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Blood survey of *Babesia spp* and *Theileria spp* in Mono's cattle, Benin

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A cross-section study was carried out in the Mono district in Benin to establish the different proportions of *Babesia* and *Theleria* in ticks and blood. Blood samples were collected between October and November, 2008, a period of relative abundance of ticks. The investigation covered nine herds (villages), including the farm of Kpinnou, in an agro-ecological zone where the people adhere to the same pastoral-farming tradition. In total, 756 ticks and 36 drops of blood on filter paper were taken from 36 bovines at a rate of 4 per village. Using morphological identification, identified 166 *Rhipicephalus* spp., 185 *Amblyomma* spp. and 405 *Rhipicephalus* (*Boophilus*) *microplus* individuals were collected, corresponding to 53.57% of the vectors. Molecular diagnosis confirmed the results of the morphological identification. Molecular methods were also used for detecting *Babesia* and *Theileria* in the bovine blood samples. Different parasites have been identified in varying prevalences: *Babesia bigemina* (13.89%), *Babesia bovis* (5.56%), *Theileria mutans* (22.22%), *Theileria ovis* (5.56%) and other *Babesia* species (22.22%). No data exists so far for the district, but the results of this study are very similar to those obtained in the neighboring districts.

Key words: Cattle, ticks, *Rhipicephalus microplus*, *Babesia bigemina*, *Babesia bovis*, Benin.

INTRODUCTION

Tick-borne diseases (TBD) such as babesiosis and theileriosis (Uilenberg, 1995) infect domestic livestock and wild animals in most regions of the world, causing problems of veterinary, medical, and economic

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Table 1. Geo-reference of the sampling points.

S/N	District	Villages	Breeders' names	Longitude	Latitude
1	Lokossa	Adjacome	Nompke Herbert	1.730194	6.646250
2	Houeyogbe	Zoungbonou	Amadou Hamidou	1.814611	6.556194
3	Come	Akodéha	Abe Come	1.911611	6.455278
4	Athieme	Sazouekpa	Kpachavi Sessouvi	1.743111	6.593833
5	Lokossa	Ouèdemè	Gnindokponou Ferdinand	1.679222	6.706083
6	Athieme	Awame`	Hanz Dalmeda	1.672139	6.609250
7	Come	Tovi	Hamidou Soumanou	1.850944	6.420194
8	Houeyogbe	Salahoue´	Désire Lakossa	1.808250	6.599611
9	Athieme	Kpinnou	Proj. of Liv. Dev3.(PDE3)	1.788083	6.566972

importance (de la Fuente et al., 2008). Although there have been a number of studies on the prevalence of tick-borne pathogens in vertebrate hosts and tick vectors in Benin, little information is available about the frequency of ixodid tick species and the prevalence of TBD in most areas of the country (Madder et al., 2012; De Clercq et al., 2012). In Benin, as in the majority of the African countries, the economic losses due to TBD and tick infestations are not known since the incidence/ prevalence of such diseases has not been quantified. The control of TBD and tick rests on human, technical and especially financial potentialities that enable it possible to make a choice of a method of fight. Unfortunately methods incomplete or non-existent of control remain a practice in the majority of breedings. Few stockbreeders use acaricides, and the few stockbreeders get to use it badly, creating problems of resistance of the vectors (Achukwi et al., 2001). This attitude reinforces the presence of the ticks and thus diseases which they transmit.

To make an attempt to improve local breeds through the importation of highly productive dairy cattle have often resulted in high mortality from TBD (Loria et al., 1999). For the development and implementation of control strategies, it is important to know and understand the epidemiology of these pathogens in the respective geographical regions. The fight against this scourge requires a thorough knowledge of the various blood parasites and their vectors encountered in animals. The impact of these diseases on animals such as abortions, falling weight and reduced milk production is reported by many authors (Stachurski et al., 1988; Teglas et al., 2005).

The objective of this study is to establish the different proportions of *Babesia* and Theileria in ticks and in the blood of animals in the district of Mono in Benin.

