**Trypanosoma vivax, T. congolense "forest type" and T. simiae: Prevalence in domestic animals of sleeping sickness foci of Cameroon**

**INTRODUCTION**

Animal trypanosomosis constitutes a serious handicap to animal husbandry in all regions of sub-Saharan Africa infested by tsetse flies (Vaucel et al., 1963). The infection agents are protozoans of the genus *Trypanosoma*. In tsetse infected areas, species and subspecies *T. congolense*, *T. vivax*, *T. simiae* and to a less extend *T. b. brucei* are pathogenic parasites to animals, especially Suinae and domestic ruminants (Omeke, 1994; Reinfenberg et al., 1997). Most of these trypanosomiasis species pathogenic to animals are transmitted by tsetse flies. However, *T. vivax* can be mechanically transmitted by Tabanids or Stomoxys in Africa, and it is presumed to be transmitted only mechanically in areas not infested by tsetse flies (Gardiner & Wilson, 1987; Gardiner, 1989; D'Amico et al., 1996). Moreover, experimental studies have demonstrated the possibility of mechanical transmission of *T. congolense* and *T. brucei* species by tabanids and Stomoxys (Mihok et al., 1995; Sumba et al., 1998). However, the importance of such transmission under natural conditions is still under debate (Desquesnes & Dia, 2003).

In animals, infection with trypanosome may result in a chronic, debilitating, emaciating and often fatal disease but the outcome of the infection differs substantially between trypanosome species or subspecies, between livestock species and within a livestock species among...
breds depending on the challenge and virulence of the strains (Connor & Van den Bossche, 2004). Due to their frequencies, pathogenicity and consequence on productivity, *T. congolense* and *T. vivax* are the principal trypanosomes in domestic ruminants (Wellede et al., 1983; Trail et al., 1991).

Desoxyribonucleic acids (DNA) probes have allowed the identification of four different sub-species of *T. congolense* in different ecological zones: *T. congolense* “forest type”, *T. congolense* “savannah type”, *T. congolense* “Kilifi type” and *T. congolense* “Tsavo” (Majiwa et al., 1985; Masiga et al., 1992; Clausen et al., 1998; Majiwa et al., 1993). These variants of *T. congolense* are pathogenic and develop high parasitaemia accompanied by anemia and leucopenia (Sidibe et al., 2002; Bengaly et al., 2002a, b). However, there are clear differences in the pathogenicity among the various types of *T. congolense* and even within one single type. For example, experimental studies comparing the virulence of one strain of each subgroup in mice and cattle have shown differences between the subgroups with the *T. congolense* strain of the Savannah subgroup being the most virulent (Bengaly et al., 2002a, b). Moreover, substantial differences in the virulence of *T. congolense* strains of the “savannah” subgroup, isolated in one geographical area from a single host species have also been recently reported (Masumu et al., 2006). For *T. vivax* infections, it is well known that animals infected by this trypanosome support better the infection because their genetic diversity is more limited than that of *T. congolense* and *T. brucei* (Authié et al., 1999). However, fulminant and hemorrhagic forms of *T. vivax* that cause death or abortion have been described (Hudson, 1944; Olubayo et al., 1985). *T. congolense* and *T. vivax* species have been shown to cause serious infections in horses and asses (Faye et al., 2001; Dhollander et al., 2006). *T. simiae* has been reported as a parasite of suidae (Stephen, 1966).

