Original Research Article

Anaplasma spp and other blood parasites in cattle in ~~of~~ the department Kounahiri (Côte d'Ivoire)

ABSTRACT

Aims: This study was initiated to reduce the economic losses caused by parasitic diseases, which are most often characterized by a drop in productivity or significant mortality.
Place and Duration of Study: This work took place from May 2020 to December 2022 in the department of Kounahiri.
Methodology:400 randomly selected cattle were used for the samples. Using a lancet, the ear was pricked to draw blood while immobilizing the animal. A drop was then placed on a slide and another slide was beveled at an angle of around 30° and brought into contact with the drop of blood. The smear is then taken on clean, dry slides. The smears are fixed in 95% methanol and stained with Giemsa diluted 1:10 in distilled water, then observed under a light microscope.
Results: The results showed that three types of blood parasites were identified through microscopic observations. The respective infection rates were 87.25% for Anaplasma, 64% for Babesia, and 21.75% for Theileria. Among the observed species of blood parasites, Anaplasma marginale was the most widespread with a significant infection prevalence of 77.5% in cattle. Furthermore, within all age groups, this pathogen (Anaplasma marginale) had the highest infection rate. The respective prevalence rates were 73.58% in cattle aged between 01-12 months, 78.90% in those of 13-24 months, and 78.92% in cattle aged more than 25 months. No difference in infection rates was observed based on the age of the animals. Additionally, no difference was detected in the seasonal distribution of Anaplasma marginale. The infection prevalencies during the two seasons were 73% at the beginning of the rainy season and 82% at the beginning of the dry season.
Conclusion: It will be essential to promote training for livestock farmers in terms of livestock health management.

Keywords: Côte d'Ivoire, blood parasite, Anaplasma marginale, cattle, infection

1. INTRODUCTION

In addition, most of these haemoparasitosis are transmitted by ticks. They can have a negative impact on animal health, the livestock industry and, sometimes, on humans. Bovine blood parasitic diseases, such as babesiosis, theileriosis, and anaplasmosis, are widely distributed throughout tropical and sub-tropical regions including Côte d'Ivoire. Infections can be deadly to farm animals but are also known to be the cause of fever, anorexia, jaundice, increased abortion rates, and sterility (1). *Anaplasma marginale* infection can also lead to reduced milk production, abortion in pregnant females and, in extreme cases, death of the animal (2). According to studies carried out by Zafar et al (3), ticks of the genus Dermacentor, Ixodes, Amblyomma and Rhipicephalus are important vectors that

cause a great deal of damage to cattle in the Punjab region of Pakistan. This was the case in recent studies by ~~Nyabongo *et al*~~ (4), in which numerous cases of co-infection by Babesia spp, Theileria spp and Anaplasma spp were reported in cattle. A variety of parasite species belonging to these genera are common and widespread blood parasites worldwide, with significant economic, medical, and veterinary consequences (5). Although some are benign or low pathogenic, others can cause a wide range of symptoms and even death. A range of prevalence of the piroplasmids in different animals was recorded worldwide (6-7).Accordingly, there have been many reports of Babesia spp., Theileria spp.,and Anaplasma spp. co-infections in cattle (4). Most of these haemoparasitic diseases aretick-borne diseases. They can be adversely impacting animal health, the livestock industry, and on occasion, human beings (1). These haemoparasitosis caused by protozoa (Theileria and Babesia) and bacteria (Anaplasma/Ehrlichia) pose a serious challenge to the health and well-being of livestock, particularly in tropical and subtropical regions. In Côte d'Ivoire, the distribution of hosts, pathogens and vectors overlap (8). As a result, a parasitological study was conducted throughout the country (Côte d'Ivoire) to map the distribution of tick-borne haemoparasites. Furthermore, the prevalence obtained was relatively low (less than 10%). ~~This~~ despite the emergence of R. microplus, one of the potential vectors of anaplasmosis and babesiosis (9). This is despite the emergence of R. microplus, one of the potential vectors of anaplasmosis and babesiosis (9). Furthermore, data on haemoparasites in Côte d'Ivoire are scarce and limited to a few departments within the different districts (10;11-12). On the diagnostic front, microscopic examination of blood smear has been widely used to detect the piroplasm stage of parasites in erythrocytes (13). However, due to the low productivity caused by blood parasitism, the benefits derived from the cattle industry remain very low. In order to ensure that cattle farming is conducted in a sensible and sustainable manner, it is therefore necessary that the diversity of Anaplasma and other blood parasites be documented for the control of the causative pathogens. To the best of our knowledge, fewer previous reports illustrate the distribution and microscopic identification of haemoparasites in cattle. This study was initiated to reduce the economic losses caused by parasitic diseases, which are most often characterized by a drop in productivity or significant mortality. This will be necessary to characterize the various haemoparasites in these animals in this locality and improve cattle productivity while effectively combating food insecurity.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Study site

