

RESEARCH PAPER

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Prevalence of *Anaplasma marginale* and *Ehrlichia ruminantium* in wild grasscutter' specific ticks in southern Côte d'IvoireZahouli Faustin Zouh Bi^{*1}, Alassane Toure², Yatanan Casimir Ble³, Yahaya Karamoko²¹Laboratoire de Biologie et Cytologie Animale, Centre de Recherche en Ecologie,
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Côte d'Ivoire**Key words:** *Anaplasma marginale*, *Ehrlichia ruminantium*, Ticks, *Thryonomys swinderianus*, Côte d'IvoireDOI: <https://dx.doi.org/10.12692/jbes/27.4.21-27>

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ABSTRACT

Ticks are vectors for numerous pathogens in mammals. Two species i.e., *Ixodes (I.) aulacodi*, *Rhipicephalus (R.) simpsoni* are specifically found in the cane rat, a large rodent whose meat is appreciated by ivorian population. To reduce the risk of contamination of hunters and consumers, two pathogens, *Anaplasma marginale* and *Ehrlichia ruminantium*, were sought in 1,017 ticks (717 *I. aulacodi* and 300 *R. simpsoni*) collected from 150 wild grasscutters from 8 regions in southern Côte d'Ivoire. Analyses were carried out at CIRDES in Bobo-Dioulasso by semi-nested PCR. *I. aulacodi* carried *A. marginale* with an overall prevalence of 1.13%, while *R. simpsoni* did not host it. Regarding *E. ruminantium*, its prevalence was 2.28% for *I. aulacodi* and 10.80% for *R. simpsoni*. Concerning regions, *A. marginale* was found in *I. aulacodi* from Agneby-Tiassa and Mé with respective prevalences of 1.85% and 6%. As for *E. ruminantium*, prevalences of 5.31%, 3.13%, 3%, 2.57%, and 2.44% were found in *I. aulacodi* respectively in regions of Agneby-Tiassa, Grands Ponts, the Autonomous District of Yamoussoukro, Béliér, and Mé. Finally, ticks of the species *R. simpsoni* carrying *E. ruminantium* were present in all regions except Loh Djiboua. The lowest prevalence was obtained in Grand Ponts (7.17%) and the highest value (20.63%) in Agneby-Tiassa. This study confirms the presence of *A. marginale* and *E. ruminantium* in grasscutters' ticks in southern Côte d'Ivoire. Further study should be considered to verify its presence and pathogenicity in grasscutters.

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INTRODUCTION

The grasscutter (*Thryonomys swinderianus*), also known as the greater cane rat, is among the largest rodents found in sub-Saharan Africa (Babarinde *et al.*, 2023). It is undoubtedly one of the most hunted and consumed game animals in most Sub-Saharan African countries (Kpassi *et al.*, 2025). Everything is eaten from this animal, which suffers from no taboos. Its excrement is used in the seasoning of certain sauces in West Africa (Quarshie *et al.*, 2023), and its fur is used as a healing agent and sponges in dermatology (Mensah and Ekué, 2003).

Caspary and Momo (1998) showed that in 1996, 33.5 millions heads of wild animals, including 8.11 million heads of grasscutters, were slaughtered in Côte d'Ivoire, which represents 40 to 60% of the sales figures for 'bush meat' in Côte d'Ivoire. Also, the meat of cane rats is the most commonly encountered bush meat in the forested south (Gonedelé *et al.*, 2016) and central parts of the country (Sikpo *et al.*, 2023). These hunted grasscutters could harbor certain pathogens that are probably dangerous for hunters and consumers. For example, Hammoudi *et al.* (2020) isolated two *Leptospira* species (*Leptospira borgpetersenii* and *Leptospira interrogans*) from wild grasscutters in Yamoussoukro, Côte d'Ivoire. Zouh Bi *et al.* (2015) and Tuo *et al.* (2020) collected ticks from wild grasscutters respectively in southern and northern Côte d'Ivoire. These ticks could be vectors of pathogens. Indeed, Adenyo *et al.* (2020) identified a bacterium of the genus *Anaplasma* in *Ixodes aulacodi*, a tick specific to the grasscutters, in Ghana. Furthermore, no previous study on the presence of pathogens in ticks infesting grasscutters and on the possible pathogenicity in grasscutters, have been conducted yet in Côte d'Ivoire. This study therefore aims to evaluate the importance of two pathogens (*Anaplasma marginale* and *Ehrlichia ruminantium*) in two species of ticks specifically infesting wild grasscutters.