MATERIALS AND METHODS

Study area

The study took place between October and November**,** 2008. Eight villages (Table 1) were randomly selected in Mono District: Salahoue, Zoungbonou, Adjacome, Ouedeme, Awame, Sazouekpa, Akodeha and Tovi (Figure 1). This area of district of Mono which houses one of the four state farms (Ferme d'Elevage de Kpinnou, FEK), is one of the twelve districts of Benin, with an area of 1396 km². It is a small farming area (less than 0.7% of cattle) and bounded on the north by the district of Couffo, west along the Togolese border, to the east by the district of Zou and south by the Atlantic Ocean. The sampling area is marked by four seasons and identical agro-ecological characteristics: a long dry season from January to March, a long rainy season which runs from April to July, a short rainy season from August to October, and a short dry season from November to December, during which a dry wind blows from the interior of the continent to the ocean (called "harmattan"). The annual change of seasons ensures the availability of natural pasture; even the artificial pasture provid**es** good nutrition for animals throughout the year.

Sampling strategy

Four animals per farm were sampled. Samples of ticks and blood were made during the period of October-November*,* 2008 since the relative abundance of ticks is highest during this period (Farougou et al., 2007). A GPS GARMIN eTrex Venture was used to record the geographic position of the sampled farm.

Ticks

Ticks were removed from the cattle by simple extraction with forceps after restraint of the animal. To standardize the sampling, collection was done by the same person. Ticks were immediately placed into a flask containing alcohol 70°, one flask for each animal and labelled with number of the animal, age, health status, name of the village and others*.*

Blood

Blood sampling was performed on the same animals immediately following tick sampling. Blood was taken from the jugular vein in labelled tubes containing 5 ml Venoject *ethylene diamine tetra acetic acid (EDTA*) in a vacuum*.* Two drops of fresh blood of approximately 50 µl was also collected in a Whatman filter paper No.3 labelled. Blood drops were dr*ied* under a mosquito net at room temperature, protected from flies and dust. After half a day of drying, the filter papers *were* carefully superimposed, always interspersed with a clean filter paper No.4. They were stapled and put into a plastic bag containing blue silica gel grains to reduce the