This work took place from May 2020 to December 2022 in the department of Kounahiri in the Béré region. Kounahiri is located 520 km far away from Abidjan. It is located in the central-northern part of Côte d'Ivoire between 7°30' and 8°10' N latitudes and 5°40' and 6°20' W longitudes, covering an area of 2,110 km², with an estimated population of 101,111 inhabitants (14). The majority of the population is rural and engaged in agriculture. The department lies between the medium forest agro-climatic zones and the low forest zone (Figure 1).

2.2 Material

Our sampling focused on zebu cattle as it is the only breed found in the livestock of this locality. Standard equipment for the identification of blood parasites was used. This equipment included an optical microscope, immersion oil, Giemsa stain, microscope slides, 95% methanol, lancet, microcapillary tube, a slide holder, and gloves.

2.3. Methods

The samples were collected during two periods (May to June 2021 and November to December 2022) due to the availability of farmers and internal transhumance movements. Our study included a total of 400 cattle, with 200 randomly selected for sampling during the dry season (November to December 2022) and 200 during the rainy season (May to June 2021). The animals were aged 01-12 months, 13-24 months and 25 months and over, and of different sexes (male and female). The samples were collected from the auricular vein. Thus, the animal was immobilized and the base of the ear was compressed. A lancet was then used to prick the ear and collect the blood sample. Blood is collected using a microcapillary tube. A drop of blood is then placed on a slide and another slide is bevelled at an angle of approximately 30°. The bevelled slide is then brought into contact with the blood drop, and as it spreads by capillary action and slides forwards, the smear is applied to clean, dry slides. The smears were fixed in 95% methanol and stained with Giemsa diluted at 1/10 with distilled water. The smears were then observed under an optical microscope to detect Anaplasma and other erythrocytic forms of piroplasm. The microscopic observation was performed at 100x magnification using immersion oil, and the results were recorded. After observing 3/4 of the observable fields, the smears were considered negative if no parasites were detected (Moretti et al., 2010). All analyses were carried out at the Korhogo Regional Laboratory (LRK), a branch of the National Agricultural Development Support Laboratory (LANADA).

2.4 Statistical Analyses

The obtained results were recorded using Excel software. Descriptive analysis was conducted to calculate prevalencies and frequencies for qualitative variables. Subsequently, the chi-square statistical test was performed using R software version 4.1.3 to compare the prevalencies and frequencies of the different parameters studied, and the obtained values (percentages) were compared against the 5% threshold.

3. RESULTS AND DISCUSSION

3.1 Parasitological research of Anaplasma spp and other haemoparasites

Microscopic examination of 400 samples (Table 1) revealed that 386 smears were positive for at least one blood parasite. The overall prevalence of cattle infected with at least one blood parasite was 96.5%, while 3.5% of animals were free from any haemoparasite. A total of 05 species belonging to 03 genera of blood parasites were identified. These included the genus Anaplasma (87.25%) with two species, A. centrale and A. marginale, with respective proportions of 60.75% and 77.5%; Babesia (64%), which comprised the species B. bovis and B. bigemina; and the genus Theileria spp. The prevalencies of B. bovis, B. bigemina, and Theileria spp were estimated at 55.75%, 26.5%, and 21.75%, respectively. The statistical analysis revealed a high significant difference (P=0.0001) among the prevalence of the haemoparasites.

