



## Serological survey of canine leptospirosis in three countries of tropical Africa: Sudan, Gabon and Ivory Coast



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### ABSTRACT

A cross-sectional survey was conducted to estimate the presence of leptospiral antibodies among 475 dogs from three countries of tropical Africa: Sudan ( $n = 62$ ), Gabon ( $n = 255$ ) and Ivory Coast ( $n = 158$ ). Sixteen reference strains belonging to seven serogroups were used as antigen in the microscopic agglutination test. Overall, considering titres  $\geq 1:40$ , 453 samples were positive towards one or several serovars of pathogenic leptospire. Focusing on high titres, i.e.  $\geq 1:320$ , the seroprevalence was 40.8%. In Gabon, the seroprevalence was higher in rural areas than in an urban environment ( $p < 0.001$ ). In Ivory Coast, the seroprevalence for serogroups Icterohaemorrhagiae and Canicola was not statistically different according to the vaccinal status. Predominant serogroups varied according to the countries but Grippotyphosa and Sejroë were the most common, while Icterohaemorrhagiae and Canicola were dominant in Sudan. In these three countries, dogs are heavily exposed to pathogenic *Leptospira* and humans living in the same environment are also at risk of infection.

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### 1. Introduction

Leptospirosis is presumed to be the most widespread zoonosis in the world [1]. The disease is caused by spirochetes of the genus *Leptospira* and more than 300 distinct leptospiral serovars of pathogenic *Leptospira* species are now recognized [2]. These are arranged in 25 serogroups based on the presence of agglutinating antibodies in analyzed sera [3]. Leptospirosis has been identified as an emerging or a re-emerging infectious disease, particularly in tropical and subtropical regions, where environmental conditions contribute to the survival and transmission of

leptospire [4,5]. Estimated 300,000–500,000 severe cases occur each year, with case-fatality reports of up to 50% [6–9]. There is limited knowledge of *Leptospira* incidence in Africa. The disease is well-known to be endemic but outbreaks can occur as in Somalia where the disease killed five Ugandan peacekeepers and affected 50 others in July 2009 [10].

Leptospirosis is also one of the most globally widespread infectious diseases in dogs. Due to their close contact, dogs pose a risk of infection for humans [2,11]. Although many prevalence studies on dogs have been done in other continents, to date, very limited data on canine leptospirosis are available in Africa.

The aim of the present study was to estimate the presence of leptospiral antibodies among dogs ( $n = 475$ ) from three countries of East, Central and West tropical Africa,

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and to identify the predominant serogroups circulating in these countries.

## 2. Materials and methods

### 2.1. Dogs and sera

From 2000 to 2013, blood samples were collected from 475 apparently healthy dogs living in East, Central and West tropical Africa: Sudan (January 2000;  $n=62$ ), Gabon (August 2003;  $n=255$ ), and Ivory Coast (December 2007 and March 2013;  $n=158$ ).

The dogs were voluntarily brought in by their owners. Breed, sex, age, way of housing, and sanitary status were recorded for each dog.

They were classified into five groups according to location, way of housing and prophylaxis (vaccinated with a bivalent vaccine or non-vaccinated).

In Sudan, dogs were living in a village of eastern Sudan ( $n=62$ ; group I). There were family dogs with free access to outside; living close to cattle and sheep (but not pigs). The climate is very hot with a dry season occurring from November to April.

In Gabon, dogs were living in Mékambo, a city of north-eastern Gabon ( $n=99$ ; group II) and different villages around ( $n=156$ ; group III). There were family dogs with free access to outside and to the plantations; dogs from the villages were also used for hunting; few farm animals could be found around. The climate is hot and humid with a long cold dry season from June to September, a short rainy season from October to November, a short dry season from December to January, and a long rainy season from February to May.