During herd control by veterinary services, *T. vivax*, *T. congolense* or *T. b. brucei* were found in pigs, ruminants and equines in West Africa (Mattioli et al., 1994; Omeke et al., 1994; Solano et al., 1999; Faye et al., 2001; Dhollander et al., 2006). In the Central African region, limited work has been conducted on animal trypanosomosis, especially in sleeping sickness foci where *Trypanosoma brucei gambiense* is the causative agent of the disease. Indeed, in the forest area of southern Cameroon where studies were undertaken in the historic sleeping sickness foci of Fontem and Mbam, *T. congolense*, *T. vivax* and *T. simiae* were identified in pigs and small domestic ruminants (Asonganyi et al., 1986; Asonganyi et al., 1990; Simo et al., 2006). Moreover, the presence of *T. brucei* s.l., *T. congolense* and *T. vivax* were reported in cattle of the pastoral zone of Adamaua (North-Cameroon) (Mamadou et al., 2006). However, most of these works were performed either on one species of domestic animals or used parasitological and immunological methods. Given the low sensitivity and specificity of these methods, it is obvious that the prevalence of different trypanosomes species or subspecies were considerably underestimated. With the development of molecular biology during the last decades, specific DNA sequences of different trypanosome species and subspecies have been identified and several PCR based methods were developed to improve the detection of various parasites. Applied in human and animal trypanosomosis, PCR appeared as a reliable, sensitive and specific techniques enabling to detect different trypanosome species and subspecies in vertebrate hosts as well as in tsetse flies (Masiga et al., 1992; Desquesnes & Tresse, 1996; Penchenier et al., 2000; Mugutu et al., 2001; Picozzi et al., 2002; Geysen et al., 2003; Delespaux et al., 2003; Gonzales et al., 2005; Cox et al., 2005). The present study reports the level of infections with *T. vivax*, *T. congolense* “forest type” and *T. simiae* in four species of domestic animals commonly found in sleeping sickness foci of forest areas of southern Cameroon.

### MATERIALS AND METHODS

#### STUDY AREA

The study was carried from 2002 to 2004 in four sleeping sickness foci of the forest region of Southern-Cameroon (Fig. 1):

- **Bipindi** (3°2’N, 10°22’E), is a historic sleeping sickness focus discovered in 1920 which has been in recrudescence recently (Grébaut et al., 2001). It lies between Lolodorf and Kribi, at 75 Km from the Atlantic Ocean. Though, *Glossina palpalis palpalis* is the dominant tsetse fly species, other tsetse fly species like *G. palli- cera*, *G. caliginea* and *G. nigrofusca* can be also found in this focus (Morlais et al., 1998; Simo et al., 2008). The domestic animals were bled in the villages Bijouka, Lambi, Memel, Bipindi-Centre and Ebimbingbang.

- **Campo** (2°20’N, 9°52’E), is a hypo-endemic focus where a few sleeping sickness patients are detected each year. It lies along the River Ntem which separates Cameroon and Equatorial Guinea and flows into the Atlantic Ocean. Several tsetse fly species including *G. p. palpalis*, and to a lesser extent, *G. pallicera*, *G. caliginea* and *G. nigrofusca* are encountered in this sleeping sickness focus (Morlais et al., 1998; Simo et al., 2008). Samples were taken from domestic animals in the villages Akak, Mabiogo, Ipono, and Campo center.

- **Fontem** (5°40’N, 9°55’E) is a sleeping sickness focus known since 1949 where the prevalence of sleeping...
sickness has reduced considerably. It has a much varied topography with numerous hills and valleys through which high speed rivers flow in the South Western Region of Cameroon. *G. p. palpalis* is the only tsetse fly species found in this area (Morlais et al., 1998; Simo et al., 2008). The focus is divided into three sub-foci (North, Center and South). In the Center sub-focus where the samples were taken (villages of Menji, Fotabong, Soko, Azi), few sleeping sickness patients were detected during the last decade. In the Northern villages (Bechati, Folepi, Besali), no patients have been detected during the last 20 years (Ebo'o Eyenga, personal communication), although in pigs, the prevalence of *T. b. gambiense* group 1 infections was 15% in 1999 (Nkinin et al., 2002).

Doumé (4°16’N, 13°25’E) is an old sleeping sickness focus in the Eastern Region of Cameroon where very few sleeping sickness patients were detected during the last decade. *Glossina fuscipes* is the main tsetse fly species found in this focus (Mbida Mbida et al., 2009). Doumé is a degraded forest zone with many rivers and vast areas of wetlands. Samples were collected from domestic animals in the villages Medim, Paki, Baillon and Loumbou.

