

PRESENCE OF MICRO-ORGANISMS IN TICKS FROM THE IVORY COAST¹

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SYNOPSIS

Ticks from seven different localities of the Ivory Coast were examined to identify the micro-organisms they carry and they transmit. Cattle ticks, *Amblyomma variegatum*, were found to be infected by a rickettsia of the spotted fever group. This rickettsia was also found in *Rhipicephalus senegalensis*, once in an area where no cattle or other domestic animals occurred. Rickettsiae possibly different from those above were also found in ticks feeding mainly or occasionally on dogs (*Rhipicephalus sanguineus*, *R. senegalensis*, *Haemaphysalis leachii*, *Haemaphysalis hoodi*), especially in the southern parts of the country. A *B. bigemina*-like babesia was found in the cattle tick *Boophilus geigy*. Although the tick is spread throughout the country, the babesia was found only in the north. *A. variegatum*, *B. geigy* and *R. sanguineus* were found to be infected by *T. theileri*-like trypanosomes, though probably not transmitted by the ticks. Three putative virus strains have been isolated, but not yet identified, from *A. variegatum* and *B. geigy* from the north, and from *R. sanguineus* from the south of the country.

INTRODUCTION

Ticks are the most important ectoparasites of livestock in tropical countries. Not only do they cause important losses in meat and hide production as a result of severe bite wounds, but also may serve as vectors of a wide range of pathogenic micro-organisms, particularly viruses, bacteria, and protozoa that may constitute limiting factors to successful cattle industry.

In West Africa, the economic importance of ticks has not as yet been thoroughly investigated. In the Ivory Coast, heavy tick infestations of cattle have been known to occur only in the northern parts. However, uncontrolled cattle importation from neighbouring countries has led to the introduction of ticks into areas that previously were relatively free of these arthropods. In view of the Ivory Coast's prime objective to increase cattle production, it is safe to speculate that tick populations also will increase to eventually reach proportions that may present health problems to both animals and man.

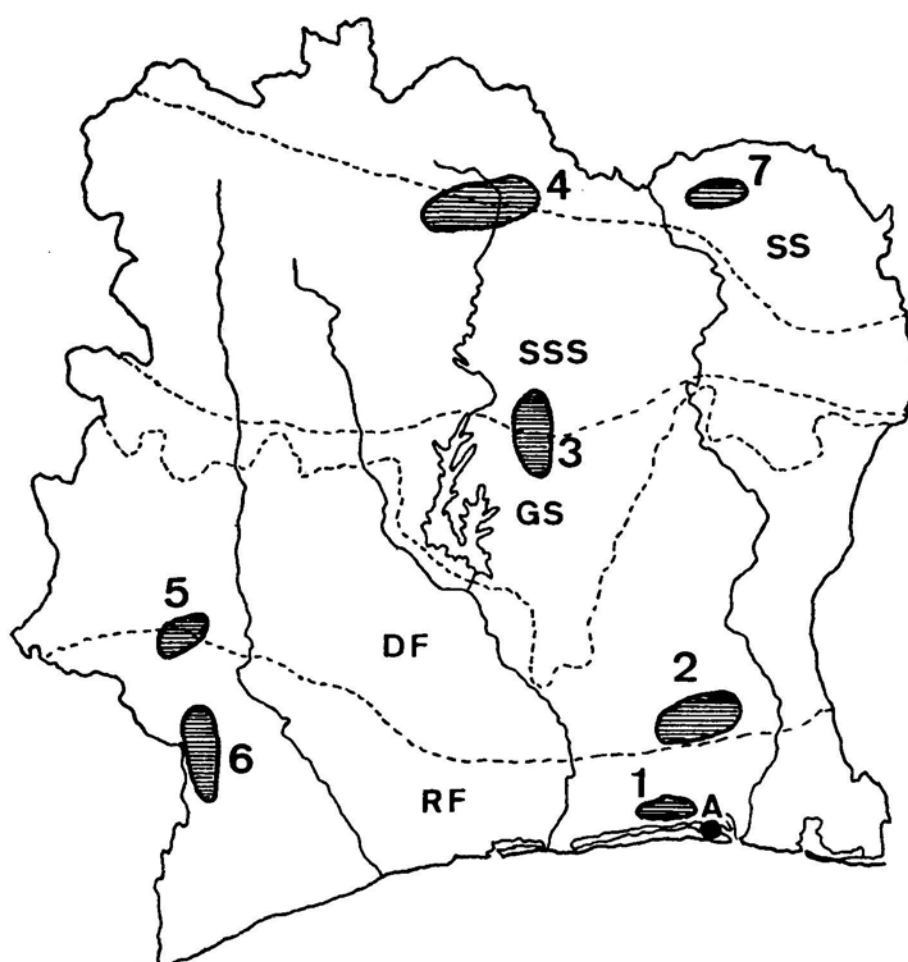
Tick-borne diseases are already prevalent in the Ivory Coast. Thus, babesiosis in cattle and dogs are common, and there is at least serological evidence of rickettsial diseases, particularly Q fever and spotted fever, in man (Gidel *et al.*²). Also the presence of a spotted fever group rickettsia in cattle tick, *Amblyomma variegatum*, from the southern parts of the country was recently demonstrated (Graf *et al.*⁴).