Table 1: Prevalence of Anaplasma spp. and other blood parasites

Species and/or Genus of Parasites	Number of Parasitized Cattle (N=400)	Prevalence (%)	P-Value
Anaplasma spp	349	87.25%a	0.0001*
Babesia spp	256	64%b	
Theileria spp	87	21.75%c	
Anaplasma centrale	243	60.75%b	
Anaplasma marginale	310	77.5%a	
Babesia bovis	223	55.75%b	

Babesia bigemina	106	26.5%c

Note: The values in the same column with different superscript letters significantly differ at the 0.05 threshold for each parameter. * highly significant

3.2 Prevalence of identified parasites by animal sex

The inventory of Anaplasma species and other associated blood parasites in cattle was also conducted, taking into account the sex of the animals. Among a total of 143 male subjects, the species A. centrale, A. marginale, and B. bovis were the most prevalent. Their respective prevalencies were 62.24% (89/143), 76.22% (109/143), and 53.8% (77/143). Notably, the species Babesia bigemina represented the lowest prevalence (26.57%), with 38 infected male cattle out of the 143 male subjects. Among the 257 females, A. centrale and A. marginale were the most numerically dominant species, with prevalencies of 78.2% and 59.9%, respectively. As for Theileria spp, it had the least infectivity (18.7%) in females (Figure 2). Overall, the chi-square test showed that the presence of parasites was not sex-related (p>0.05).

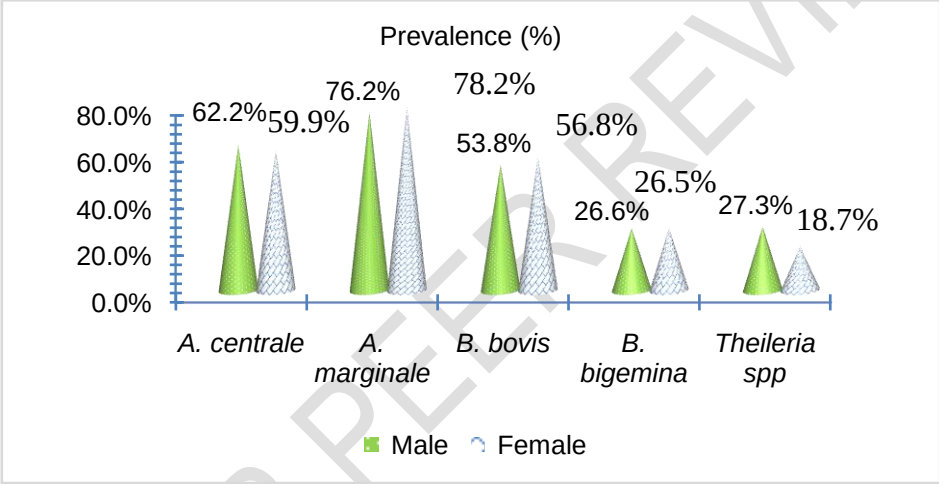

Figure 2: Variation of the prevalence of parasites by animal sex

3.3 Prevalence of parasite species according to the age of animals

The parasite A. marginale showed ~~the highest~~ respective proportions of 73.58% (youngest age group), 78.89% (juveniles), and 78.91% (adults) across all age categories. Additionally, A. centrale also recorded significant prevalence rates, estimated at 54.71% in animals aged 01 to 12 months, 67.18% in those aged 13-24 months, and 59.63% in adults (25 months and older). Similarly, 55.66% of the youngest animals (01-12 months), 53.90% of juveniles (13-24 months), and 57.22% of animals aged 25 months and older were infected with B. bovis. In contrast to these pathogens, the prevalence of B. bigemina showed lower proportions among the youngest (22.64%), 31.25% among juvenile animals, and 25.30% in those aged 25 months and older. Likewise, Theileria spp. was less represented, with distinct prevalencies of 12.26% (01-13 months), 24.21% (13-24 months), and 25.90% (25 months and older). The following graphs summarize and illustrate these details (Figure 3).

