

EPIDEMIOLOGICAL STUDY OF CANINE TRYPANOSOMOSIS IN AN URBAN AREA OF IVORY COAST

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Summary:

Following confirmed cases of trypanosomosis in military working dogs, a cross-sectional study was undertaken to evaluate the source of infection and determine the prevalence of canine infection with *Trypanosoma congolense* in the urban focus of Abidjan, Ivory Coast. Blood from 123 dogs were collected and subjected to PCR using specific primers for *Trypanosoma congolense* "forest type". In addition, an entomological study was conducted in an urban area near the forest surrounding the military camp. The observed prevalence was 30.1 % and PCR positivity to *Trypanosoma congolense* was not significantly associated with sex or age of animals. This study demonstrates the high contamination rate of dogs in enzootic zones, the potential risk of introduction of the disease in free animal populations and the ability of *Glossina palpalis* to adapt to urban areas and to transmit trypanosomosis in such areas. The factors leading to a possible emergence of canine trypanosomiasis in enzootic zones need further investigations.

KEY WORDS: *Trypanosoma congolense* "forest type", dog, *Glossina palpalis palpalis*, Ivory Coast.

Résumé :

ÉTUDE ÉPIDÉMIOLOGIQUE DE LA TRYPANOSOMOSE CANINE DANS UN FOYER URBAIN DE CÔTE D'IVOIRE
Après la confirmation de cas de trypanosomose chez des chiens militaires, une étude transversale a été entreprise afin de déterminer la source de l'infection et la prévalence de *Trypanosoma congolense* chez le chien dans le foyer urbain d'Abidjan, Côte d'Ivoire. Le sang de 123 chiens a été prélevé et analysé par PCR à l'aide d'amorces spécifiques de *Trypanosoma congolense* "type forêt". De plus, une enquête entomologique a été conduite en zone urbaine et à proximité de la forêt entourant le camp militaire. La prévalence observée était de 30,1 %; le fait d'être positif à la PCR à *T. congolense* n'était pas associé significativement au sexe ou à l'âge de l'animal. Cette étude a montré le fort taux de contamination des chiens dans cette zone enzootique, le risque potentiel d'introduction de la maladie dans une population animale naïve et la capacité de *Glossina palpalis* de s'adapter et de transmettre la trypanosomose dans un environnement urbain/péri-urbain. Des études complémentaires permettant de mieux cerner les facteurs pouvant conduire à une émergence de la trypanosomose canine en zone enzootique sont nécessaires.

MOTS CLÉS : *Trypanosoma congolense* "type forêt", chien, *Glossina palpalis palpalis*, Côte d'Ivoire.

INTRODUCTION

African trypanosomiasis due to *Trypanosoma congolense* is a major threat to livestock production in sub-Saharan Africa. It is transmitted cyclically by tsetse flies (Diptera: Glossinidae). Although the disease mainly concerns cattle, it may also infect a large range of domestic and wild animals. It has been particularly observed in military working dogs in a French squad (43rd BIMA) located in Abidjan, Ivory Coast, where several dogs died suddenly between 2000 and

2001. At this time, infection by *T. congolense* "forest type" was incriminated (Davoust *et al.*, 2006a) and it was decided to administrate an isometamidium preventive treatment to each military dog.

Hence, this disease appeared to be a significant veterinary problem for cynophilic sections in this region, and it was necessary to evaluate its origin and define a vector control strategy. In order to evaluate the risk of infection, the presence of the vector *Glossina palpalis palpalis* was studied by an entomological survey around this zone. Furthermore, the presence of infected dogs was investigated. The present study reports the results of these investigations and discusses epidemiological patterns and risk factors.

MATERIAL AND METHODS

The climate of the area is characterised by a rainy season during approximately four months and by a dry season covering the remaining months. Temperatures vary between 21 and 33 °C and

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humidity between 80 and 90 %. The data were collected between November and December 2003, during a cross-sectional survey of the dog population of Abidjan (Ivory Coast). Consenting owners provided the age and sex of each animal and the study mainly concerned watch dogs which are more exposed to the disease because of their outdoor way of life. A total of 123 dogs were sampled. The sampling involved more male dogs (65.9 %) than females (34.1 %). Young dogs (< 3 years) were more numerous (54.5 %) than older dogs (45.5 % for 3-8 years dogs and 4.1 % for dogs older than nine years). From each of the 123 animals, blood was sampled on Whatman filter paper N° 4 and kept frozen before DNA extraction.

The entomological survey was carried out within the 43rd BIMA by placing 22 Vavoua traps (Laveissière and Grébaut, 1990) during three different periods (April 2003, November 2003 and March to May 2004) in order to capture the seasonal variations in the vector density. Three traps were placed around the kennel, three around the horses, and the others along the perimeter of the BIMA, particularly on the east side near the forest. Flies were collected every day during four consecutive days. Insects were counted, sex was recorded, and they were dissected daily in order to determine their age and detect the presence of trypanosomes. For the dissection, the mouthparts were first removed to prevent contamination from the midgut, and dissecting instruments were cleaned between each organ dissection with sodium hypochlorite followed by rinsing in sterile water. Each organ (mouthparts, salivary glands, midgut) was put into a separate eppendorf tube containing 30 µl sterile distilled water.