The biology of ticks, their economical role and their epidemiological significance in the Ivory Coast are the subjects of research at the "Centre Suisse de Recherches Scientifiques" in Abidjan. The present paper describes the Centre's additional observations concerning the role of these arthropods as possible vectors and reservoirs of microbial agents.

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Prospected sites

- 1 Adiopodoumé
- 2 Agboville
- 3 Foro
- 4 Korhogo
- 5 Guiglo
- 6 Tai
- 7 Wango-Fitini

- A Abidjan
 RF Rain Forest
 DF Deciduous Forest
 GS Guinea-type Savanna
 SSS Sub-Sudan-type Savanna
 SS Sudan-type Savanna

MATERIAL AND METHODS

Ticks were collected from domestic animals (cattle, sheep, horses, dogs, poultry) or from vegetation in 7 different localities between August 1979 and April 1980. After taxonomic identification, all live specimens were examined by hemolymph test (Burgdorfer¹) for microscopically detectable microbial agents. For this purpose, one of two preparations of hemolymph obtained by amputation of the distal portion of one or more tick legs, was stained by Gimenez³, the other by Giemsa for microscopic evaluation for rickettsiae and protozoan agents, respectively. Ticks found positive for rickettsiae were dissected and preparations from their hypodermis and malpighian tubules were treated by direct immunofluorescence with an anti-*Rickettsia rickettsii* conjugate (RMSF No 288, Rocky Mountain Laboratory) to determine whether the rickettsial agent present was a member of the spotted fever group (SFG). Ticks infected with protozoan agents were also dissected for the preparation of tissue smears of salivary glands, malpighian tubules, intestinal diverticula, ovary or testes. Engorged females harbouring microbial agents were allowed to oviposit before their tissues and eggs were microscopically examined. Finally, pools of ticks from each collection site were forwarded to the "Institut Pasteur de Côte d'Ivoire" where they were examined for tick-borne viruses.

Table 1 Ticks, hosts and prospected sites

| Sites | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|--------|---|---|-------------|------|---|---|---|
| Ticks: | | | | | | | |
| A.v | C | C | C, S, D, F, | C, S | C | – | – |
| B.a | – | – | – | C | – | – | – |
| B.d | H | – | – | – | – | – | – |
| B.g | C | C | C | C, S | C | – | – |
| H.h | – | – | – | – | – | P | – |
| H.l | D | D | – | – | – | D | – |
| H.t | – | C | – | C | – | – | – |
| R.sa | D | D | D | D | – | – | D |
| R.se | – | – | D, F | – | – | – | F |

Ticks

| | |
|------|-----------------------------------|
| A.v | <i>Amblyomma variegatum</i> |
| B.a | <i>Boophilus annulatus</i> |
| B.d | <i>Boophilus decoloratus</i> |
| B.g | <i>Boophilus geigy</i> |
| H.h | <i>Haemaphysalis hoodi</i> |
| H.l | <i>Haemaphysalis leachii</i> |
| H.t | <i>Hyalomma truncatum</i> |
| R.sa | <i>Rhipicephalus sanguineus</i> |
| R.se | <i>Rhipicephalus senegalensis</i> |

Sites

| | |
|---|--------------|
| 1 | Adiopodoumé |
| 2 | Agboville |
| 3 | Foro |
| 4 | Korhogo |
| 5 | Guiglo |
| 6 | Tai |
| 7 | Wango-Fitini |

Hosts

| | |
|---|-------------------|
| C | Cattle |
| S | Sheep |
| H | Horse |
| D | Dog |
| P | Poultry |
| F | Free-living stage |

RESULTS

A total of 3,472 ticks belonging to 5 genera and 9 species were collected in the seven localities indicated in Map 1 in relation to their vegetation. The tick species encountered, their distribution per locality, and their host associations are given in Table 1. Microscopic examination of tick hemolymph revealed as outlined below the presence of rickettsiae, babesiae and trypanosomes in some of these ticks.

