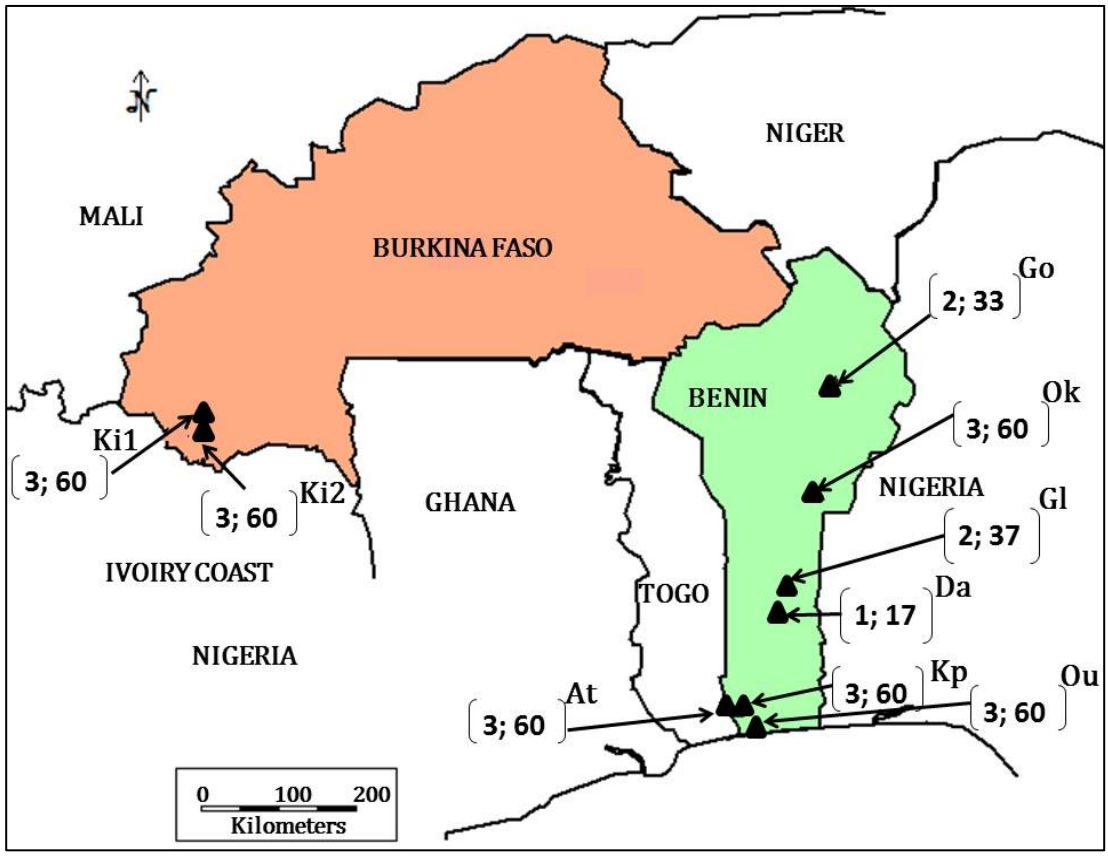


**Figure 1:** Sampling geography (The sampled localities are represented by triangles annotated respectively with the first two letters of the locality names. At, Kp and Ou refer thus to the southern Beninese localities of Athiémé (N 6.5864; E 1.6653), Kpinnou (N 6.5681; E 1.7810) and Ouidah (N 6.3336; E 2.0064), respectively. Da and Gl refer to the central Beninese localities of Dassa (N 7.7500; E 2.1830) and Glazoué (N 7.9707; E 2.2489). Ok and Go refer to the northern Beninese localities of Okpara (N 9.3050; E 2.7314) and Gogounou (N 10.7383; E 2.9233), respectively. Ki1 and Ki2 respectively correspond to two cattle herds managed by Peulh populations in Kimini (Kimini1: N 10.0716; W 4.808; Kimini2: N 10.0857; W -4.778). Letters are followed by two numbers (in square brackets) indicating the number of sampled infrapopulations and the sampling sizes per locality, respectively).

## RESULTS

### Congruency among markers

Significant heterozygote deficit was evidenced on multi-locus genotypes among infrapopulations ($F_{is}$ = 0.161; P<0.0001) (Figure 2). This pattern was separately supported by four individual markers (L22: $F_{is}$ =0.43; P<0.0001- L29: $F_{is}$ = 0.183; P<0.0001- L33: $F_{is}$ =0.405; P<0.0001- BmC07: $F_{is}$ =0.055; P<0.05) (Figure 2). Spearman's correlation computed between $F_{is}$ values and the null alleles frequencies estimated under the assumption of Hardy-Weinberg equilibrium were significantly (P<0.0001) positive. If the tick populations were at Hardy-Weinberg equilibrium, then 8% of $F_{is}$ variations would result from null alleles.