MATERIALS AND METHODS

Study areas

Côte d'Ivoire, a country located in the northern hemisphere of West Africa, is between the tropic of Cancer and the Equator, precisely between 4th and

10th degree of latitude north, and 2nd and 8th degree of longitude west. The study has been carried on ticks collected on wild grasscutters from eight regions of the south: Abidjan and Yamoussoukro Districts, Agnéby-Tiassa, la Mé, Grands Ponts, Lôh Djiboua, Sud Comoé and Belier regions are located in forest zone with high rainfall. These regions were chosen because the grasscutter meat was widely consumed by the population.

Study hosts and tick sampling

This study was conducted on 1017 ticks belonging to two species: *Ixodes aulacodi* (717 ticks) and *Rhipicephalus simpsoni* (300 ticks). They were collected from 150 dead wild grasscutters from above-mentioned regions. Grasscutters were hunting products intended for consumption. They were bought in bushmeat markets of each region.

Ticks' collection and identification

Ticks were collected from April 2010 to October 2012 and conserved in a bottle with a hermetic latch containing 70% Ethanol. Their identification has been performed at the Veterinary Central Laboratory of Bingerville (LCVB). It is based on the morpho-anatomic characteristics (Arthur, 1956 ; Walker *et al.*, 2003; Chitimia-Dobler *et al.*, 2016). All ticks were conveyed in the acarology section of CIRDES in Bobo-Dioulasso, for confirmation.

Ticks' pools

The search for Pathogens in ticks was carried out by semi-nested PCR at the Molecular Biology Laboratory of CIRDES in Bobo-Dioulasso. Given the high number of ticks, they were pooled (Katholi *et al.*, 1995) to minimize cost and duration of the work. Indeed, the pool size (K) which is equal to 3 was chosen according to the formula of Katholi and Unnasch (2006):

$$K = \frac{1.5936 - p}{p}$$

p = The observed field prevalence

Since there are no data available for this parameter for grassland ticks, the expected prevalence was estimated to be 50%; hence K = 3.

DNA extraction

DNA extraction was performed on 339 ticks' pools. Prior to extraction, five-minute scrubbing of ticks in sterile PBS was carried out to remove traces of ethanol that would inhibit PCR. Ticks were then weighed and a mass between 25 and 40mg was used for DNA extraction using 5% chelex resin (100). Indeed, the ticks were cut into a petri dish and the pieces were placed in a sterile conical tube of 1.5mL capacity using the scalpel blade. Then 1mL of PBS-saponin 0.5% was added.

The preparation was incubated at 4°C overnight. After incubation, it underwent centrifugation at 14000rpm for 3min. The supernatant was discarded and then 1mL of PBS was added. The preparation was passed in the vortex and incubated at 4°C for 2 hours. After incubation, the preparation was centrifuged for 1min at 14000rpm and the supernatant were discarded. The pellet was ground with 250µL of 5% Chelex (100). The ground obtained was incubated in a thermocycler (PCT-100) according to the following program: 56°C for 1 hour and 94°C for 30min. At the end of the incubation, it was centrifuged for 5min at 14000rpm and the supernatant were carefully recovered in a new sterile conical tube of capacity 1.5mL.

A final centrifugation for 5min at 14000rpm was carried out and the supernatant was carefully recovered in a new sterile conical tube. The extract was stored at 4°C for short-term use.

Performing PCR

Anaplasma marginale

Anaplasma marginale detection was made in the Biometra thermocycler by semi-nested PCR by targeting the Msp5 gene (De Echaide *et al.*, 1998). Primers targeting this gene were: Msp5 external Forward: 5'-GCATAGCCTCCGCGTCTC-3', Msp5 internal Forward: 5'-TACACGTGCCCTACCGAGTTA-3' and Msp5 external Reverse: 5'-TCCTCGCCTTGGCCCTCAGA-3' (De Echaide *et al.*, 1998). For each of the two steps (PCR1 and PCR2), the reaction was carried out in a reaction medium of 25µL containing 200µM of dNTP and 3mM of MgCl₂. The Msp5 extFOR and Msp5 extREV primers were used at a final concentration of 2ng/µL for PCR1.