In these four sleeping sickness foci where cattle is rare, pigs, sheep, goats and chickens are kept to meet dietary, ceremonial and commercial needs. Dogs serve as pets and hunting companions.

**BLOOD COLLECTION FROM ANIMALS**

Domestic animals were sampled during five field surveys: one was done at Doumé (October 2002), one at Bipindi (July 2003), one at Fontem (October, 2003) and two at Campo (April 2003 and June 2004). During each survey, about one in three animals that has spent at least three months in the focus was sampled and bled with the cooperation of the owners, but some dogs did not cooperate. Goats and sheep were bled from the jugular vein; pigs from the sub-clavicular vein and dogs from the cephalic vein. The blood was put in EDTA coated tubes, labeled and preserved at 4 °C for molecular analyses.

All pigs and dogs sampled in this study were of a local breed, originating from a mixture of different breeds. The sheep and goats are Dwarf breeds (Djallonke West-African Dwarf for sheep and Guinea goat), which are known to be trypanotolerant.

**EXTRACTION AND AMPLIFICATION OF DNA**

DNA was extracted from the samples using the kit “Ready Amp Genomic DNA purification system” (PRO-MEGA) essentially as described by Penchenier et al., (2000). The supernatant containing DNA was stored at -20 °C or used directly for PCR.

Amplification of trypanosome DNA was conducted using the primer pairs TCF1 (GGACACGCCAGATTCTT) / TCF2 (GTTCTCGCACCAAATCCAAC), TVW1 (CTGAGTGTCCATGTCCCAC) / TVW2 (CCACCA-GAACACCAACCTGA), and TSM1 (CCGGTCAAAAACGTCAATT) / TSM2 (AGTCGCCGGAGTGCATT) specific for *T. congolense* “forest type”, *T. vivax* and *T. simiae* respectively (Masiga et al., 1992). The amplifications were conducted in a total volume of 25 µl containing 2.5 µl of PCR buffer 10X [10 mM Tris – HCl (pH 9.0), 50 mM KCl, 3 mM MgCl₂], 15 picomoles of each primer, 200 µl of deoxynucleotide-triphosphate (dNTP), one unit of Taq DNA polymerase (Appligene-Oncor, USA), sterile water and 3 µl DNA extract. Amplification involved pre-denaturation at 94 °C for 3 min 30 s followed by 40 cycles of denaturation at 94 °C (30 s), hybridization of primers at 60 °C and elongation at 72 °C for 1 minute, then final elongation at 72 °C for 5 min. The amplification products were resolved on 2% agarose gel containing ethidium bromide (0.3 µg/ml). DNA bands were visualized under ultraviolet (UV) light.
STATISTICAL ANALYSES

The proportions of animals infected by different trypanosome species were compared between animals and localities using Chi-square ($\chi^2$) test of the Statistix Computer program.

RESULTS

Fig. 2 shows an example of profiles obtained after resolution of PCR products from the amplification of T. vivax DNA. Tables I and II report the number of animals analyzed and the PCR-positive samples by animal species and localities. Although only the results of three trypanosomes species (T. vivax, T. congolense “forest type” and T. simiae) are reported in this study, T. brucei s.l. and T. b. gambiense group 1 (human infective trypanosome) were also investigated and the results are reported in Njiokou et al. (2010). These authors showed that 19.88 % and 3.08 % of the animals were infected by T. brucei s.l. and T. b. gambiense respectively. T. b. gambiense were harboured by pigs, already known to be reservoir hosts, but also by goats and sheep, pointing out their contribution to the epidemiology of HAT. The prevalences significantly varied according to the animal species and the focus, in connection with the level of endemicity of HAT. In this study, 27.08 % (237/875) of animals analyzed were infected by at least one of the three trypanosome species (Tables I and II). The levels of infection differed significantly between animal species ($\chi^2 = 77.92; p < 0.0001$) and between localities ($\chi^2 = 52.89; p < 0.0001$).