Computing the values taken by $F_{is}$ values on the clusters identified with BAPS4 resulted nonetheless in the disappearance of all heterozygote deficits but those associated to markers L22 and L33 (Figure 2).

### Population structure

There was a significant differentiation between herds ($F_{Herd\text{-}total}$=0.008; P= 0.001) but

apparently not between infrapopulations within herds ($F_{Host\text{-}herd}$ = 0.011; P= 0.08). This pattern persisted when restricting the dataset to Benin ($F_{Herd\text{-}totalBENIN}$=0.001, P= 0.001; $F_{Host\text{-}herd}$ = 0.009, P= 0.195) but not in Burkina Faso (($F_{Herd\text{-}totalBF}$=0.002; P= 0.20; $F_{Host\text{-}herd}$ = 0.019; P= 0.12). Interestingly, removing markers L22 and L33 rendered significant the genetic differentiation between infrapopulations within herds both on the entire dataset ($F_{Host\text{-}herd}$ = 0.02; P= 0.009) and on the sampling performed in Burkina Faso ($F_{Host\text{-}herd}$ = 0.02; P= 0.018). Complementarily, the hierarchical analysis evidenced significant genetic differentiation between Benin and Burkina Faso ($F_{countries}$ = 0.02; P= 0.04). Furthermore, significant heterozygote deficits relatively to Hardy-Weinberg expectations were observed over infrapopulations (-0.06 < $F_{is\_infrapopulation}$ < 0.325; P=0.0001) or cattle herds (-0.06< $F_{is\_herds}$< 0.292; P <0.0001). These signals disappeared when the analysis was performed on the clusters identified by BAPS4 (-0.159 < $F_{is\_clusters}$ < 0.088). Meanwhile, the pairwise estimates of $F_{st}$ among cattle herds remained very low whenever they were significantly non-null (Table 1).

**Sex-biased dispersal**

Even if no linkage disequilibrium was observed between the sex determining locus and any of the microsatellite markers, the values taken by the $F_{is}$ parameter significantly differ between sexes ($F_{is\_FEMALES}$ = 0.09 *Versus* $F_{is\_MALES}$ = 0.22; P=0.0001). Two other cases indicating higher dispersal in male than in female ticks were observed at Okpara (AIc test; P = 0.01) and Kimini1 (Fst test; P = 0.04).

**Assignment probabilities, population effective sizes and migration pattern**

Ninety three percent (93%) of the genotyped ticks were correctly assigned to their sampling infrapopulations according to GENCLASS2. This probability rose to 96.9% or 99.6% when considering the sampling herd or the clusters identified by BAPS4, respectively.

Tick population effective sizes in cattle herds ranged from 52 reproducers in Gogonou to 188 in Kimini1 with most other herds hosting ~100 tick reproducers (Table 2).

The local recruitment rates were maximal in Athieme and Kimini2 (over 99% and equal to 98%, respectively) and ranged from 67% to 72% elsewhere (Table 2). Athieme was the cattle herd which were the most likely to act as an immigrating source: i.e., the Athieme tick population provided 30% of the migrants reaching any other Beninese localities while all other putative migration rates remained below 1% (Table 2). In Burkina Faso, the migration rates between the neighbour herds appeared asymmetrical with 27% estimated from Kimini1 to Kimini2 but 0.16% from Kimini2 to Kimini1. The lowest migrations rates were those estimated between both countries (Table 2).

**Mutation/drift and migration/drift balances**

All the tick samples look at mutation/drift equilibrium irrespectively of the nature of the assumed mutation models (Table 3). Migration/drift balance resulted in signals of isolation by distance characterized by slopes b ranging from b = 0.00150295 (significantly non-null; P = 0.02) among Beninese samples (Figure 3) to b = 0.0041874 (significantly non-null; P< 0.0001) over the entire dataset. Such slopes translate in the mean estimates of neighbourhood sizes (De: $\sigma^2 = 1/(4\pi b)$) equal to 53 or 19 reproducing adults on the Beninese dataset or the entire dataset, respectively.