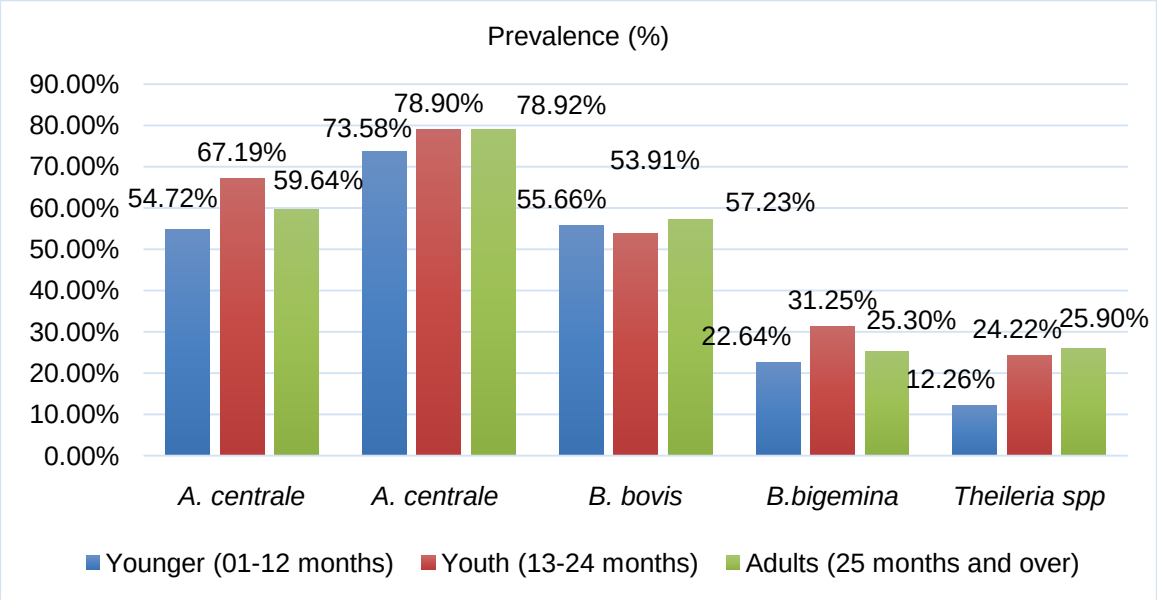

Figure 3: Prevalence of parasite species according to the age of animals

3.4 Prevalence of parasites according to the physiological stage of cattle

Anaplasma centrale was the most infective parasite in yearling bulls (70.58%), while it showed the lowest prevalence in calves (48.93%). As for the infection caused by A. marginale, it was highly significant at all stages, with a clear predominance in yearling bulls (77.77%). On the other hand, the haemoparasite B. bovis was more prevalent in cows, with a prevalence of 59.50% of infected cows. Additionally, heifers recorded a significant infection of B. bigemina with a proportion of 32.46%. Unlike other parasites, infections caused by Theileria spp were very low (Table 2). The statistical analysis showed a significant difference (P=0.0001) in parasite prevalence with respect to the physiological stage and sex.

Table 2: Prevalence of parasites according to the gender and physiological stage of cattle.

	Different blood parasites observed					
Physiological Stage	Anaplasma Centrale	Anaplasma Marginale	Babesia Bovis	Babesia Bigemina	Theileria Spp	P-Value
Calves N=47	48.93%a (23/47)	70.21%a (33/47)	51.06%a (24/47)	21.27%b (10/47)	10.63%b (5/47)	0.0001*
Female calves N=59	59.32%a (35/59)	76.27%a (45/59)	59.32%a (35/59)	23.72%b (14/59)	13.55%b (8/59)	0.0001*
Young bulls N=51	70.58%a (35/51)	80.39%a (41/51)	58.82%a (30/51)	29.41%b (15/51)	35.29%b (18/51)	0.0001*
Heifers N=77	64.93%a (50/77)	77.92%a (60/77)	50.64%a (39/77)	32.46%b (25/77)	16.88%c (13/77)	0.0001*
Bulls N=45	66.67%a (30/45)	77.77%a (35/45)	51.11%a (23/45)	28.88%b (13/45)	35.55%b (16/45)	0.0001*
Cows N=121	57.02%a (69/121)	79.33%a (96/121)	59.50%a (72/121)	23.96%b (29/121)	22.31%b (22/121)	0.0001*

Note: The values in the same row with different superscript letters significantly differ at the 0.05 threshold for each parameter. * Highly significant

3.5 Seasonal variation in the prevalence of Anaplasma spp and other associated blood parasites

The diversity of haemoparasite species encountered in the cattle farms in the department of Kounahiri during the rainy and dry seasons remains completely identical, with the only difference being their observed proportions (Figure 4). During both seasons, most of the infections were caused by the pathogen A. marginale responsible for bovine anaplasmosis, with respective prevalencies of 82% at the beginning of the dry season and 73% at the beginning of the rainy season. In contrast, Theileria spp had the least impact on animals in this locality, with low seasonal prevalencies of 20% and 24%, respectively, at the beginning of the rainy season and the beginning of the dry season. The statistical analysis does not reveal any significant difference (P=0.05) between the action of parasites during the seasons.