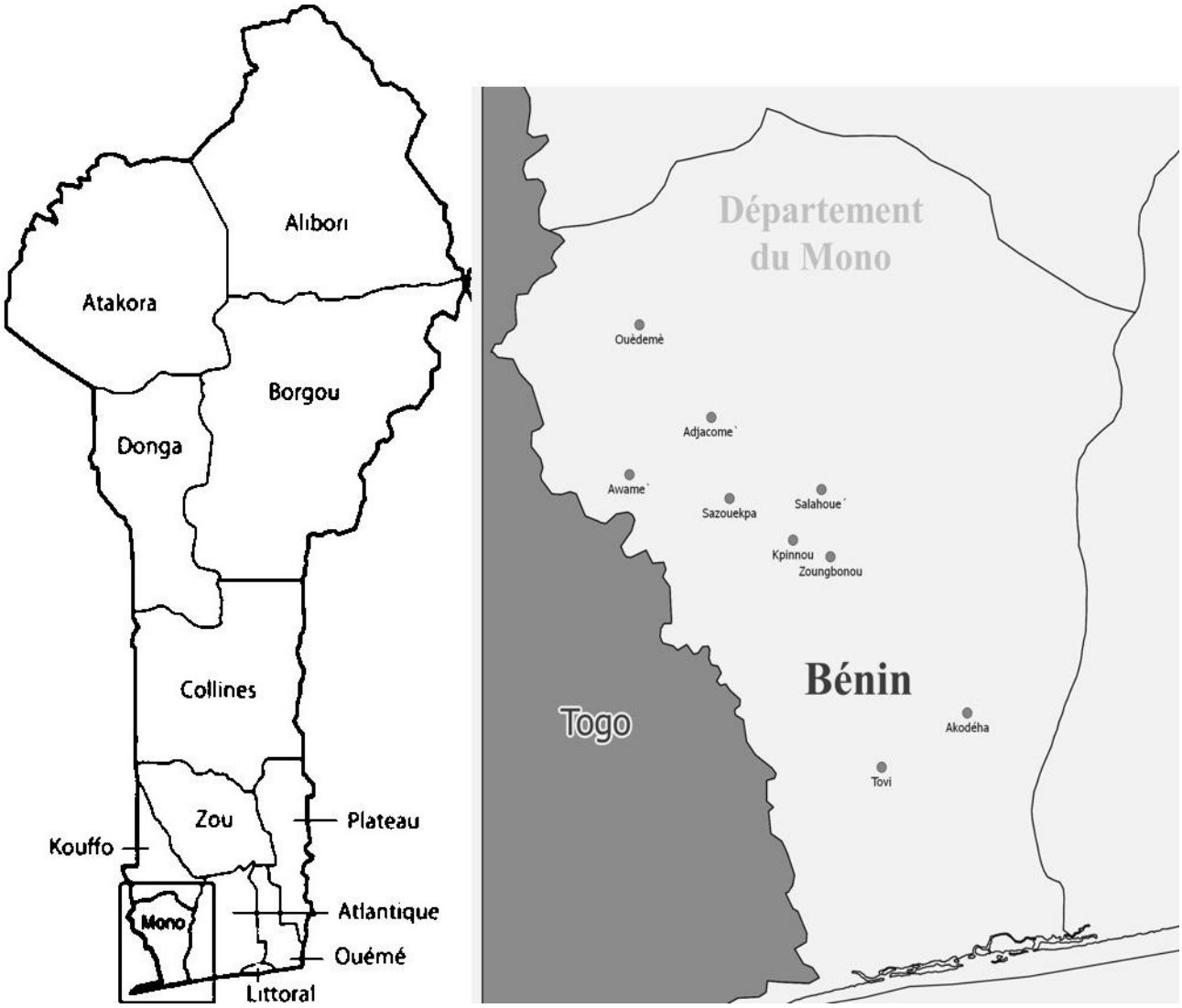

Figure 1. Left the 12 departments of Benin, right the location of the collection sites in Mono.

moisture absorption by the filter paper. Samples on filter paper were stored at -20°C.

Routing samples

At the end of each day of sampling, the samples were transported in a cooler to the Veterinary Diagnostic Laboratory of Bohicon (LADiVet) for storage. At the end of the field mission, all samples were gathered and sent to the laboratory of the Institute of Tropical Medicine in Antwerp, Belgium, accompanied by a Health certificate.

Laboratory analysis

Tick identification

The collected ticks were first identified up to genus level using a Zeiss Stemi 2000 stereomicroscope. Only *Rhipicephalus* (blue) ticks were further identified up to species level with a microscope (Zeiss Axioscope, at magnification 1009). The main characteristics used for identification were the hypostome dentition, presence or absence of the ventro-internal protuberance bearing setae, shape of the adanal plates and their internal and external spurs and the presence of scalelike external spurs on coxae 2 and 3 of the female ticks. Several identification keys were used (Walker et al., 2000; Madder et al., 2012). For confirmation of the identification of *Rhipicephalus* (*Boophilus*) ticks the traditional polymerase chain reaction (PCR) was used.

Pathogen detection

Two DNA analysis were made. First, bovine blood drops on filter paper were screened for *Babesia* and Theileria and secondly, DNA was extracted from the ticks to confirm microscope identification on the one hand, and to screen them for pathogens on the other hand. Two methods were used, being the PCR and the *restriction fragments length polymorphism* (RFLP). For DNA extraction of the ticks of the species *Rhipicephalus* (*Boophilus*), the technique of