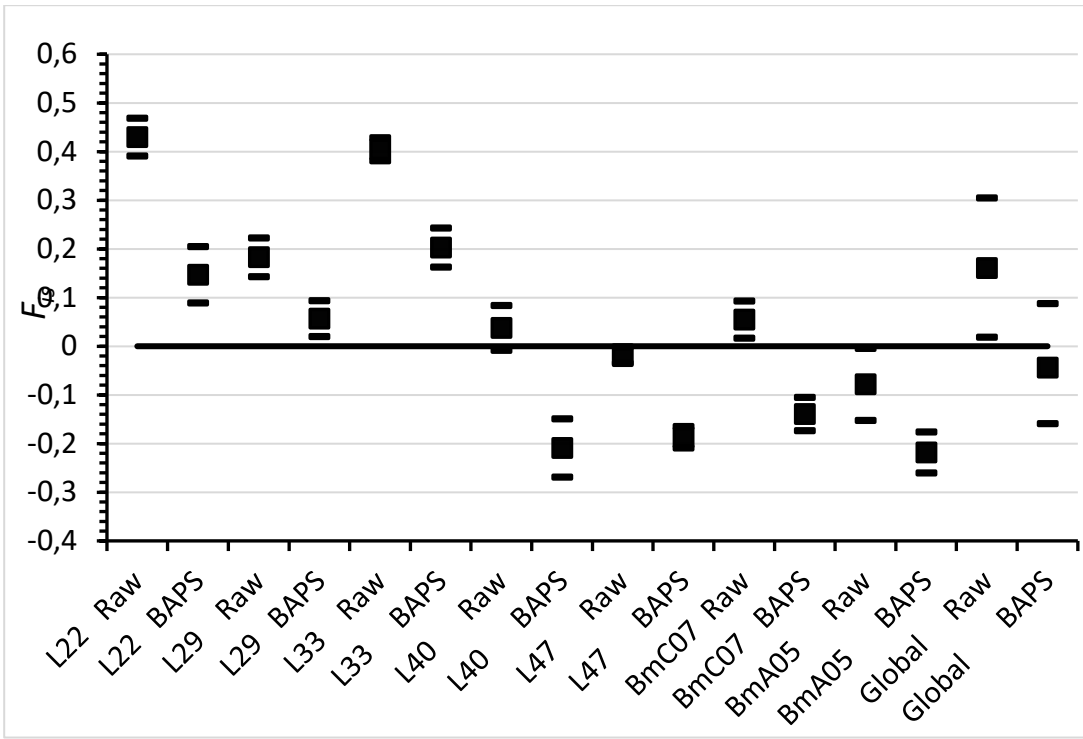


**Figure 2:** Variation in $F_{is}$ estimates (Means and confidence intervals are pictured for the per-locus and multi locus estimates after computations over the infrapopulations sampled on cattle herds (raw) or among the clusters identified by BAPS4 within these infrapopulations (BAPS)).

**Table 1:** Pairwise Fst estimates (Significantly positive values appear in bold characters).

| | **Athieme** | **Kpinnou** | **Okpara** | **ouidah** | **Gogounou** | **Bétécoucou** | **Glazoue** | **Kimini1** |
|---|---|---|---|---|---|---|---|---|
| Kpinnou | 0.002 | | | | | | | |
| Okpara | 0.003 | 0.001 | | | | | | |
| ouidah | **0.003**** | **0.005**** | 0.002 | | | | | |
| Gogounou | 0.015 | 0.01 | 0.01 | 0.009 | | | | |
| Bétécoucou | 0.000 | 0.000 | 0.006 | 0.005 | 0.017 | | | |
| Glazoue | 0.002 | 0.003 | 0.000 | 0.000 | 0.006 | 0.005 | | |
| Kimini1 | **0.027**** | **0.016*** | **0.024**** | **0.021**** | 0.029 | 0.022 | 0.015 | |
| Kimini2 | **0.023**** | **0.007*** | **0.021**** | **0.021**** | **0.024**** | 0.02 | 0.013 | 0.008 |

**Table 2:** Migration rates among localities (Bold characters refer to resident rates (i.e., the proportion of non-emigrating ticks). 95% confidence intervals of population effective sizes and migration rates are given in parentheses)).

