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Seroprevalence of *Anaplasma* spp in dwarf goats in central Côte d'Ivoire

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Abstract

The study aimed to detect anaplasmosis in dwarf goat farms in order to improve their health and their production. To do this, 270 sera of dwarf goats from a secondary-prefecture in central Côte d'Ivoire was tested by competition ELISA (cELISA). The overall infection rate was estimated at 20.74%. The study showed that seroprevalence of species of *Anaplasma* is influenced by the zone and the season. However, the seroprevalence of *Anaplasma* spp is specific neither to the sex nor at the age of the dwarf goats. The present study indicates that the dwarf goat can be implied in the transmission of *Anaplasma* genus. From where need for integrating the dwarf goats in the fight against the haemoparasitoses.

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Introduction

Bacteria of the Anaplasmataceae family are compulsory intracellular Gram-negative bacteria (Dumler *et al.*, 2005). Anaplasmosis due to *Anaplasma* spp is a non-contagious vector-borne bacterial infection that infects domestic animals (De Sousa *et al.*, 2012) and is mainly targeted at blood cells such as red blood cells, leukocytes and platelets (Dumler, 2005) .The main vectors are ticks, mainly *Ixodes ricinus* in Europe, North Africa and the Middle East, *Ixodes Scapularis* in North and Central America, *Ixodes pacificus* in South America, *Ixodes persulcatus* in Japan and several other tick species (Sarih *et al.*, 2005). *Anaplasma* spp infection results in febrile syndrome in ruminants with apathy, decreased appetite, arthritis, edema of the distal extremities of the limbs, weight loss, sometimes haemorrhage in the mucous membranes or Skin, abortions and immune suppression (Dumler, 2001).

The detection of anti-*Anaplasma* spp is an excellent method of serological detection of infection and most often involves competition ELISA (Palmer *et al.*, 1994). The detection of an increase in the antibody title between the acute phase And the convalescent phase of the disease may be useful for the diagnosis of anaplasmosis in the absence of microscopic tests and molecular. The dwarf goat (*Capra hircus*) is a domestic mammal belonging to the family Bovidae and the genus *Capra*. In central Côte d'Ivoire, where goats are heavily represented, they are an important source of income for the rural population (FAO, 2015).

In Côte d'Ivoire, work on *Anaplasma* spp. remain very limited and have mainly concerned cattle, while a recent study has shown seropositivity to the *Anaplasma* genus in goats in Italy (Torina *et al.*, 2007). The present study was carried out with the aim of revealing the existence of *Anaplasma* spp in the dwarf goat farming in central Côte d'Ivoire.

Materials and methods

Study zone

This study was carried out from december 2015 to september 2016 in Béoumi region in central Côte d'Ivoire in humid and coastal West Africa, between the tropics of cancer and the equator. This sub-prefecture (Fig. 1) covers a zone of 1.780 km².

Fig. 1. Map of the study zone (Source: Maphill, 2011).

The population is estimated at more than 73.475 inhabitants (RGPH, 2014). The relief consists of a set of trays. The climate is characterized by two seasons (a dry season and a rainy season), with rainfall between 900 and 1200 mm/year (FAO, 2005). Two rivers irrigate the sub-prefecture, namely Bandama River and Kan River, which is its tributary.

Sample

The blood of hundred and seventy (270) dwarf goats, including 16 adult males, 110 adult females, 56 young males and 88 young females were collected (Table 1).

Blood samples

The blood samples were taken from the jugular vein. About 3 to 3.5 ml of blood are collected in the dry tubes. The dry tubes containing the blood collected are slightly inclined for 30 minutes to 1 hour, this process will evacuate sera after retraction of the clot. The clot removed, the sera is centrifuged at 3000 rpm for 5 min and stored in microtubes to be placed in the freezer at -20 ° C.

Competition ELISA

The detection of anti-*Anaplasma* antibodies was carried out using the kit cELISA; VMRD Inc., Pullman, WA, USA. This kit was used to detect antibodies against *Anaplasma* spp, following the manufacturer's instructions. This assay specifically detects the presence of sera antibodies that target the MSP5 protein of *Anaplasma* spp (Knowles *et al.*, 1996; De la Fuente *et al.*, 2005a). A negative control and a positive control provided in the kit were added to each assay. This test was performed according to methods of De la Fuente *et al.*, (2003, 2005b). The average of negative controls must have an optical density between [0.40 and 2.1].