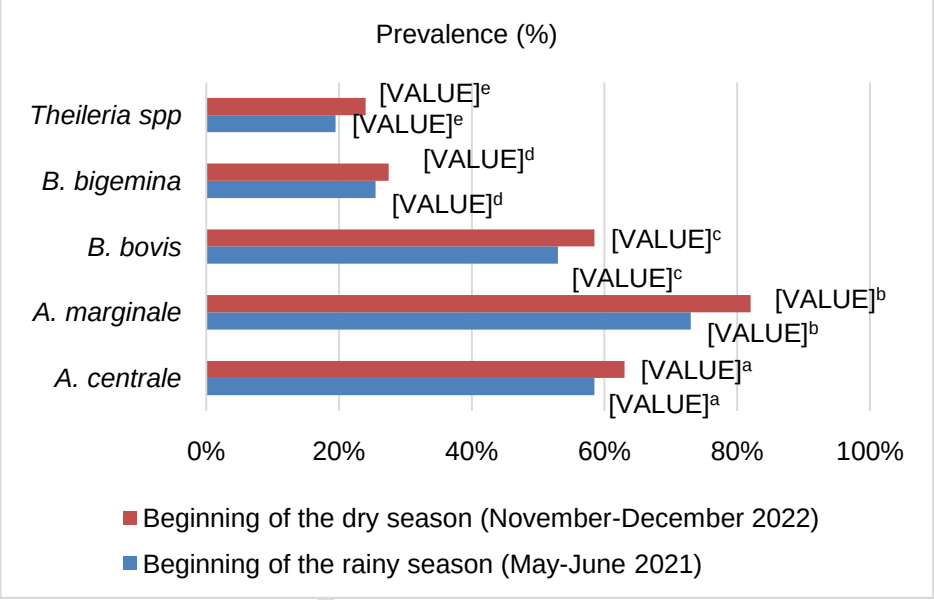

Figure 4: Seasonal dynamics of Anaplasma spp and other associated parasites
Note: Values with different letters indicate a significant difference in the prevalence ofidentified parasite species according to the season.

3.6 Discussion

Overall, 96.5% of cattle in this department were infected by at least one haemoparasite. Vector diversity and the lack of an appropriate prophylaxis programme would explain this high rate of spread. Our investigations corroborate those of Aké-Bogni et al (9), according to whom a total of 86.1% (155/180) of cattle are infested by one or more haemoparasite species. The search for Anaplasma spp and other haemoparasites revealed a significant infection rate of 77.5% for A. marginale in bovine samples from Kounahiri. This high prevalence could be attributed to the lack of preventive treatment by farmers. Furthermore, it may be explained by the absence of holding facilities, as well as the size and weight of the animals, which can make the administration of medications (acaricides and other veterinary care) more challenging. Our results are in agreement with those of Turi et al. (15), who reported an infection rate of 41.6% for A. marginale in cattle samples from the Lakki Marwat and Peshawar districts in Pakistan. Additionally, Farooq and Ashraf (17) reported significant infection rates of 18.3% for A. marginale in samples from cattle in three distinct areas of the Khyber Pakhtunkhwa province and 8.6% for A. marginale in cattle from the Layyah district in Pakistan.

The results concerning the variation in parasite prevalencies with respect to the sex of the animals indicated a significant infection rate of A. marginale (53.8%) in males and (59.9%) in females. Consequently, both male and female animals would be susceptible to tick infestations and other vectors, which explains the increased risk of transmission. Our results are not identical to the findings of Yéo et al. (18) on ruminant haemoparasites in the Korhogo and Sinématiali departments. According to these authors, 54.41% of females and 16.18% of males were infected with A. marginale. Our results are in line with the work conducted by Djakaridja et al. in the Northern and Central regions of Côte d'Ivoire, where they obtained an infection rate of 78.76% for A. marginale in 178 females and a 73.13% infection rate for the same parasite in a population of 98 males (11).