In Ivory Coast, dogs were living in Abidjan and in two close cities of South Ivory Coast. There were family and police dogs living in cities. The coastal and central region has a long dry season from December to April and a short dry season from August to September as well as a long rainy season from May to July and a short rainy season from October to November. Dogs from Ivory Coast were divided into a vaccinated ( $n=93$ ; IV) and a non-vaccinated group ( $n=65$ ; V).

No symptoms of disease were identified in any of the dogs included in the study.

### 2.2. Laboratory diagnostics

After centrifugation, sera were stored at  $-20^{\circ}\text{C}$  before sending to France for microscopic agglutination tests (MAT) in the French animal *Leptospira* laboratory in Nantes and later in Lyon. The MAT is the serological reference test, particularly appropriate for carrying out epidemiological studies [4]. The MAT was performed using different serovars of pathogenic *Leptospira* species: Icterohaemorrhagiae, Copenhageni, Australis, Bratislava, Munchen, Autumnalis, Pyrogenes, Canicola, Grippotyphosa, Vanderhoedoni, Panama, Zanoni, Sejroe, Saxkoebing, Hardjo, and Wolffi, belonging to height serogroups: Australis, Autumnalis, Icterohaemorrhagiae, Canicola, Grippotyphosa, Sejroë, Panama, and Pyrogenes (Table 1).

The choice of the serovars used for each serogroup was based upon the experience of the laboratory. According to observations recorded in the French animal *Leptospira* laboratory in Nantes and later in Lyon, titres as low as 1:40 were considered as evidence of past exposure to the contact while titres  $\geq 1:320$  were considered as evidence of recent contact or vaccination (usually under 2 months) or current infection [12].

The titre for a serogroup was given by the highest titre of the contributing serovars and the global titre for leptospirosis was given by the titre of the serogroup with the highest titre.

### 2.3. Statistical analysis

The results were analyzed by  $2 \times K$  contingency tables of exposure variables. Odds ratios (OR), 95% confidence intervals (CI) and  $p$  values were calculated separately for each variable using the Epi info™ (version 5.01, CDC Atlanta, USA). The Chi-square, or the Fisher's exact test if appropriate, was used to evaluate associations ( $\alpha=5\%$ ). Differences were considered statistically significant when  $p \leq 0.05$ .

## 3. Results

On the 475 blood samples tested, 95.4% [CI<sub>95</sub>: 93.4–97.2] were found to be positive to one or more serovars when a cut off of 1:40 was applied.

Focusing on high titres ( $\geq 1:320$ ), the prevalence rate was significantly different: 74.2% in group I (Sudan), 9.1% in group II (Gabon, Mékambo), 34.6% in group III (Gabon undetermined villages), 58.1% in group IV (Ivory Coast, vaccinated dogs), and 47.7% in group V (Ivory Coast, non-vaccinated dogs).

Leptospiral seroprevalence (titres  $\geq 1:320$ ) according to the different groups is shown in Fig. 1.

Prevalence in males and females was not statistically different and no effect of age was found among highly positive dogs ( $p > 0.05$ ).

The distribution of different *Leptospira* serogroups among the seropositive animals according to groups is presented in Fig. 2 (titres  $\geq 1:40$ ) and in Fig. 3 (titres  $\geq 1:320$ ).

## 4. Discussion

### 4.1. Prevalence

Over 95% of the tested animals are positive (titres  $\geq 1:40$ ) towards one or several serovars of pathogenic leptospires. Most of the dogs reacted to several serogroups, which can be a result of diverse exposures or more probably the result of cross reactions between serogroups. Such cross reactions between different serogroups for low and even higher titres has been reported during the MAT testing [12].

Additionally, 40.8% [CI<sub>95</sub>: 36.4–45.2] of animals are positive on high titres ( $\geq 1:320$ ), without evidence of clinical leptospirosis.

Dogs are not usually considered as a reservoir for *Leptospira*, except for Canicola [4,12], thus the high prevalence found in this study is likely a result from a heavy exposure

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