The average of positive controls must have an inhibition greater than 30%. Test is declared negative if the inhibition percentage is less than 30%.

$$\text{Inhibition percentage (\%)} = \frac{\text{optical density of sample}}{\text{optical densityof negative controls}}$$

Prevalence

Prevalence is a type of epidemiological indicator that characterizes the status of a disease at a given time or date. The prevalence of adult males, adult females, young males, and young females was calculated according to the following formula:

$$\text{Prevalence} = \frac{\text{Number of positive samples}}{\text{Number of samples tested}}$$

Statistical treatments

Excel software (Microsoft Office, 2007) was used to process the collected data. Statistical comparisons of the different proportions were determined using the SPSS software. The chi-square test determined whether there was a significant difference between observations at the 5% threshold of p values. This test was also used for the comparison of the percentages. It is based on the calculation of a value of Chi- square.

Results and discussion

Prevalence of Anaplasma spp

Anti *anaplasma* antibodies were detected in the serum of dwarf goats during serological tests. On 270 serums tested, 56 were positive to *Anaplasma* spp with prevalence of 20.74% (Table 2).

Serological survey revealed the presence of anti-*Anaplasma* antibodies in 56 dwarf goats out of a total of 270 subjects tested (20.74%). This result testifies to the sensitivity of this animal species to infection with *Anaplasma* spp., Although no clinical signs have been found.

There is less work on anaplasmosis in small ruminants and none has yet specified the prevalence of *Anaplasma* spp by the serological test method in dwarf goats in Côte d'Ivoire. On the other hand, the genus *Anaplasma* was detected in goats in Sicily (Italy) by the same diagnostic technique (cELISA). Indeed, Torina *et al.* (2007) studies on parasitism of domestic animals gave a prevalence of 45.45% in goats infested with *Anaplasma* spp.

Recently in Corsica Dahmani *et al.*,(2016). obtained 100% of goats infested with *Anaplasma* spp with another technique (qPCR).

Table 1. Dwarf goats taken by zone.

	Adult male	Adult female	Young male	Young female
Zone far from watercourses (Zone A)	8	55	28	44
Zone near watercourses (Zone B)	8	55	28	44
Total	16	110	56	88

The different prevalences obtained in Sicily and Corsica are higher than those obtained during the study. These differences could be explained, on the one hand, by the mode of rearing which differs from one side to the other. In Sicily and Corsica, the samples were taken from goats in enclosures where the risk of transmission is much higher compared to the goats taken from the wanderings in a traditional breeding system. On the other hand, Dahmani *et al.* (2016) work focused on a very small number (5 goats tested in about 50) and used a much more sensitive diagnostic technique (qPCR) compared to the technique used during the study (cELISA). Specificity and sensitivity of the diagnostic system used by Dahmani *et al.*, (2016) and its ability to identify parasites at very low levels in the goat's DNA would explain the high prevalence obtained in Corsica.

Table 2. Prevalence of *Anaplasma* spp by zone.

	ZONES		
	Far from a watercourses (A)	Near a watercourses (B)	Total
Number of tested serum	135	135	270
Number of positive serum	11	45	56
Prevalence (%)	8.15[a]	33.33[b]	20.74

The exponents a and b on the same line indicate a significant difference ($p<0.05$).

Prevalence of Anaplasma spp by zone

Data from the cELISA tests showed that dwarf goats infested with *Anaplasma* spp originated mostly from zones bordering rivers.

The prevalence was 33.33% near watercourses. This value was statistically higher than that obtained in the zone away from the rivers (8.15%) according to the chi-square test ($p <0.05$) (Table 2).

56 samples were detected positive for the cELISA test with an overall prevalence of 24.7%. The prevalence in watercourse zones (33.33%) is higher than the prevalence (8.15%) observed in remote zones of watercourses. The surroundings of these rivers represent a microclimate in which is maintained moisture favorable to the survival of certain vectors responsible for anaplasmosis.

Table 3. Prevalence of *Anaplasma* spp by season.

	Zone A	Zone B	Total
Positive serum in dry season	(2/67)	(8/67)	(10/134)
	2.98	11.94	7.46[a]
Positive serum in rainy season	(9/68)	(37/68)	(46/136)
	13.23	54.41	33.82[b]

The exponents a and b on the same column indicate a significant difference ($p<0.05$)

This would explain the many cases of goats infested in these places. These results are consistent with observations by Memeteau *et al.*, (1998) who reported that the risk of infestation is higher in the presence of a thick vegetal mat and especially of uncultivated natural vegetation. Estrada Pena (2001), Estrada Pena *et al.* (2004) also reported that ticks responsible for anaplasmosis had a preference for zones around rivers.

However, infested goats have been detected far from watercourses where the climate is dry. This is due to certain species of vectors that are better adapted to zones where hard leaf and thick cuticle plants predominate, which serve as habitat for their survival and multiplication. However, the difference between these two zones is due to some vectors of anaplasmosis as ticks proliferate more in wetland than any other zone (Morel, 2003).

Table 4. Prevalence of *Anaplasma* spp by sex.