T. vivax was the most predominant trypanosome in animals with a global infection rate reaching 20.91 % and more precisely 36.15 % in pigs, 18.18 % in goats, 8.61 % in sheep and 2.7 % in dogs. T. congolense “forest type” was identified in 11.42 % of the animals, and 19.86 %, 10.22 %, 8.1 % and 3.37 % for pigs, goats, dogs and sheep respectively. T. simiae was rare (0.34 %) and was diagnosed only in two (0.75 %) goats and one (0.37 %) sheep. The frequency of T. vivax and T. congolense “forest type” differed significantly between animal species and between localities ($p < 0.0001$). The comparison of T. vivax infection rates in different pairs of animal species showed significant differences, except for the sheep/dog pair. Significant differences were also observed for the T. congolense “forest type” infection rates for the pig/goat, pig/sheep and goat/sheep pairs.

The comparison by locality showed that animals from Bipindi and Fontem-Center are more frequently infected with T. vivax and T. congolense “forest type”. However, the level of infection of these trypanosome species does not differ significantly between these two localities ($\chi^2 = 0.44; p = 0.5$) and ($\chi^2 = 0.78; p = 0.37$) respectively. Animals from Doumé were significantly less infected by T. vivax and T. congolense “forest type” than those of the other localities. Significant differences were observed between the T. vivax infection rates in Bipindi and Campo ($\chi^2 = 7.72; p = 0.005$) as well as between the T. congolense “forest type” infection rates in Fontem-Center and Campo ($\chi^2 = 5.64; p = 0.017$). However, animals from Bipindi and Fontem-Center are more infected by T. vivax and T. congolense “forest type” respectively.

Mixed infections were found in 32 animals including 26 pigs and 6 goats carrying both T. congolense “forest type” and T. vivax; one sheep carried both T. vivax and T. simiae; and one goat carried triple infections of T. congolense “forest type”, T. vivax and T. simiae (Table III). When taking into account the occurrence of T. b. gambiense revealed in Njiokou et al. (2010), three other double infections with T. b. gambiense group 1 and T. vivax are identified in one sheep and one goat while T. b. gambiense group 1 and T. congolense “forest type” were identified in one sheep. Furthermore, one goat was infected with four trypanosomes including T. b. gambiense group 1, T. vivax, T. congolense “forest type” and T. simiae.

DISCUSSION

In this study, PCR revealed infections with T. congolense “forest type”, T. vivax and T. simiae in the domestic animals, thus confirming the circulation of these parasites in the sleeping sickness foci of southern Cameroon as previously reported in tsetse flies (Morlais et al., 1998), wild animals (Herder et al., 2002; Njiokou et al., 2004; Njiokou et al., 2006) and pigs (Penchenier et al., 1996; Simo et al., 2006). The infection rate of 27.08 % is in line with results obtained in pigs, small domestic ruminants, and dogs in the Central and West African regions (Asonganyi et al., 2002).
The high prevalence of *T. vivax* corroborates other findings in domestic animals (Bourzat & Gouteux, 1990; Dadah et al., 1997; Kalu et al., 2001), also out of any sleeping sickness areas. The high prevalence of *T. vivax* may result from the level of pathogenicity of this trypanosome, which is generally low and better controlled by animals (Stephen, 1970; Authié et al., 1999). It may result also from the mechanical transmission, which has not been reported in the other species studied here, except in certain extend *T. congolense*.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>NE</th>
<th>TCF</th>
<th>TVW</th>
<th>TSM</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs</td>
<td>307</td>
<td>61</td>
<td>111</td>
<td>0</td>
<td>133</td>
</tr>
<tr>
<td>Goats</td>
<td>264</td>
<td>27</td>
<td>48</td>
<td>2</td>
<td>69</td>
</tr>
<tr>
<td>Sheep</td>
<td>267</td>
<td>9</td>
<td>23</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>Dogs</td>
<td>37</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>875</td>
<td>100</td>
<td>183</td>
<td>3</td>
<td>237</td>
</tr>
</tbody>
</table>

\[\chi^2\] p 39.5 0.0001 76.15 0.0001 77.92 0.0001

Table I. – Number and percentage of animals infected with *T. congolense*, *T. vivax* and *T. simiae*.