| | **Emigration sources:** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Athieme | Kpinnou | Ouidah | Dassa | Glazoue | Okpara | Gogounou | Kimini1 | Kimini2 |
| Ne | 104 | 148 | 109 | 3368 | ∞ | 83 | 52 | 188 | 96 |
| (95% CI) | (36;∞) | (48;∞) | (40;∞) | (16;∞) | (81;∞) | (25;∞) | (18;∞) | (33;∞) | (28;∞) |
| **Immigration targets:** | | | | | | | | | |
| Athieme | **0.99** | 0.001 | 0.001 | 0.003 | 0.003 | 0.001 | 0.001 | 0.001 | 0.002 |
| | **(0.997; 0.9998)** | ($10^{-09}$; 0.007) | ($2.10^{-10}$; 0.009) | ($10^{-5}$; 0.014) | ($6.10^{-6}$; 0.014) | ($10^{-10}$; 0.008) | ($5.10^{-10}$; 0.007) | ($10^{-10}$; 0.009) | ($5.10^{-10}$; 0.012) |
| Kpinnou | 0.24 | **0.67** | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 | 0.078 |
| | (0.17; 0.29) | **(0.667; 0.690)** | ($10^{-05}$; 0.015) | ($9.10^{-06}$; 0.014) | ($4.10^{-05}$; 0.015) | ($10^{-05}$; 0.015) | ($10^{-05}$; 0.013) | ($10^{-05}$; 0.014) | (0.029; 0.134) |
| Ouidah | 0.30 | 0.002 | **0.67** | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
| | (0.26; 0.33) | ($10^{-06}$, 0.014) | **(0.667;0.690)** | ($4.10^{-06}$; 0.012) | ($10^{-06}$; 0.014) | ($10^{-06}$; 0.012) | ($10^{-06}$; 0.013) | ($10^{-06}$, 0.012) | ($10^{-05}$, 0.059) |
| Dassa | 0.12 | 0.008 | 0.044 | **0.68** | 0.009 | 0.101 | 0.008 | 0.009 | 0.014 |
| | (0.04; 0.22) | ($2.10^{-05}$, 0.044) | ($6.10^{-04}$; 0.130) | **(0.667;0.723)** | ($2.10^{-05}$; 0.045) | (0.039; 0.184) | ($2.10^{-05}$; 0.040) | ($2.10^{-05}$, 0.041) | ($3.10^{-05}$, 0.072) |
| Glazoue | 0.064 | 0.008 | 0.095 | 0.005 | **0.68** | 0.102 | 0.006 | 0.006 | 0.037 |
| | (0.02; 0.12) | ($2.10^{-05}$, 0.036) | (0.046; 0.155) | ($2.10^{-05}$; 0.022) | **(0.667;0.702)** | (0.046; 0.178) | ($10^{-05}$; 0.027) | ($2.10^{-05}$, 0.028) | ($10^{-03}$, 0.097) |
| Okpara | 0.31 | 0.002 | 0.003 | 0.003 | 0.003 | **0.67** | 0.002 | 0.002 | 0.015 |
| | (0.279; 0.330) | ($10^{-07}$, 0.013) | ($10^{-07}$; 0.014) | ($4.10^{-06}$; 0.013) | ($8.10^{-06}$; 0.012) | **(0.667; 0.686)** | ($10^{-07}$; 0.013) | ($10^{-06}$; 0.012) | ($10^{-06}$; 0.034) |
| Gogonou | 0.24 | 0.010 | 0.009 | 0.007 | 0.008 | 0.007 | **0.72** | 0.012 | 0.008 |
| | (0.17; 0.29) | ($10^{-06}$, 0.051) | ($10^{-06}$, 0.049) | ($2.10^{-05}$, 0.035) | ($10^{-05}$, 0.036) | ($10^{-06}$, 0.041) | **(0.669; 0.776)** | ($3.10^{-05}$; 0.066) | ($10^{-06}$, 0.040) |
| Kimini1 | 0.036 | 0.004 | 0.003 | 0.004 | 0.004 | 0.004 | 0.003 | **0.68** | 0.27 |
| | (0.002, 0.093) | ($10^{-06}$, 0.022) | ($10^{-06}$, 0.021) | ($2.10^{-05}$, 0.018) | ($4.10^{-05}$, 0.017) | ($10^{-06}$, 0.022) | ($10^{-06}$, 0.016) | **(0.667; 0.703)** | (0.210; 0.320) |
| Kimini2 | 0.012 | 0.002 | 0.002 | 0.003 | 0.003 | 0.001 | 0.001 | 0.002 | 0.98 |
| | ($10^{-09}$, 0.075) | ($10^{-09}$, 0.011) | ($10^{-09}$, 0.011) | ($2.10^{-06}$, 0.015) | ($10^{-06}$, 0.013) | ($10^{-09}$, 0.0092) | ($10^{-09}$, 0.010) | ($10^{-09}$, 0.010) | (0.913; 0.996) |

**Table 3:** Absence of bottleneck signatures (IAM, SMM and TPM refer to the infinite allele mutation model, single-step mutation model and the mixed mutation model respectively. Proportion of SMM in TPM is 70%).

| Herd | IAM | | | TPM | | | SMM | | |
|---|---|---|---|---|---|---|---|---|---|
| | He<Heq | He>Heq | P | He<Heq | He>Heq | P | He<Heq | He>Heq | P |
| Athieme | 3 | 4 | 0.47 | 4 | 3 | 0.95 | 4 | 3 | 0.98 |
| Kpinnou | 5 | 2 | 0.96 | 6 | 1 | 0.99 | 7 | 0 | 1 |
| Okpara | 1 | 6 | 0.15 | 3 | 4 | 0.66 | 5 | 2 | 0.97 |
| Ouidah | 2 | 5 | 0.34 | 5 | 2 | 0.96 | 7 | 0 | 1 |
| Gogounou | 2 | 5 | 0.23 | 6 | 1 | 0.98 | 6 | 1 | 1 |
| Kimini1 | 5 | 2 | 0.77 | 6 | 1 | 0.99 | 7 | 0 | 1 |
| Kimini2 | 3 | 4 | 0.29 | 3 | 4 | 0.77 | 6 | 1 | 1 |