PCR2 was performed with the Msp5 extREV primer at the same final concentration (as in PCR1) and the Msp5 intFOR primer at 168nM. During PCR1, 1µL of DNA was added to the reaction medium while in R2 it was 1µL of the PCR product of PCR1 that constituted the substrate. The PCR1 program consisted of three phases. The first step consisted of an initial denaturation at 94°C for 3min. The second stage consisted of 40 cycles of 94°C for 50s, 60°C for 50s and 72°C for 50s. Finally, the third step which is the final elongation was done at 72°C for 10min. The same program was used for PCR2 except for the hybridization temperature of the primers which was 58°C. For each test, a positive control (purified DNA from *Anaplasma marginale*) was added to the samples to be analyzed. The expected PCR products were 456bp for PCR1 and 343bp for PCR2.

Ehrlichia ruminantium

The detection of *E. ruminantium* in DNA extracted from ticks was made by semi-nested PCR amplification of a 279bp fragment within the open reading phase of the 1306bp of the conserved pCS20 gene (Martinez *et al.*, 2004). The pair of external primers used in PCR1 was AB128: 5'-ACTAGTAGAAATTGCACAATCTAT-3' and AB130 : 5'-ACTAGCAGCTTTCTGTTCAGCTAG-3'. This pair amplified a fragment of about 380bp. As for PCR2, the pair of internal primers used was AB128: 5'-ACTAGTAGAAATTGCAGCTAG-3' and AB129: 5'-TGATAACTTGGTGCGGGAAATCCTT-3'. This pair of primers allowed for the amplification of the 279bp fragment of interest. PCR reaction was carried out, for both PCR1 and PCR2, in a reaction volume of 24µL per tube containing 670mM of Tris-HCl pH 8.8; 160 mM of (NH₄)₂SO₄; 0.1% Tween 20; 200µM dNTPs; 1.5mM MgCl₂; 0.2µM of each primer; and 0.4 U/µL Taq. For PCR1, 1µL of sample DNA was added to the reaction mixture, whereas for PCR2, it was 1µL of the PCR product from PCR1 that completed the final volume to 25µL. For each test, a positive control (purified DNA from *E. ruminantium*) and a negative control (sterile double distilled water) were added to the series of samples to be analyzed.

The pCS20 amplification program in the first round (PCR1) consisted of three steps. The first step involved an initial denaturation at 94°C for 3min. The second

step comprised 35 cycles of 94°C for 45 seconds, 50°C for 45 seconds, and 72°C for 45 seconds. Finally, the third step (the final elongation) was done at 72°C for 10 minutes. The same program was used for the PCR2 except for the primer hybridization temperature which was at 55°C and the number of cycles in the second step which increased to 40.

Electrophoresis and visualization of PCR products

For electrophoresis, horizontal gel of 1.5% agarose prepared with TBE1X buffer (45mM Tris base, 45mM boric acid, 1mM ethylenediaminetetraacetic acid [EDTA], pH 8.0) was used. To visualize nucleic acids that migrated, 1µL/100mL of ethidium bromide (BET) solution (10mg/mL) was added to the gel composition. The migration lasted for 1h and 30mn at 100 Volts. DNA fragments were visualized in ultraviolet light and photographed.

Statistical analysis

The Office Excel 2007 spreadsheet on Windows was used to enter data, calculate prevalence of pathogens in each tick species. The prevalence of each tick species was calculated using the formula described by Katholi *et al.* (1995) and later applied by Touré *et al.* (2012) :

$$P (\%) = 1 - n \sqrt{\frac{K}{M}}$$

With K being the number of pools negative for semi-nested PCR, M being the total number of pools, and n being the size of pools, was used.

Statistical comparison of this prevalence was made with the Chi-square test at the 5% threshold using STATISTICA 7.1 software.