<table>
<thead>
<tr>
<th>Locality</th>
<th>NE</th>
<th>TCF</th>
<th>TVW</th>
<th>TSM</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bipindi</td>
<td>204</td>
<td>30</td>
<td>65</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>Campo</td>
<td>310</td>
<td>32</td>
<td>65</td>
<td>2</td>
<td>82</td>
</tr>
<tr>
<td>Fontem-center</td>
<td>154</td>
<td>28</td>
<td>44</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Doumé</td>
<td>207</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>875</td>
<td>100</td>
<td>183</td>
<td>3</td>
<td>237</td>
</tr>
</tbody>
</table>

\[\chi^2\] p 18.3 0.0001 54.6 0.0001 52.89 0.0001

Table II. – Number and percentage of animals infected with *T. congolense*, *T. vivax* and *T. simiae* in various localities.

<table>
<thead>
<tr>
<th>Type of infections</th>
<th>Trypanosome identified</th>
<th>Pigs</th>
<th>Goats</th>
<th>Sheep</th>
<th>Dogs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>TCF</td>
<td>70</td>
<td>31</td>
<td>11</td>
<td>1</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>TVW</td>
<td>19</td>
<td>17</td>
<td>6</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>TSM</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Double</td>
<td>TCF et TVW</td>
<td>26</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>TVW et TSM</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

TCF: *Trypanosoma congolense* “forest type”; TVW: *Trypanosoma vivax*, TSM: *Trypanosoma simiae*. 

Table III. – Type, nature and number of infections revealed by PCR in different domestic animal species.

1986, 1990; Makumyaviri et al., 1989; Omeke, 1994; Penchenier et al., 1996; Dadah et al., 1997; Kalu et al., 2001), also out of any sleeping sickness areas.

The high prevalence of *T. vivax* corroborates other findings in domestic animals (Bourzat & Gouteux, 1990; Dadah et al., 1997; Kalu et al., 2001) as well as wild animals (Komoin-Oka et al., 1994; Truc et al., 1997; Herder et al., 2002; Njokou et al., 2004; Njokou et al., 2006) of the Central and West Africa regions. This high prevalence of *T. vivax* may result from the level of pathogenicity of this trypanosome, which is generally low and better controlled by animals (Stephen, 1970; Authié et al., 1999). It may result also from the mechanical transmission, which has not been reported in the other species studied here, except in certain extend *T. congolense*.
The identification of *T. vivax* in pigs is in accord with results obtained by Ng’ayo et al. (2005) in East Africa. Using specific primers, these authors showed that *T. vivax* infections are frequent in pigs and goats. Pennenier et al. (1996) and Simo et al. (2006) also found numerous *T. vivax* infections in pigs from Cameroon, by PCR. These results need to be confirmed by other techniques because it is generally accepted that pigs are refractory to infections with *T. vivax* (Taylor & Authié, 2004). The fact that *T. vivax* is detected only by PCR based methods and not by parasitological techniques could be explained by “transient” infections. Moreover, with the development of new genotyping tools that do not require isolation of trypanosomes, it is urgent to characterize *T. vivax* circulating in pigs as well as in other vertebrate hosts in order to investigate if some strains might be infective to pigs and others non-infective. Indeed, previous studies have suggested genetic variation in *T. vivax* populations that renders some isolates more pathogenic than others (Mwongela et al., 1981; Welde et al., 1983; Roeder et al., 1984).

The lower prevalence of *T. congolense* “forest type” with respect to *T. vivax* in domestic animals may result from higher parasitemia in *T. congolense* infections, accompanied by serious anemia, which leads to the rapid death of the host animal (Sidibe et al., 2002; Bengaly et al., 2002a, b).