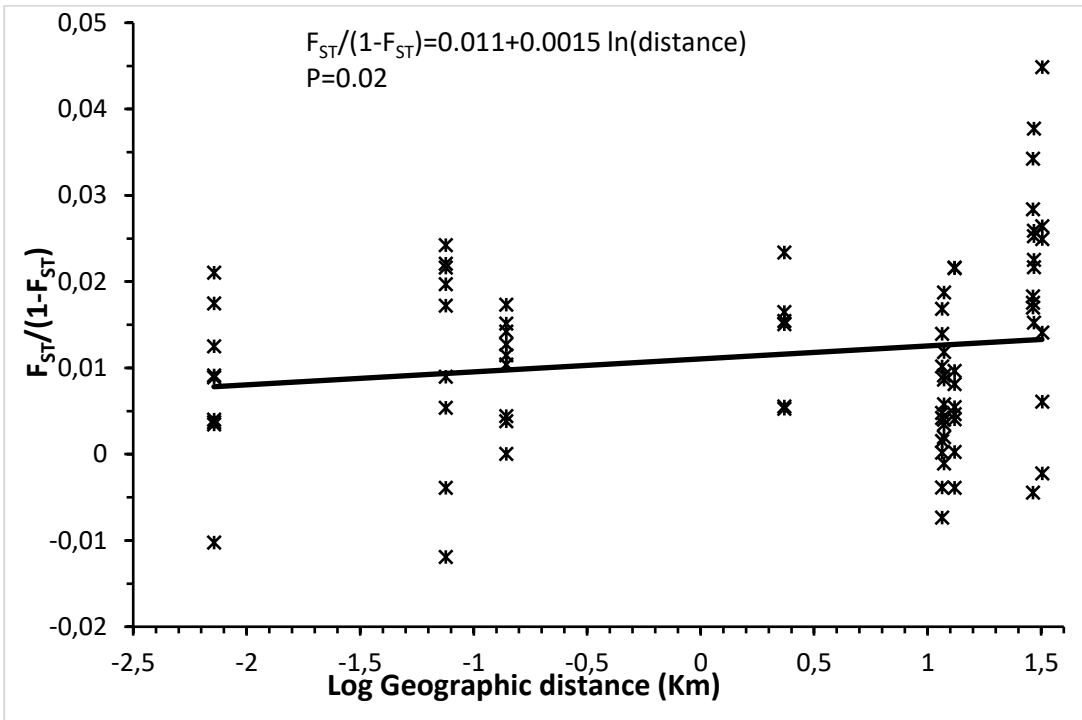


**Figure 3:** Isolation by distance among the infrapopulations sampled on Beninese cattle herds.

## DISCUSSION

### Requirements of new markers to characterize each invasive wave

The microsatellites developed from *R. microplus* ticks of Australian and Mexican origins (Chigagure et al., 2000) were not suitable for characterizing the population processes at play along the invasion of New Caledonia (Koffi et al., 2006b); this lead thus to the development of new microsatellite markers. Later on, the characterization of the genetic population structure of *R. microplus* in South Africa had again required the development of a new set of microsatellite markers (Boucher, 2013). The same scenario has been observed in West Africa (Biguezoton et al., *In press*). Interestingly enough, such a repetition is disconnected from the phylogeographical divergence between, on the one hand, *R. australis* (i.e. the member of *R. microplus* complex that has colonized the Pacific, (Labruna et al., 2009)) and, on the other hand, *R. microplus* (Burger et al., 2014). The present population genetics analysis of *R. microplus* in West Africa provided support for lower null alleles' frequencies at markers BmA05 and BmC07 -initially developed on *R. australis* ticks- than on the markers developed on *R. microplus* indeed. The quasi-absence of polymorphism *R. microplus* observed on maternally inherited markers (mitochondrial genes and bacterial genes of the *Coxiella*-like endosymbionts, Binetruy, Duron & Chevillon, unpublished results) interestingly contrasts with the nuclear polymorphism on the microsatellite flanking regions that is responsible for such recurrent difficulties to avoid high frequencies of null alleles when addressing a new invasive wave of these ticks.

### Support for *R. microplus* recruitments during transhumance

Several indicators converged for supporting the hypothesis of population admixture within the infrapopulations defined by individual-steers. First, the signals of statistical linkage disequilibrium among pairs of loci observed among the sampled infrapopulations did disappear when re-computed on the clusters identified by BAPS4 (Figure 2). Second, the heterozygote deficiencies observed on sampled infrapopulations did also drastically decrease when re-computed on the clusters identified by BAPS4. This is exactly the patterns expected if the BAPS4-identified clusters would represent distinct population origins of the *R. microplus* ticks co-infesting the same steer (generating thus Walhund effects within infrapopulations). Moreover, the high estimate in effective population size driven from Kimini1 (188 *Versus* ~80-to-100; Table 2) was associated with a moderate estimate of the self-recruitment rate (0.68 versus 0.993 in Athieme where Ne ~104; Table 2) and with low estimate in the emigration rate toward the neighbour herd Kimini2 (0.002 in Table 2; geographic distance of about 30 km at sampling time). As the method used for estimating effective population sizes is based on the examination of linkage disequilibrium patterns, such estimates would be inflated in presence of Walhund effects. It is noteworthy that Kimini1 and Kimini2 are two herds reared by distinct Peulh families and that Peulh societies have evolved for long cultural practices on cattle production that include long-distance transhumance (Djenontin, 2010). Therefore, the patterns above take sense if we assume that the herders who are managing either Kimini1 or Kimini2 did not follow exactly the same routes along the transhumance event that had preceded our sampling.