	Zone A		Zone B		Total
	Dry season	Rainy season	Dry season	Rainy season	
Positive male	(0/18)	(3/18)	(0/18)	(9/18)	12/72
	0	16.67	0	50	16.67[a]
Positive female	(3/49)	(5/50)	(8/49)	(28/50)	(44/198)
	6.12	10	16.33	56	22.22[b]
Total	(3/67)	(8/68)	(8/67)	(37/68)	(56/270)
	4.48	11.76	11.94	54.41	20.74

The exponents a and b on the same column indicate a significant difference ($p<0.05$).

Prevalence of Anaplasma spp *by season*

Anaplasma spp infection rate was lower in the dry season (7.46%) compared to that reported during the rainy season (33.82%) (Table 3). According to the Chi-square test, values observed were statistically different from each other at the 5% threshold ($p <0.05$).

Prevalence of *Anaplasma* spp in goats is much higher in the rainy season than in the dry season. It is 33.82% against 7.46%. Infested goats were much more prevalent during the rainy season than during the dry season. This is due to the large number of vectors during the rainy season. This preponderance of vectors would be linked to two phenomena which would favor them during the rainy season and hostile during the dry season. These are first extreme temperatures (very high or very low) during the dry season. Secondly, the destruction of microenvironments due to bush fires which are almost frequent in savannah zones. When living conditions are unfavorable in an zone, the vectors move away or adapt, or remain in a slowed-down state without activity.

But when conditions return to favorable conditions, they return to life and activity, hence the high prevalence during the rainy season. These results are consistent with Boyar *et al.* (2004), who justified the difference between the two seasons by the scarcity of wood near the pasture in the dry season.

Prevalence of Anaplasma spp *by sex*

Data from cELISA tests showed that male and female goats were infected with *Anaplasma* spp. However, there is preponderance in females (Table 4). Thus, significant differences were observed between the proportion of females (22.22%) and males (16.67%). Prevalence is higher in female goats than in male goats. It is 22.22% for females compared with 16.67% for males. Majority of dwarf goats whose serum was detected positive in the cELISA test were female goats. This difference in proportion between females and males could be explained by the fact that female goats do not undergo or undergo very little reform.

Prevalence of Anaplasma spp by age

Adult goats and goats were found among dwarf goats infested with *Anaplasma* spp.

Table 5. Prevalence of *Anaplasma* spp by age.

	Zone A		Zone B		Total
	Dry season	Rainy season	Dry season	Rainy season	
Positive young	(0/36)	(1/36)	(1/36)	(11/36)	13/144
	0	2.78	2.78	30.56	9.03[a]
Positive adult	(3/31)	(7/32)	(7/31)	(26/32)	(43/126)
	9.68	21.87	22.58	81.25	34.13[b]
Total	(3/67)	(8/68)	(8/67)	(37/68)	(56/270)
	4.48	11.76	11.94	54.41	20.74

The exponents a and b on the same column indicate a significant difference ($p<0.05$).

Infestation rates were (34.13%) for adults and (9.03%) for kids (Table 5). The prevalence of infected adults is significantly different from that of kid-sized children at the 5% threshold ($p < 0.05$).

It is the adult goats and the young males that are much reformed or who are sacrificed much during the ceremonies. In fact, many female goats (198) were recorded in relation to male goats (72) in this study. However, the results obtained are different from those of Hungerford *et al.* (1997) who reported that males were more commonly affected than females. One possible explanation is that estrogens in abundance in females would reduce rickettsiemia. Prevalence is higher in adult goats than in young goats.

It is 34.13% for adults as against 9.03% for young people. This difference in the proportion between goats and young goats may be explained by the fact that goat breeding is seasonal. When the forage decreases or fails, the latter give little or no energy to reproduction. Goats are generally pregnant during the forage abundance and parturition occurs during the dry season. However, during this season, the vectors due to the unfavorable conditions are in a state of life slowing down, hence the low infection observed in young goats. However, young goats have natural resistance during their young age, due to the many antibodies present in colostrum which provide a defense against certain bacteria (Kocan *et al.*, 2003). Young animals (up to 12 months of age) are naturally resistant to the disease, whereas adults after the age of three develop a severe form of the disease that can cause death (Pailley, 2007).

Conclusion

At the end of our study, we can say that cELISA diagnostic tests used in this study showed the existence of *Anaplasma* spp in the serum of the dwarf goats with a prevalence of 20.74%. All of the overall values obtained in this study varied considerably by zone, season, sex and age. Prevalence was much more significant in the zones along the rivers and in the rainy season. Females and adults were the most infected in this study. However, this seroprevalence at *Anaplasma* spp is not related to the sex or age of the dwarf goats. In general, dwarf goats are involved in the transmission cycle of anaplasmosis, which is a constraint on the development of livestock. Hence, the need to integrate the dwarf goat into programs to combat parasitic diseases.

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