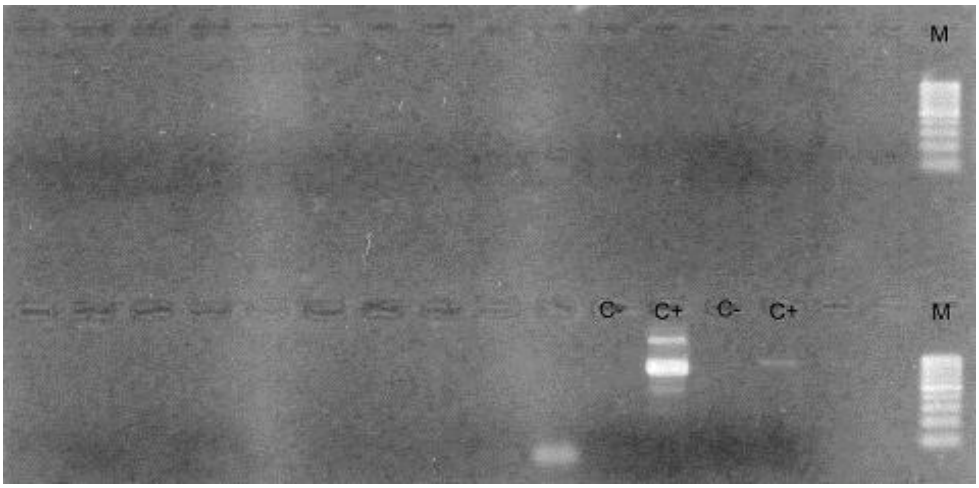

Figure 2. PCR product from the extraction of *Rhipicephalus* (B) microplus on 2% agarose gel.

Boom is used (Boom et al., 1990). For the DNA extraction of the blood drops, the technique used was the saponin-PBS 0.5% extraction. For pathogen detection a semi nested PCR or PCR 2nd round was performed, which yields a good test specificity. The pairs of primers BabF3/BabR2 and BabF3/BabR3 were used in each amplification of haemoparasites. The pair of primers BoopITS2F/BoopITS2R was used in the reaction of amplification (First-round PCR) of *Rhipicephalus* (*Boophilus*) DNA *(Lempereur, 2008).* The positive samples were tested again with the RFLP with a specific enzyme of restriction which makes it possible to make a cleavage (cut) in amplicons obtained with the PCR during a digestion at a temperature indicated. The reading of the results was made with UV. BseDI, MspI, DdeI and AluI are the enzymes used during various RFLP. The remaining of the samples not identified *were* cloned and sequenced at the University of Antwerp in Belgium.

Data analysis

Data analyses were performed with Excel 2003. Maps were made using Quantum GIS software (1.8.0).

RESULTS

Tick identification

In total, 756 ticks were collected from 36 cattle animals in nine different villages in the Mono district. Two genera were identified morphologically: *Amblyomma spp*. (24.47%) and *Rhipicephalus spp*. (75.53%). Among them, all blue ticks collected were identified as *Rhipicephalus microplus* (53.57%), and the results of the morphological identification are similar to those obtained with PCR concerning the confirmation of the identification of *Rhipicephalus (Boophilus)* ticks. This sampled tick species are those well known to the zone taking into account the national inventory of the species of ticks taken in 19 localities of Benin and the District of Mono (Vercruysse et al., 1982).

Babesia species identification in blood

Babesia spp. was detected in 25 from the 36 sampled bovines. *restriction fragment length polymorphism (*RFLP*)* was performed in the positive samples. This freezing shows profiles which are identical with controls of *Babesia bigemina*, which is very similar to parasites like Theileria (Figure 2). The results obtained with the enzyme of DdeI restriction present identical profiles for all samples. This enzyme does not allow *one* to distinguish the various species of Babesia or Theleria (Gragnon, 2005). This was also the case for the enzyme of AluI restriction. With the enzyme of MspI restriction, the results showed four groups of similar samples (Figure 3), of which *B. bigemina* and *Babesia bovis* are quite distinct. The remaining of the samples not identified was cloned and sequenced at the University of Antwerp in Belgium. After cloning and sequencing, these samples showed different profile of either *Theileria ovis* or *Theileria mutans*. After them, a representative of the non-specific samples of eight samples was made *and* tested with the enzyme BseDI. This complementary test made it possible to identify samples similar to Babesia but different from *B. bigemina* and from *B. bovis*. In all things considered, *B. bigemina* (5), *B. bovis* (2), *T. mutans* (2), *T. ovis* (8) and *Babesia spp* (8) were identified. The relative prevalence of parasites is shown in Figure 4. The present study shows a prevalence of 5.56% for *B. bovis*; 13.89% for *B. bigemina*; 5.56% for *T. mutans*, 22.22% for *T. ovis* and 22.22% for *Babesia spp*.