The very low prevalence of *T. simiae* as already reported in pigs (Pennenier et al., 1996; Simo et al., 2006) and wild animals (Herder et al., 2002; Njokou et al., 2004) of several sleeping sickness foci indicates a low transmission of this parasite in various localities of Cameroon. The absence of *T. simiae* in pigs of various areas of Cameroon is likely due to its high pathogenicity because pigs infected with this trypanosome species would probably not survive. Our results corroborate those obtained by Simo et al. (2006) in pigs of the Fontem sleeping sickness focus of Cameroon.

Our results also showed a significant difference in the prevalence of *T. vivax* and *T. congolense* “forest type” between the different animal species. Pigs are more infected than goats, sheep and dogs; this could either be indicative of a higher susceptibility of pigs to *T. vivax* and *T. congolense*, or of a higher frequency of contact with the tsetse fly vector. Indeed, it has been shown that *G. p. palpalis* takes more blood meals from pigs than from goats and sheep and rarely on carnivores (Dagnogo et al., 1985; Sané et al., 2000; Spáth, 2000; Simo et al., 2008). The keeping of pigs in sties near habitations exposes them to more contacts with peri-domestic tsetse flies (Frézil et al., 1980) than animals that roam freely. Finally, the highest prevalences of *T. vivax* and *T. congolense* “forest type” in domestic animals from Bipindi and Fontem-Centre respectively, confirm the results reported in wild animals from Bipindi (Herder et al., 2002; Njokou et al., 2004) and in domestic animals from Fontem (Asonganyi et al., 1990) and indicate their intense transmission in these sleeping sickness foci.

The presence of *T. vivax* and *T. congolense* in all the localities studied is not only indicative of the ubiquity of the trypanosomes, but also of the presence of an appropriate vector in the foci. Their prevalence in Bipindi and Fontem where *G. p. palpalis* represents about 100 % is higher than in Campo where *G. p. palpalis* represents only 56 % and Doumé where *G. fuscipes* is the only tsetse fly species found (Mbida Mbida et al., 2009).

Our results on mixed infections in pigs corroborate those of Jamonneau et al., (2004) who reported a high proportion of mixed infection in pigs of the Bonon sleeping sickness focus in Côte d’Ivoire and those of Simo et al., (2006) in pigs of the Fontem sleeping sickness focus in Cameroon. These results are in line with those obtained in tsetse flies (Morlais et al., 1998; Masiga et al., 1996; Reifenberg et al., 1997; Malele et al., 2003). Indeed, entomological studies showed that infected tsetse flies from the sleeping sickness foci of Cameroon (Morlais et al., 1998) and other zones of Africa (Masiga et al., 1996; Reifenberg et al., 1997; Malele et al., 2003) frequently harbour more than one trypanosome species. This demonstrates the high probability of tsetse flies to transmit several trypanosome species to vertebrate hosts. Up till now, the interaction and the evolution of different trypanosome species or subspecies in the infected hosts remain not yet well understood. Experimental studies have shown that primary infection with *T. congolense* prevents the establishment of a second strain of *T. congolense* (Morrison et al., 1982) while an animal already infected with *T. congolense* becomes refractory to *T. brucei* s.l. infections (second infection), but not *T. vivax* (Dwinger et al., 1989). Results of our study, especially the low proportion of mixed infection including *T. congolense* “forest type” and *T. b. gambiense* (see also Njokou et al., 2010 data) and the considerable numbers of mixed infections involving *T. vivax* and *T. congolense* “forest type” play in favor of these experimental studies. However, in the field conditions where tsetse flies are infected by several trypanosome species, it is obvious that several trypanosome species or subspecies are transmitted to the same hosts. In such conditions, the prevalence of each species of trypanosome in the mixed infections may reflect probably the prevalence of this trypanosome species in the locality. Our results on the mixed infections of several trypanosomes species in vertebrate hosts suggest further investigations on the establishment and the evolution of different trypanosomes species or subspecies in the vertebrate...
hosts. Such investigations may enable to understand some aspects of the Human African Trypanosomiasis as well as Animal African Trypanosomiasis. For example, these investigations may enable to know if some trypanosome species can prevent the establishment and the evolution of T. b. gambiense and if genetic exchanges between the same trypanosome species can occur in vertebrate hosts.

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