RESULTS

Global prevalence of *Anaplasma marginale* and *Ehrlichia ruminantium* in ticks

The Msp5 semi-nested PCR was used to detect ticks' infestation by *A. marginale*. Positive samples in Msp5 were those giving bands at 343bp at the same height as the positive control as shown in Fig. 1. For the positive samples in semi-nested PCR pCS20 concerning *E. ruminantium*, the bands appear at 279bp at the same height as the positive control (Fig. 2).

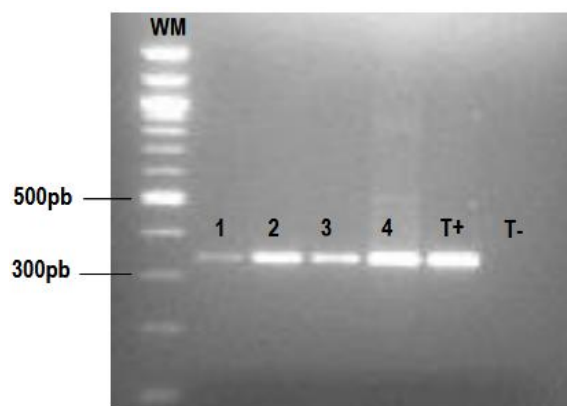


Fig. 1. Agarose gel electrophoresis (1.5%) of Msp5 gene amplification products

WM = weight marker; samples 1 to 4 are positive ; T+ : positive control ; T- : negative control.

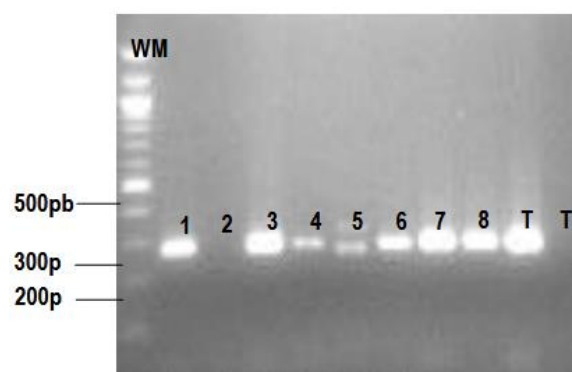


Fig. 2. Agarose gel electrophoresis (1.5%) of pCS20 gene amplification products

WM = Molecular weight marker of size 4Kb; samples 1, 3, 4, 5, 6, 7 and 8: tick samples containing the DNA fragment of *E. ruminantium* of size 279bp; sample 2: tick sample lacking the DNA fragment of *E. ruminantium*; T+: positive control; T-: negative control.

The Fig. 3 presents the global prevalence of *A. marginale* and *E. ruminantium* obtained in the different tick species. Of the two hundred and thirty-nine (239) pools of *I. aulacodi* ticks analyzed, eight (8) were carriers of *A. marginale*. This gives an overall prevalence of 1.13%.

As for the species *R. simpsoni*, no sample harbored *A. marginale*. Regarding *E. ruminantium*, the prevalence in the species *I. aulacodi* was 2.28% (16 tick pools carrying the 279bp fragment of *E. ruminantium*). This value was lower than that

obtained for *R. simpsoni* (10.80%) (29 tick pools carrying the 279bp fragment of interest).

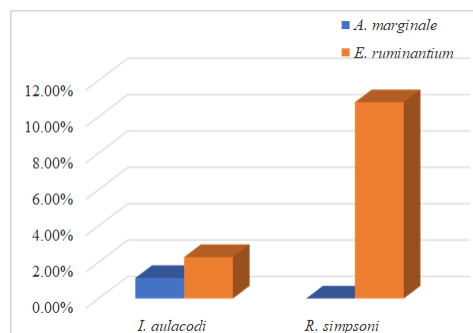


Fig. 3. Global prevalence of *A. marginale* and *E. ruminantium* in each tick species

Prevalence of *Anaplasma marginale* and *Ehrlichia ruminantium* in ticks according to the regions

Ticks hosting the sought-after pathogens had been found in different regions of the study. Indeed, *A.*

marginale had been found in ticks of the species *I. aulacodi* in two regions (Agneby-Tiassa and Mé) with respective prevalences of 1.85% and 6%. On the other hand, no ticks of the species *R. simpsoni* were found to harbor this pathogen in any region (Table 1).