In the laboratory, blood impregnated Whatman papers were placed in an eppendorf tube containing 1 ml of a Chelex 100® 5 % (w/v) solution. The tube was then heated at 55 °C for one hour and 30 minutes at 95 °C. After centrifugation at 15,000 rpm for 10 minutes, the supernatant containing the DNA was diluted (1/10, 1/100) and used directly as template for PCR. Dilution was performed to avoid inhibition of the enzyme Taq DNA polymerase due to blood residues. Primers specific for *T. congolense* "forest type" (Masiga *et al.*,

1992) were used. Amplifications were performed in a final volume of 50 µl containing 2 µl of diluted DNA extract (template), 10 mM Tris-HCl (pH 9), 50 mM KCl, 3 mM MgCl₂, 20 picomoles of each primer, 200 µM of each dNTP and one unit of Taq DNA polymerase (Appligene-Oncor, USA). One denaturing step at 94 °C for five minutes was followed by 40 amplification cycles. Each cycle was constituted by a denaturation step at 94 °C for 30 seconds, an annealing step at 55 °C for 30 seconds and an extension step at 72 °C for one minute. This was followed by a final extension at 72 °C for 10 minutes. PCR products were separated on 2 % agarose gel and stained with ethidium bromide (0.3 µg/µl). Polymerase chain reaction on tsetse samples was performed with a simple 1 % Chelex 100® 5 % treatment used to obtain DNA for PCR reactions, as in Solano *et al.*, 1999. The following primers were used: TBR1-2 specific for *T. brucei*, TCF 1-2 specific for *T. congolense* forest type, TCS 1-2 specific for *T. congolense* Savannah and TV1-2. specific for *T. vivax*. Odds Ratios (OR) for each risk factor and Khi² test were calculated with Epi-Info®. The level of significance was set at 5 %.

RESULTS

The prevalence obtained in the whole population studied was 30.1 % (25.1-35 %). The 11 dogs sampled inside the BIMA were all negative but had recently arrived from France and received an isometamidium treatment at their arrival. There was no association between positivity and the sex of the animals (OR = 0.67, IC_{95%} = [0.28-1.62]) nor with their age (p = 0.311).

The results of the entomological survey during the three periods are summarized in table I. *Glossina palpalis palpalis* was the only captured species. They were present in high densities, mainly in the traps situated on the east side of the BIMA near the forest, but very few around the kennel and the horses (< 0.2 fly/trap/day). Density was lower in November 2003, probably because of an unexpected spreading of insecticide per-

		April 2003	November 2003	March 2004	April 2004	May 2004
Density		5.92	1.1		2.95	
Sex-ratio (F/M)		1.33	1.15		1.32	
Number of tsetse flies	Captured	427	88	374	236	170
	Dissected	62	58		248	
	Infected	16	12		52	
Infection rate (%)		25.8	20.7		20.96	

Table I. – Results of the different tsetse trapping period: details of sampled flies and infection rates (density is expressed in number of tsetse flies/trap/day).

formed at the first day of the collection. During 2004, the density progressively decreased from March to May. Trypanosome infection rate in the flies was high in the three periods, ranging between 20.7 and 25.8 %. PCR analysis showed that the parasites identified belonged to the species *T. congolense* "forest type" and *T. vivax* but not to *T. congolense* "savannah type" nor to *T. brucei*.

DISCUSSION

Dogs are susceptible to several trypanosomes, mainly *T. cruzi* causing Chagas disease in America, and also *T. congolense*, *T. brucei* and *T. evansi* in Africa (Abenga *et al.*, 1999; Matete, 2003). Infections with *T. brucei* appear to be the most pathogenic (Nwosu & Ikeme, 1992; Matete, 2003). *T. congolense* infection in dogs may occur in an acute, chronic or subclinical form (Losos & Ikede, 1972; Sayer *et al.*, 1979; Davoust *et al.*, 2006a). The main symptoms of the acute form are not pathognomonic but hyperthermia, anorexia, anemia, adenomegaly, splenomegaly, haemorrhages, oedematous swelling in different part of the body and neurological signs have been reported (Harrus *et al.*, 1995; Davoust *et al.*, 2006a). Specific anti-trypanosome treatments are based on the use of trypanocidal drugs, mainly isometamidium, which can also be used for chemoprophylactic purpose (Davoust *et al.*, 2006b).

In our study, although all dogs sampled were apparently healthy, the prevalence rate of parasitemic animals was high. This may be attributed to the fact that they were infected by *T. congolense* of the Forest type, which has been reported to be poorly virulent and gave low parasitemias in mice and cattle (Bengaly *et al.*, 2002a, b). Hence, the parasitemia may be low and cyclic for indigenous dogs so they tend to be chronic carriers and may serve as a reservoir for infection (Hörchner *et al.*, 1985). In contrast, non-indigenous dogs may show a persistent high parasitemia and die rapidly after infection. Few imported cases of *T. congolense* infection have been reported during the past 20 years (Harrus *et al.*, 1995; Davoust *et al.*, 2006b; Gow *et al.*, 2007). The acute flare up of chronic disease could be due to the stress caused by environmental changes or an underlying disease process.

The prevalence observed in this study may be overestimated since 95 % of the dogs sampled were watch dogs and thus were probably more exposed to the disease than pet dogs. Sex and age do not seem to be risk factors for this disease. PCR is a very sensitive tool for estimation of prevalence of trypanosomiasis (Solano *et al.*, 1999). The major problem is to know whether dogs found positive with this technique will become ill and when they will show the first signs of the disease.

Glossina palpalis palpalis were present in high densities and the rate of infected flies was high during the three periods of sampling. This observation confirms the ability of *G. palpalis* to adapt to big urban centers such as Abidjan where it is also able to transmit trypanosomosis, provided microclimatic conditions and host availability are met. It also strengthens the need to monitor a potential risk of transmission of sleeping sickness, such epidemics having been described in other big towns (Kinshasa, see Ebeja *et al.*, 2003).

CONCLUSION

This study demonstrates the high contamination rate of dogs by *Trypanosoma congolense* in enzootic zones, and the risk they could represent if they were introduced in disease free animal populations. It also emphasizes the need for routine quarantine, including health checks and eventually chemoprophylactic treatments for dogs coming from enzootic zones.

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