DISCUSSION

Ticks identification

All the collected tick species were already reported in the analyzed geographical region (Vercruysse et al., 1982).

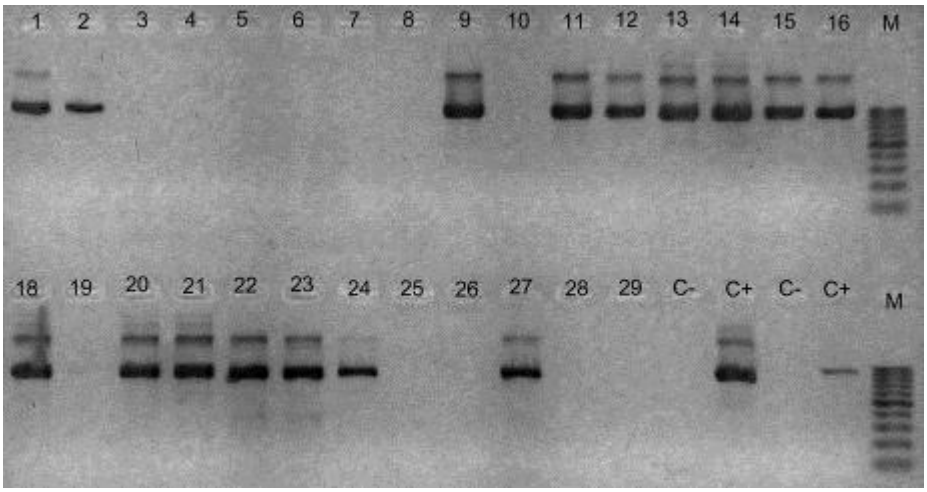

Figure 3. PCR products of bovine blood after migration on agarose gel 2%.

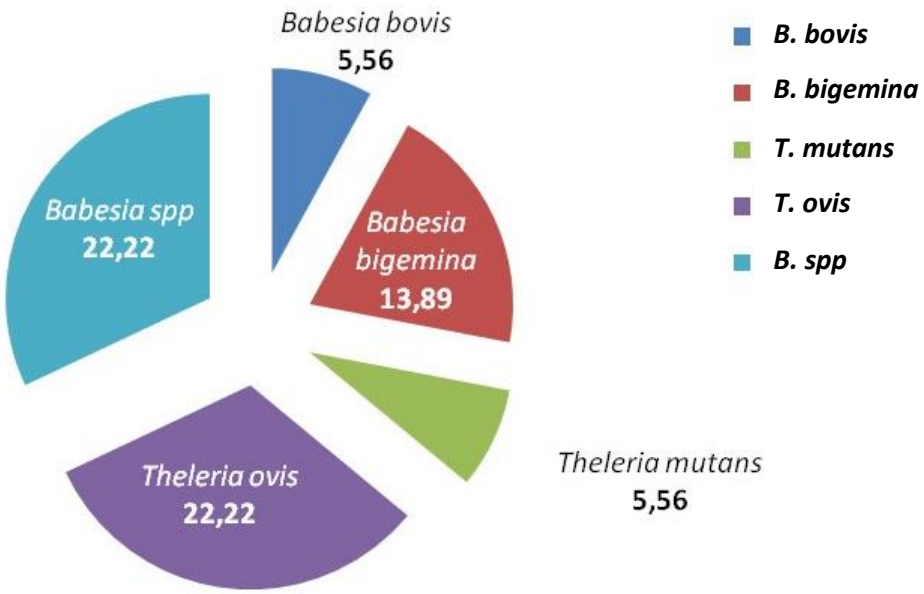

Figure 4. Respective prevalence of different haemoparasites in the blood samples.