The very high level of *R. microplus* self-recruitment estimated in the Kimini2 herd (0.98; Table 2) provided further support to the hypothesis of tick recruitment along transhumance event. Such a high estimation indicates that none of the samples presently analysed borne similar genotypes that those from the Kimini2 sample. This makes sense given that the present sampling was mostly performed in Benin but that the cattle herds such as Kimini2 (i.e., located in Burkina Faso in rainy seasons) move in dry seasons to Ivory Coast rather than to Benin.

Overall, the signal of genetic differentiation observed between Beninese samples and the two samples from Burkina Faso (Table 1) is thus very likely to translate

the difference among the ticks recruited in dry seasons in either mid-to-south Benin or Ivory Coast, respectively. This hypothesis could be easily tested by adding to the analysis several *R. microplus* populations from Ivory Coast.

**The relative importance of herds and individual steers in *R. microplus* genetic structure in Benin**

Eight years after the *R. microplus* introduction in south-Benin (Madder et al., 2012), the *R. microplus* populations collected over the entire country appear to have both reached mutation/drift and migration/drift equilibriums. This contrasts with the situation observed in New Caledonia: 70 years after the tick introduction in the island, a bottleneck signature was still visible on the microsatellite polymorphism (Koffi et al., 2006a). Additional work is needed to decipher whether such a contrast relies on a difference in the demographic growth at the early times of invasion and/or on biases resulting from differences in either markers polymorphism level or in the delay separating the tick introduction and the population genetics analysis.

The estimates of population effective sizes driven by Waples et al. (2014) and the estimates of neighbourhood sizes driven by the signal of isolation by distance observed among Beninese samples were remarkably similar: Ne ranges from 52 to 148 while De. $\sigma^2 = 53$ in Benin (Table 2). These Ne estimates computed within cattle herds in Benin are intermediate to those computed in New Caledonia on cattle herds (Ne ~1 000) and on rusa deer (Ne ~10) (De Meeûs, 2012). This makes sense considering that (i) the European cattle races reared in New Caledonia ('charolais' and 'limousin') are well-known for their inability to control their *R. microplus* burden via immunity, and (ii) the African cattle displayed a peak in genetic diversity in West Africa (Hanotte et al., 2002), including probably at the loci involved in immune response against ticks. Interestingly too, the two state farms Kpinnou and Okpara were not associated with far highest Ne estimates than the other Beninese cattle herds. This may indicate that opposite consequences between the absence of admixture in these *R. microplus* populations (given enclosure of these herds contrarily to all others) and the persistence of higher *R. microplus* burdens in dry seasons on these state farms herds contrarily to all others (Biguezoton et al., 2016).

In Benin, the cattle herd represent the main level defining *R. microplus* population genetic structure. This was also the case in New Caledonia where all herds remained enclosed in private pastures (Koffi et al., 2006a) but not in the Republic of South Africa along the Kruger Park where *R. microplus* populations were delimitated by individual steers (Boucher, 2013). Such differences highlight how differences in farming practices do determine the population processes of cattle parasites.

The examination of migration pattern among Beninese cattle herds (hence among Beninese populations of *R. microplus*) strengthens the importance of farming practices onto *microplus* demography. Contrarily to any other locality, Athieme appeared as a very likely source of *R. microplus* immigrants for any Beninese herd (Table 2: from 0.1% to 1% *Versus* ~30%). This particularity does not arise from history since *R. microplus* introduction did not take place in Athiémé but in the Kpinnou state farm (Madder et al., 2012). The most parsimonious explanation relies on the one hand, on the suitability of the South-Beninese climate for *R. microplus* all year around and on the other hand, the higher economic resources of the Kpinnou state farm relatively to Athiémé (allowing improved control of tick burden all year around via chemical tick-control but also closer and most frequent examination of each individual steer in Kpinnou).

**Conclusion**

The present results evidenced that to be efficient tick control programs should primarily focus on the cattle coming back north after transhumance because they could harbour *R. microplus* ticks that they might have recruited in more southern localities. Such a focus involves the use of different chemicals to treat these returning cattle relatively to the

chemicals used in tick control programs in the southern areas from where cattle had passed the last dry seasons.

