

Sub-clinical infection of dogs from the Ivory Coast and Gabon with *Ehrlichia*, *Anaplasma*, *Mycoplasma* and *Rickettsia* species

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In 2003, we carried out a field survey in order to investigate the significance of sub-clinical tick-borne bacterial infections in African dogs from the Ivory Coast ($n = 137$) and Gabon ($n = 255$). So far, only limited data on this topic are available in Africa when compared with the USA and Europe, and in most of the cases, serological techniques have been applied [1]. In this study, we used PCR amplification and DNA sequencing to screen different tick-borne bacterial pathogens in the blood of dogs.

The dogs from the Ivory Coast were resident in the capital Abidjan and were used for surveillance purposes by protection companies. They were kept in 16 different kennels and received regular medical prophylaxis including vaccinations and external anti-parasitic treatments. Dogs from Gabon were recruited in 15 small villages in the area of Ogooué-Ivindo, situated in the north-eastern part of the country. They were non-kennelled companion animals and received no specific medical prevention. All the dogs appeared healthy at the time of the examination. All Gabonese dogs were infested by fleas and some of them by *Haemaphysalis leachi* ticks, while no ectoparasites were found in dogs from the Ivory Coast. Blood was collected in EDTA-anticoagulated tubes kept deep-frozen at -20°C until further processing.

In the laboratory, DNA extraction was carried out using QIAamp DNA Mini Kits (Qiagen Ltd, Crawley, UK) according to the manufacturer's recommendations. Briefly, blood samples were digested for 10 min with a mixture of proteinase K and detergents at 56°C to liberate host and

pathogen DNA. DNA was extracted from 200 μL of blood and purified DNA was eluted in 100 μL of low ionic strength buffer, pH 8.0. DNA from individual dogs was screened by PCR, using specific primers, for the presence of *Ehrlichia* and *Anaplasma* species (Ivory Coast), and *Ehrlichia* and *Anaplasma* species, *Mycoplasma* spp. and *Rickettsia* spp. (Gabon). PCR assays amplified a 350 base pair fragment of the 16S rRNA gene for *Ehrlichia*/*Anaplasma* species (primer sequences: ggtaccyacaagaagtcc and tagcactcatcgtttacagc), 16S rRNA gene for *Mycoplasma* spp. (primer sequences: atacggccatattcctacg and tgctccaccactgttca) and ompB gene for *Rickettsia* spp. (primer sequences: gacaattaatcggtagcgg and tgcatcagcattaccgcttcg). Sequencing was required to further characterise positive results for *Ehrlichia*/*Anaplasma* PCR and limited sequencing was performed for *Mycoplasma* spp.

Overall, 10/392 (2.6%) of the tested dogs were positive for *E. canis* and 5/392 (1.3%) for *A. platys* (Table 1). The majority of them was from the Ivory Coast; 10 were positive for *E. canis* and two for *A. platys*. In Gabon, only three dogs were positive for *A. platys* and none was positive for *E. canis*. In this latter country, a high proportion of dogs (114/255, 44.7%) was positive for *Mycoplasma* spp. Sequencing of 10 *Mycoplasma* positive samples revealed four *Mycoplasma haemocanis*, one *Mycoplasma haemofelis*, and five samples of a novel *Mycoplasma*. No sample was positive for *Rickettsia* spp. The three Gabonese dogs positive for *A. platys* were co-infected with *Mycoplasma* spp. No *E. canis* and *A. platys* co-infections were present in the Ivory Coast dogs.

Ehrlichia canis is responsible for canine monocytic ehrlichiosis, a widespread infectious disease in the distribution area of its vector *Rhipicephalus sanguineus*, the brown dog tick. Interestingly, in a previous seroprevalence survey of *E. canis* infection in the same population of dogs [1], 67.8% of

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No conflicts of interest declared.

Table 1. Molecular detection of bacterial tick-transmitted pathogens in dogs of western Africa

	<i>Ehrlichia canis</i>	<i>Anaplasma platys</i>	<i>Mycoplasma</i> spp.	<i>Rickettsia</i> spp.
Ivory Coast Abidjan <i>n</i> = 137	10/137 7.3%	2/137 1.5%	Not-detected	Not-detected
Gabon Ogooué-Ivindo area <i>n</i> = 255	0 0%	3/255 1.2%	114/255 44.7%	0 0%
Total <i>n</i> = 392	10/392 2.6%	5/392 1.3%	114/255 44.7%	0 0%

dogs in the Ivory Coast were positive and only 3.1% in Gabon, while our molecular study found 7.3% and 0%, respectively. Moreover, the average titres of positive samples from the Ivory Coast dogs were much higher than those from the Gabonese dogs. The absence of *E. canis* DNA among 255 dogs in Gabon is consistent with the low prevalence of its vector, *R. sanguineus*.

Rhipicephalus sanguineus is also considered as being the main vector of *A. platys*, the cause of canine infectious cyclic thrombocytopenia, although naturally infected dogs do not necessarily exhibit clinical signs [2]. *Anaplasma platys* has already been isolated from a dog in the Democratic Republic of the Congo [2]. The presence of *A. platys* in the Gabon dog population despite the apparently low exposure to *Rhipicephalus* ticks requires further investigation. However, our study suggests that specific testing for *A. platys* is recommended when a clinical suspicion of *E. canis* infection is not confirmed.

Mycoplasma haemocanis is responsible for anaemia in dogs but clinical signs mainly appear in those that are immuno-compromised or splenectomised [3,4]. The prevalence of *Mycoplasma* infection in the Gabonese dogs of this study is high and this may be explained by persistent infection, which has been previously reported in dogs [4]. Transmission by *R. sanguineus* is suspected [3] but this does not correlate with the high prevalence of *Mycoplasma* infection in an environment of low *R. sanguineus* exposure. The dogs

sampled in Gabon were parasited by fleas and *Haemaphysalis leachi* ticks, and the role of these arthropods in the transmission of *Mycoplasma* spp. should be further investigated. The finding of novel haemoplasma species, including one with high similarity to *M. haemofelis*, in this dog population, requires further investigation in light of the new canine haemoplasma species being recognised elsewhere [5]. The high level of *Mycoplasma* infection in Gabon could be responsible for an increased morbidity and mortality, when combined with stress or in the case of co-infection with other arthropod-transmitted infections. In our study, the three dogs positive for *A. platys* were also infected by *Mycoplasma* spp.

Overall, the high prevalence of sub-clinical canine tick-borne bacterial infections from these two African countries indicates the circulation of these agents, more particularly *Mycoplasma* spp.; however, the transmission biology may differ from those described previously.

ACKNOWLEDGEMENTS

The authors thank Dr Philippe Parola for his excellent cooperation. This research was supported in part by Merial SAS.

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