Authors classified the species by numerical order of importance: *Rhipicephalus* (*Boophilus*) *microplus*, Amblyomma variegatum, *Rhipicephalus* (*Boophilus*) *geigyi*, *Rhipicephalus* (*Boophilus*) annulatus and *Rhipicephalus senegalensis*. This classification was confirmed in a recent study of ticks *(*Farougou et al., 2007; Madder et al., 2012*).* The resistance of animals with respect to the ticks is a very significant factor, because the tick must fix itself on the host during 3 days at least before *Babesia* pathogens be transmitted. At the same moment, tick can be rejected or not *in order* to be able to nourish itself. Another significant factor which influences the level of infection of the ticks is the parasitemy of the host. During latent infections, the parasitemy is tiny and a very small proportion of the ticks is infected (Marcotty, 2009). During strong parasitemies, much of Boophilus females die because of the intestinal lesions caused by the parasite, which decreases also the rate of infection.

Babesia pathogen detection in tick

The high rate of *R. microplus* recorded on animals should give hope for an increase of blood pathogens in ticks. But unfortunately the polymerase chain reaction (PCR) which

is a significant and specific diagnosis test (Devos, 2001), judicious to detect the parasites even in weak proportion, did not give any results, all samples were negative. However the same ticks DNA extracts were used to highlight the species of *Rhipicephalus* (*Boophilus*). This shows that the DNA extractions of the tick samples do contain DNA strings. The negative results can be explained in several ways. The periodic treatment of breedings against trypanosomes in programs of disease prevention constitutes an obvious reason of the very weak parasitemy and absence of the blood pathogens DNA in PCR samples. The vector concerned here has a monoxen cycle and both Babesia species are transmitted by various manners, on the level of the vectors as well as on the level of the hosts. *B. bovis* is transmitted by the larval stage and adults to animals considered as sinks on which they are obligatory reinfected. Actually, the parasitemy is weak in the endemic zones and the probability of the adults infecting themselves is even weaker. This low level of infection justifies the result obtained. In the case of *B. bigemina*, the transmission is not only transovarial as at *B. bovis*, but also transstadial and it is only the adults and the nymphs that transmit parasites to animals. In this case, if the females ticks are lesser infected, then the probability of having the infected larvae and other stages is very weak. The result is identical to the precedent. The method of DNA extraction of the ticks is not effective for obtaining the DNA of haemoparasites. Parasits are as small and numerically weak as one needs a method of extraction that is much more specific. It is as possible as trypanocides the almost quarterly treatments of the bovines in the zone of sampling hustled the cycle of haemoparasites returning not infected the parasites.

Babesia pathogen detection in host

The PCR/RFLP was used to highlight the various parasites of blood (Devos, 2001). The various parasites observed are well-known in the zone of collection and the animals which are there profit from a stable endemicity compared to the affections which they cause (Farougou et al.., 2007; Pangui and Salifou, 1992). The proportions described for these parasites are almost similar to those obtained for our collection. That of *B. bovis* seems to be a little low (5.56%) compared to that of 14.58% observed by Pangui and Salifou (1992) using microscopy which is less sensitive and less specific than the molecular method. The weak rate obtained for *B. bovis* can be explained by the fact that the blood test was done in the chin-strap and not in the blood capillaries, place of concentration of the parasite, contrary to *B. bigemina* which prefers young red blood corpuscles in general blood circulation (Figueroa and Camus, 2003).