Concerning *E. ruminantium*, prevalences of 5.31%, 3.13%, 3%, 2.57%, and 2.44% were obtained in ticks of the species *I. aulacodi* respectively in Regions of Agneby-Tiassa, Grands Ponts, Autonomous District of Yamoussoukro, Bélier and Mé. Ticks of the species *R. simpsoni* carrying the DNA fragment of *E. ruminantium* were present in all regions except for the Lôh Djiboua area. The prevalence varied from one region to another. The lowest value was obtained in Grand Ponts Region (7.17%) while the highest value (20.63%) was recorded in Agneby-Tiassa Region (Table 1).

Table 1. Prevalences (%) of *Anaplasma marginale* and *Ehrlichia ruminantium* in different tick species

Regions	<i>Ixodes aulacodi</i>		<i>Rhipicephalus simpsoni</i>	
	<i>Anaplasma marginale</i>	<i>Ehrlichia ruminantium</i>	<i>Anaplasma marginale</i>	<i>Ehrlichia ruminantium</i>
Abidjan	(0/27) 0%	(0/27) 0%	(0/12) 0%	(4/12) 15.66%
Agneby-Tiassa	(2/55) 1.85%	(8/55) 5.85%	(0/21) 0%	(10/21) 20.63%
Belier	(0/42) 0%	(3/42) 2.57%	(0/13) 0%	(3/13) 9.14%
Grands Ponts	(0/14) 0%	(1/14) 3.13%	(0/11) 0%	(2/11) 7.17%
Lôh Djiboua	(0/26) 0%	(0/26) 0%	(0/10) 0%	(0/10) 0%
Mé	(5/30) 6%	(2/30) 2.44%	(0/10) 0%	(3/10) 12.64%
Sud Comoé	(0/17) 0%	(0/17) 0%	(0/11) 0%	(3/11) 12.21%
Yamoussoukro	(0/26) 0%	(2/26) 3%	(0/12) 0%	(4/12) 14%

Note : A tick sample consists of a pool of three ticks

DISCUSSION

During this study, the semi-nested PCR revealed the presence of *A. marginale* in *I. aulacodi* while it was absent in *R. simpsoni*. This may be due to the fact that this pathogen is generally transmitted by ticks of *Ixodes* genus. These results were in line with the findings of Adenyo *et al.* (2020) who found the bacterium of the *Anaplasma* genus in *I. aulacodi* in Ghana while it was absent in *Rhipicephalus* sp. The low prevalence of *A. marginale* in *I. aulacodi* (1.18%) and its absence in *R. simpsoni* could also be explained by the fact that *A. marginale* preferentially infects cattle. Ticks carrying this pathogen may have become infested by taking their blood meals from other animal species before attaching to grasscutters.

Regarding *E. ruminantium*, samples from both tick species examined carried the pathogen. Adenyo *et al.* (2020) also found the genus *Ehrlichia* in *I. aulacodi*. However, they did not find it in ticks of the genus *Rhipicephalus*.

Considering regions, it should be noted that the pathogen *A. marginale* had only been found in the species *I. aulacodi* in two regions (Agneby-Tiassa and Mé). This is likely explained by the occurrence of a major tick vector of this pathogen in these areas. (*Rhipicephalus* (*Boophilus*) *microplus*). As for *R. simpsoni*, positive samples were distributed across all regions of the study except for that of Lôh Djiboua. *E. ruminantium* is generally transmitted by ticks of

Amblyomma genus (Walker *et al.*, 2003). Consequently, it is found throughout the range of this genus of ticks (Biguezoton *et al.*, 2016). Indeed, these ticks are found in almost all regions of Côte d'Ivoire.

No prior study has been conducted on the specific ticks of cane rats concerning the role of vector for this pathogen. These results therefore represent the first information regarding the vectors of this pathogen in grasscutters.

CONCLUSION

This study revealed the presence of *A. marginale* and *E. ruminantium* in tick species specific to grasscutters in southern Côte d'Ivoire. The tick *I. aulacodi* harbored both pathogens while *R. simpsoni* only harbored *E. ruminantium*. These pathogens could be dangerous for grasscutters. It would be wise to study their presence and pathogenicity in grasscutters in the future.

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