Table 2 Rickettsiae and “rickettsia-like” organisms (rickettsia-like organisms bold typeface)

| Ticks | A.v | | | B.a + g | | | B.d | | | H.h | | | H.l | | | H.t | | | R.sa | | | R.se | | |
|------------------|-----|----|------|---------|---|---|-----|---|---|-----|---|-------------|-----|---|-------------|-----|---|---|------|----|-------------|------|------|------|
| | E | I | % | E | I | % | E | I | % | E | I | % | E | I | % | E | I | % | E | I | % | E | I | % |
| Adiopo- doumé | 321 | 10 | 3,12 | 184 | – | – | 34 | – | – | – | – | – | 98 | 3 | 3,06 | – | – | – | 360 | 6 | 1,67 | – | – | – |
| Agboville | 68 | 1 | 1,47 | 30 | – | – | – | – | – | – | – | – | 86 | – | – | 6 | – | – | 75 | 5 | 6,67 | – | – | – |
| Foro | 167 | 7 | 4,19 | 58 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 48 | – | – | 118 | 3 | 2,54 |
| Korhogo | 280 | 9 | 3,33 | 113 | – | – | – | – | – | – | – | – | – | – | – | 28 | – | – | 39 | – | – | – | – | – |
| Guiglo | 139 | 5 | 3,60 | 73 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Tai | – | – | – | – | – | – | – | – | – | 610 | 1 | 0,16 | 10 | – | – | – | – | – | 32 | 2 | 6,25 | – | – | – |
| Wango-Fitini | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 3 | – | – | 58 | 3 | 5,17 |
| Total | 975 | 32 | 3,28 | 458 | – | – | 34 | – | – | 610 | 1 | 0,16 | 194 | 3 | 1,55 | 34 | – | – | 557 | 13 | 2,33 | 176 | 6 | 3,41 |
| | | | | | | | | | | | | | | | | | | | | | | 4 | 2,27 | |

Ticks

| | |
|---------|---|
| A.v | <i>Amblyomma variegatum</i> |
| B.a + g | <i>Boophilus annulatus</i> + <i>geigy</i> |
| B.d | <i>Boophilus decoloratus</i> |
| H.h | <i>Haemaphysalis hoodi</i> |
| H.l | <i>Haemaphysalis leachii</i> |
| H.t | <i>Hyalomma truncatum</i> |
| R.sa | <i>Rhipicephalus sanguineus</i> |
| R.se | <i>Rhipicephalus senegalensis</i> |

E number of examined ticks
I Number of infected ticks
% percentage of infected ticks

a) **Rickettsial organisms:** As summarized in Table 2, fifty-nine (1,9%) of 3,038 ticks contained rickettsia-like organisms indistinguishable from each other by conventional microscopy. Thirty-eight of these (32 *A. variegatum*; 6 *R. senegalensis*) were infected with a rickettsia that completely and strongly reacted with the anti-*R. rickettsii* conjugate, whereas the remaining 21 positive ticks (13 *R. sanguineus*; 4 *R. senegalensis*; 3 *H. leachii*; 1 *H. hoodi*) contained rickettsiae that exhibited a particulate staining pattern.

Table 3 Babesiae and trypanosomes (trypanosomes bold typeface)

| Ticks | A.v | | | B.a + g | | | B.d | | | H.h | | | H.l | | | H.t | | | R.sa | | | R.se | | |
|--------------|-----|---|------|---------|---|---------------|-----|---|---|-----|---|---|-----|---|---|-----|---|---|------|---|------|------|---|---|
| | E | I | % | E | I | % | E | I | % | E | I | % | E | I | % | E | I | % | E | I | % | E | I | % |
| Adiopo- | 193 | 1 | 0,52 | 186 | - | - | 33 | - | - | - | - | - | 51 | - | - | - | - | - | 293 | - | - | - | - | - |
| doumé | | | | | | | | | | | | | | | | | | | | | | | | |
| Agboville | 70 | - | - | 31 | - | - | - | - | - | - | - | - | 84 | - | - | 4 | - | - | 74 | 1 | 1,35 | - | - | - |
| Foro | 91 | - | - | 57 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 50 | - | - | 117 | - | - |
| Korhogo | 109 | - | - | 519 | 8 | 1,54 | - | - | - | - | - | - | - | - | - | - | - | - | 42 | - | - | - | - | - |
| Guiglo | 28 | - | - | 72 | 1 | 1,39 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Tai | - | - | - | - | - | - | - | - | - | 368 | - | - | 9 | - | - | - | - | - | 32 | - | - | - | - | - |
| Wango-Fitini | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 86 | - | - |
| Total | 491 | 1 | 0,20 | 865 | 8 | 0,92 | 33 | - | - | 368 | - | - | 144 | - | - | 4 | - | - | 491 | 1 | 0,20 | 203 | - | - |
| | | | | | | 1 0,12 | | | | | | | | | | | | | | | | | | |