## COMPETING INTERESTS

The authors declare that they have no competing interest.

## AUTHORS' CONTRIBUTIONS

ASB, HA, SF, CC designed the study. HA, ASB, SZ coordinated and participated to field sampling. ASB and SZ identified the collected ticks to species. ASB and VN performed the genotyping. ASB and CC analyzed data and wrote the first draft. All authors read and approved the final version of the manuscript.

## ACKNOWLEDGMENTS

We acknowledge all the livestock keepers who were involved in tick sampling. We are also grateful to the responsible of the state farms in Benin for their supports in *R. microplus* collection.

## REFERENCES

Achi LY, Boka M, Biguezoton A, Yao PK, Adakal H, Kande S, Azokou A, Koffi LS, Akoto PR, Kone M. 2022. Resistance of the cattle tick Rhipicephalus microplus to alphacypermethrin, deltamethrin and amitraz in Côte d'Ivoire. *Int. J. Biol. Chem. Sci.*, **16**(3): 910-922. DOI: https://dx.doi.org/10.4314/ijbcs.v16i3.2.

Adakal H, Stachurski F, Chevillon C. 2013a. Tick control practices in Burkina Faso and acaricide resistance survey in *Rhipicephalus* (*Boophilus*) *geigyi* (Acari: *Ixodidae*). *Exp. Appl. Acarol.*, **59**(4): 483–491. DOI: https://doi.org/10.1007/s10493-012-9610-5.

Adakal H, Biguezoton A, Zoungrana S, Courtin F, De Clercq EM, Madder M. 2013b. Alarming spread of the Asian cattle tick *Rhipicephalus microplus* in West Africa—another three countries are affected: Burkina Faso, Mali and Togo. *Exp. Appl. Acarol.*, **61**(3): 383-386. DOI: 10.1007/s10493-013-9706-6.

Beerli P, Felsenstein J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proc. Nat. Acad. Sci.*, **98:** 4563–4568. DOI: https://doi.org/10.1073/pnas.081068098.

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 & Vectors*, **9**(1): 43. DOI: https://doi.org/10.1186/s13071-016-1305-z.

Biguezoton AS, Adakal H, Farougou S, Chevillon C. *In press*. Development of new microsatellite markers for the characterization of Rhipicephalus microplus population in West Africa. *Int. J. Biol. Chem. Sci.*

Boucher F. 2013. Génétique des populations de la tique invasive du bétail Rhipicephalus (Boophilus) microplus en Afrique du Sud. Master 1 Thesis. Montpellier, France. P.86

Burger TD, Shao R, Barker SC. 2014. Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, Rhipicephalus (Boophilus) microplus, contains a cryptic species. *Mol. Phylogenet. Evol.*, **76**: 241–253. DOI: 10.1016/j.ympev.2014.03.017.

CEDEAO, CSAO/OCDE. 2008. Élevage et marché régional au Sahel et en Afrique de l'Ouest Potentialités et défis. CSAO-OCDE / CEDEAO, Paris, France.

Chevillon C, Koffi BB, Barré N, Durand P, Arnathau C, de Meeûs T. 2007. Direct and indirect inferences on parasite mating and gene transmission patterns: Pangamy in the cattle tick Rhipicephalus (Boophilus) microplus. *Infect. Genet. Evol.*, **7**: 298–304. DOI: 10.1016/j.meegid.2006.11.007.

Chigagure NN, Baxter GD, Barker SC. 2000. Microsatellite loci of the cattle tick Boophilus microplus (Acari: Ixodidae).

*Exp. Appl. Acarol.*, **24**: 951–956. DOI: 10.1023/a:1010732024895.

Corander J, Marttinen P. 2006. Bayesian identification of admixture events using multilocus molecular markers. *Mol. Ecol.*, **15**: 2833–2843. DOI: https://doi.org/10.1111/j.1365-294X.2006.02994.x.

De Clercq EM, Vanwambeke SO, Sungirai M, Adehan S, Lokossou R, Madder M. 2012. Geographic distribution of the invasive cattle tick Rhipicephalus microplus, a country-wide survey in Benin. *Exp. Appl. Acarol.*, **58** : 441–452. DOI: 10.1007/s10493-012-9587-0.

De Meeûs T. 2012. Initiation à la génétique des populations naturelles : applications aux parasites et à leurs vecteurs. IRD, Marseille.

De Meeûs T, Humair P-F, Grunau C, Delaye C, Renaud F. 2004. Non-Mendelian transmission of alleles at microsatellite loci: an example in Ixodes ricinus, the vector of Lyme disease. *Int. J. Parasitol.*, **34**: 943–950. DOI: 10.1016/j.ijpara.2004.04.006.

Djenontin J. 2010. Dynamique des stratégies et des pratiques d'utilisation des parcours naturels pour l'alimentation des troupeaux bovins au Nord-Est du Bénin. PhD Thesis. Université de Abomey-Calavi, Abomey-Calavi, p. 214.