The originality of the data of this study is explained by the fact that no data on the haemoparasites existed for the area of collection. All the investigations former on these haemoparasites were interested in other districts of Benin and almost never to that of Mono. The primers used for the amplification of the Babesia revealed the presence of Theileria *(Gragnon, 2005).* The cloning-sequencing of not identified samples showed profiles specific with the cleavage of the enzymes of restriction used during the RFLP. This made it possible to highlight Theileria. It is of Theileria mutans and a strong specific proportion of Theileria: one suspects a mutant form of T. mutans and of T.ovis in the blood of the sampled bovines.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

Achukwi MD, Tanya VN, Messine O, Njongmeta LM (2001). Comparative study of the infestation of Namchi (Bos taurus) and Ngaoundere Gudali (Bos indicus) cattle by *Amblyomma variegatum* adult ticks. Revue d'élevage et de médecine vétérinaire des pays tropicaux 54(1).

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van PM, vander NJ (1990). Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28:495-503.

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(4):441-52. DOI 10.1007/s10493-012-9587-0

de la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM, Sonenshine DE (2008). Overview: Ticks as vectors of pathogens that cause disease in humans and animals. Front. Biosci. 13:6938-6946.

Devos J (2001). *Babesia divergens*: comparaison de l'intérêt de l'IFI et de la PCR pour la réalisation d'enquêtes épidémiologiques. Animales Tropicales. Institut de Médecine Tropicale, Anvers.

Farougou S, Tassou AW, Tchabode DM, Podekon M, Boko C, Youssao AKI (2007). Tiques et hémoparasites du bétail dans le nord-Bénin. Rev. Méd. Vét. 158(8-9):463-467.

Figueroa J, Camus E (2003). Babésioses. Lefevre PC, Blancou J, Chermette R. Principales maladies infectieuses et parasitaires du bétail. Europe et régions chaudes. Lavoisier, Paris, Tome 2:1569-1583.

Gragnon BG (2005). Prévalence de l'anaplasmose et de la Babesiose Bovines en zone forestiere de la Cote d'Ivoire. Travail de fin de formation pour l'obtention de grade de Master of Science (M.Sc) en Santé Animales Tropicales. Institut de Médecine Tropicale, IMT, Anvers. 50:0-115.

Lempereur L (2008). Identification by morphological and PCR-RFLP approaches of Boophilus ticks from West Africa with special interest

in *Boophilus microplus*. Edition Travail de fin de formation pour l'obtention de grade de Master of Science (M.Sc) en Santé Animales Tropicales. Institut de Médecine Tropicale. IMT, Anvers.

Loria GR, Riili S, Vitale F, Greco A, Sparagano O (1999). Clinical and laboratory studies on theileriosis outbreaks in Sicily, Italy. Parasitologia 41:63-68.

Madder M, Adehan S, De Deken R, Adehan R, Lokossou R (2012). New foci of *Rhipicephalus microplus* in West Africa. Exp. Appl. Acarol. 56(4):385-390.

Pangui LJ, Salifou S (1992). Enquêtes parasitologiques sur les hémoparasites des bovins transmis par les tiques au Bénin. Rev. Mond. Zootechnol. 73:48-50.

Stachurski F, Barre N, Camus E (1988). Incidence d'une infestation naturelle par la tique *Amblyomma variegatum* sur la croissance des bovins et des caprins créoles. Rev. Elev. Méd. Vét. Pays Trop. 41:395-405.

Teglas M, Matern E, Lein S, Foley F, Mahan SM, Foley J (2005). Ticks and tick-borne disease in Guatemalan cattle and horses. Vet. Parasitol. 131:119-127.

Uilenberg G (1995). International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health. Vet. Parasitol. 57(1):19-41.

Vercruysse J, Lafia S, Camicas JL (1982). Les tiques (Amblyommidae) parasites des bovins en République Populaire du Bénin. Rév. Elev. Méd. Vét. Pays Trop. 35(4):361-364.

Walker AR, Bouattour A, Camicas JL, Estrada-Pena A, Horak I, atif A, Egram R, Reston PM (2003). Ticks of domestic animals in Africa: A guide to identification of species. Edition Bioscience Reports. Scotland.