Ticks

A.v *Amblyomma variegatum*

B.a + g *Boophilus annulatus* + *geigy*

B.d *Boophilus decoloratus*

H.h *Haemaphysalis hoodi*

H.l *Haemaphysalis leachii*

H.t *Hyalomma truncatum*

R.sa *Rhipicephalus sanguineus*

R.se *Rhipicephalus senegalensis*

E number of examined ticks

I number of infected ticks

% percentage of infected ticks

b) **Protozoan agents:** Of the 2,599 ticks examined for protozoan agents, 8 *Boophilus geigy*, all from the Korhogo collection site, were found infected with a babesia, and one specimen each of *A. variegatum*, *B. geigy*, and *R. sanguineus* contained trypanosomes (see Table 3). Babesia vermicules were abundant in the hemolymph but scarce in the smears of hypodermis, salivary glands and ovaries. Vermicules were also found in squash preparations of eggs laid by infected females.

The mean size of the hemolymph forms was about 9,5 μ , with a range of 6-12 μ . This suggests the presence of *Babesia bigemina*, for which Riek⁶ reports a length ranging from 9-13 μ . Identification of the protozoan is in progress.

As shown in Table 3, trypanosomes were found in each one of the following ticks, *A. variegatum*, *B. geigy*, and *R. sanguineus*. Epimastigotes were highly abundant in the hemolymph of the three species. Amastigotes were found in the hypodermis of *R. sanguineus* only. Ovaries and salivary glands were not infected and no infective metacyclic form was found.

Appearance and size of the epimastigotes didn't differ from one tick to the other. Total length was about 28,5 μ , with a range of 22-32 μ and in this regards, the trypanosomes didn't differ significantly from *Trypanosoma theileri* - like forms described by Krinsky and Burgdorfer.⁵

c) **Viruses:** Of 56 tick pools tested so far, 3 (1 pool of *R. sanguineus* from Agboville; 1 pool of *A. variegatum* and 1 pool of *B. geigy* from Korhogo) induced in suckling mice reactions suggestive of virus isolation. Isolation efforts and additional tick testing are in progress.

DISCUSSION

The finding of SFG rickettsiae in 59 of 3.038 ticks from different localities in the Ivory Coast confirms previous observations (Graf *et al.*⁴) and suggests widespread distribution of these organisms in that country. Whether these rickettsiae represent *R. conorii*, the causative agent of boutonneuse fever, or closely related organisms, must await their isolation and identification by sophisticated serologic and/or biochemical procedure. Morphologic characterization by ordinary light microscopy, and staining reactions with nonspecific conjugates are of little value in differentiating members of this antigenically heterogeneous groups of organisms.

The majority of rickettsiae-infected ticks, 32 of 59, were represented by the cattle tick, *A. variegatum*. This tick, along with other *Amblyomma* spp. was previously shown to be the main vector of an unidentified SFG rickettsia in

Ethiopia (Burgdorfer *et al.*^{1a}). It is of interest that in that study, as in the present one, other cattle ticks were free of rickettsiae.

The presence of a SFG rickettsia in *R. senegalensis* from an area free of cattle, and in ticks that principally (*R. sanguineus*, *H. leachii*) or occasionally (*R. senegalensis*, *H. hoodi*) feed on dogs, strongly suggests this agent to be of different ecology than that present in *A. variegatum*. This is also indicated by the staining reactions with the anti-*R. rickettsii* conjugate; the rickettsiae in contrast to those in *A. variegatum* and in *R. senegalensis* stain in an incomplete fashion, i.e. only part of their antigenic make-up reacts with the labelled antibodies.

To the best of our knowledge, this is the first report of *Babesia* in ivorian ticks and certainly for the first time in *B. geigy* which replaces *B. decoloratus* in West Africa. According to veterinarians, *B. bigemina* and *B. bovis* are prevalent in cattle in the northern parts of the Ivory Coast where the latter species appears to be more abundant. The size of the protozoa in *B. geigy* suggests that the organism belongs to the former species.

There was no evidence of *B. canis* in the relatively large numbers of *R. sanguineus* and *H. leachii* examined. This is surprising in view of the fact that canine babesiosis, according to local veterinarians, is common throughout the Ivory Coast.

Detection of trypanosomes in one specimen each of *A. variegatum*, *B. geigy* and *R. sanguineus* is in support of previous reports (reviewed by Krinsky and Burgdorfer²) of infection of ticks with this parasite. However, like some of the previous authors, we recorded only epimastigotes and dividing forms in the hemolymph. Absence of infective forms either in the hemolymph or in the tissues of salivary glands and ovary, suggests that these ticks are not involved in the transmission of these trypanosomes.

Further investigations, particularly isolation and identification of the organisms encountered are necessary to determine their significance as pathogens for animals and man.

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