Regarding the diagnosis of parasite species based on the age of the animals, Anaplasma marginale prevailed at 78.90% among the young cattle, followed by Babesia bovis (57.23%), and Babesia bigemina (25.90%) among adult bovines. Several factors could explain this; firstly, young cattle have a diet primarily composed of milk, which may not contain all the necessary nutrients to strengthen their immune system. As a result, calves may be more susceptible to vector infestations. Additionally, young cattle are not treated with antiparasitic agents until a certain age, as these products can be harmful to their health. These results are similar to those obtained by Canever et al. (19) in southern Brazil, where they found that Babesia bigemina, Anaplasma marginale, and Babesia bovis were widespread, with respective infection rates of 63.6%, 60.6%, and 18.2%. According to the researchers, the low prevalence of infection might be related to low parasitaemia during the acute phase of the disease. An important incidence of 52.5% of cattle affected by anaplasmosis was reported in Aberdeen Angus cattle in New Valley, Egypt (20) following a diagnostic study.

At the physiological stage of cattle, high parasite burdens were recorded in yearling bulls. The infection prevalencies were distinctly 70.58% for A. centrale, 80.39% for A. marginale, and 29.41% for B. bigemina. The lack of appropriate management practices, such as pasture rotation and reducing herd density to minimize animal exposure to vectors, could explain this high parasite infection. Our results do not corroborate those of Vieira et al. (21), who observed a low infection rate of 26.76% for A. marginale and 35.21% for B. bovis in animals aged between 12 and 24 months. Another study conducted in the Balochistan province of Pakistan on the impact of parasite infestations revealed that young animals over one-year-old were the most exposed (22).

Regarding the seasonal variation of Anaplasma spp and other associated blood parasites, the pathogen A. marginale responsible for bovine anaplasmosis was most prevalent at the beginning of the rainy season and the beginning of the dry season, with respective prevalencies of 82% and 73%. A low distribution of Theileria spp, 20% and 24%, respectively, was observed at the beginning of the rainy season and the beginning of the dry season. This high prevalence can be explained by the fact that cattle are more exposed to insect bites and a high burden of tick vectors, which transmit these parasites. Additionally, the humidity may favour certain aspects of the parasites' life cycle and reproductive rate. Similar infection prevalencies have been reported in all cattle farming areas in Côte d'Ivoire, with overall prevalencies of 68.9% for A. marginale and 10% for Theileria annulata (9). Our results confirm the findings of studies conducted in the Northern and Central regions of Côte d'Ivoire, which estimated 77.7% in the North and 55% in the Center (11). Nwoha et al. (23) also found high proportions of 31% for A. marginale in Abia State, southeast Nigeria, although the proportions differ from ours.

4. CONCLUSION

This study on the parasitic status allowed for a comprehensive inventory of various haemoparasites in cattle from the Kounahiri department. Three genera and four species were identified, namely Anaplasma spp, Babesia spp, and Theileria spp. The species

included A. marginale, A. centrale, B. bovis, and B. bigemina. No trypanosomes were identified during this study. Concerning blood parasites, the genus Anaplasma was the most prevalent. Factors such as sex and season had no significant impact on the prevalence of parasites. However, the physiological stage influenced infection rates. Understanding the parasites will enable the implementation of more or less appropriate health service programs to meet the health needs of livestock, ultimately leading to increased production in all aspects.

CONSENT

By submitting this article to International Journal of Pathogen Research, we, the undersigned authors, confirm our consent to the publication of our work titled "Anaplasma spp and other blood parasites in cattle in of the department Kounahiri (Côte d'Ivoire)" in your journal. We understand and agree that the content of the article will be peer-reviewed and may undergo revisions recommended by reviewers and the editorial team to enhance its quality and relevance. By submitting this article, we declare that it is an original contribution and has not been previously published or submitted to another journal. All co-authors have significantly contributed to the research and writing of the article and have approved its submission to International Journal of Pathogen Research. If substantial revisions are suggested by peers or the editorial team, we commit to making them within the stipulated timeframe. We acknowledge that the final decision regarding the publication of the article will be made by the editorial team of International Journal of Pathogen Research. We understand that International Journal of Pathogen Research may retain the publication rights of the article, while respecting copyright and granting authors rights for reuse and non-commercial dissemination. We certify that the information provided in the article is accurate and based on verifiable data. By giving our consent for the publication of this article, we fully accept the terms and conditions of submission and publication of International Journal of Pathogen Research.

ETHICAL APPROVAL

All authors hereby declare that the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) have been followed, as well as specific national laws where applicable. All specimens have been examined and approved by a State Veterinary Doctor of the Department.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

INS : Institut Nation de la Statistique
A. marginale : Anaplasma marginale
A. centrale : Anaplasma centrale
B. bovis : Babesia bovis
B. bigemina : Babesia bigemina