Djenontin JA, Madjidou O, Houinato MR, Mensah GA, Sinsin BA. 2012. Le calendrier pastoral en élevage extensif dans le Nord-Est du Bénin: un outil de gestion du cheptel bovin de l'exploitation. *Science et Changements Planétaires / Sécheresse*, **23** : 261–270. DOI: 10.1684/sec.2012.0350.

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Mol. Ecol. Resour*., **14**: 209–214. DOI: 10.1111/1755-0998.12157.

FAO. 2009. Country Stat pour l'Afrique Sub-Saharienne. Bénin- Premier rapport panorama.

Goudet J. 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. *Mol. Ecol. Notes*, **5**: 184–186. DOI: https://doi.org/10.1111/j.1471-8286.2004.00828.x.

Goudet J, Perrin N, Waser P. 2002. Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Mol. Ecol.*, **11**: 1103–1114. DOI: https://doi.org/10.1046/j.1365-294X.2002.01496.x.

Hanotte O, Bradley D, Ochieng J, Verjee Y, Hill E, Rege J. 2002. African pastoralism: genetic imprints of origins and migrations. *Science*, **296**: 336-339. DOI: 10.1126/science.1069878.

Koffi BB, De Meeûs T, Barré N, Durand P, Arnathau C, Chevillon C. 2006a. Founder effects, inbreeding and effective sizes in the Southern cattle tick: the effect of transmission dynamics and implications for pest management. *Mol. Ecol.*, **15**: 4603–4611. DOI: 10.1111/j.1365-294X.2006.03098.x.

Koffi BB, Risterucci AM, Joulia D, Durand P, Barré N, De Meeûs T, Chevillon C. 2006b. Characterization of polymorphic microsatellite loci within a young Boophilus microplus metapopulation. *Mol. Ecol. Notes*, **6**: 502–504. DOI: https://doi.org/10.1111/j.1471-8286.2006.01295.x.

Labruna MB, Naranjo V, Mangold A, Thompson C, Estrada-Peña A, Guglielmone AA, Jongejan F, de la Fuente J. 2009. Allopatric speciation in ticks: genetic and reproductive divergence between geographic strains of Rhipicephalus (Boophilus) microplus. *BMC Evol. Biol.*, **9**: 1–12. DOI: 10.1186/1471-2148-9-46.

Madder M, Adehan S, De Deken R, Adehan R, Lokossou R. 2012. New foci of Rhipicephalus microplus in West Africa. *Exp. Appl. Acarol.*, **56**: 385–390. DOI: 10.1007/s10493-012-9522-4.

Madder M, Thys E, Achi L, Touré A, De Deken R. 2011. Rhipicephalus (Boophilus) microplus: a most successful invasive tick species in West-Africa. *Exp.*

*Appl. Acarol.*, **53**: 139–145. DOI: 10.1007/s10493-010-9390-8.

Musa HI, Jajere SM, Adamu NB, Atsanda NN, Lawal JR, Adamu SG, Lawal EK. 2014. Prevalence of Tick Infestation in Different Breeds of Cattle in Maiduguri, Northeastern Nigeria. *Bangladesh Journal of Veterinary Medicine*, **12**: 161–166. DOI: https://doi.org/10.3329/bjvm.v12i2.21279.

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: A Software for Genetic Assignment and First-Generation Migrant Detection. *J. Hered.*, **95**: 536–539. DOI: 10.1093/jhered/esh074.

Some MV, Biguezoton AS, Zoungrana S, Zoungrana A, Belem A. 2023. Epidemiology of heartwater in West Africa: similar infection rate of *Ehrlichia ruminantium* evidenced in adults of *Amblyomma variegatum* and *Rhipicephalus microplus* in peri-urban villages of Bobo-Dioulasso, Burkina Faso. *Int. J. Biol. Chem. Sci.*, **17**(2): 400-406. DOI: https://dx.doi.org/10.4314/ijbcs.v17i2.9.

Toure A, Diaha C, Sylla I, Kouakou K. 2014. Récente recomposition des populations de tiques prévalant en Côte d'Ivoire. *Int. J. Biol. Chem. Sci.*, **8**: 566–578. DOI: 10.4314/ijbcs.v8i2.15.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes*, **4**: 535–538. DOI: https://doi.org/10.1111/j.1471-8286.2004.00684.x.

Waples RS, Antao T, Luikart G. 2014. Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics*, **197**: 769–780. DOI: https://doi.org/10.1534/genetics.114.164822.

Weir BS, Cockerham CC. 1984. Estimating F-Statistics for the Analysis of Population Structure. *Evolution*, **38**: 1358–1370. DOI: 10.1111/j.1558-5646.1984.tb05657.x

Wilson GA, Rannala B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **163**: 1177–1191. DOI: 10.1093/genetics/163.3.1177.