

Research Article

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A study on African animal trypanosomosis in four areas of Senegal

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Abstract: In Senegal, several areas provide great potential for agriculture and animal production, but African animal trypanosomosis (AAT) is one of the major constraints to the development of more effective livestock production systems. A study was conducted to assess the current situation of AAT in this country. Surveys were carried out between June 2011 and September 2012 in four different areas: Dakar, Sine Saloum, Kedougou region and Basse Casamance in several animal species: dogs (152), donkeys (23), horses (63), sheep (43), goats (52) and cattle (104), distributed in the four sites. Molecular tools (PCR) indicated 3.4% positive animals including dogs, donkeys, a goat and cattle. The savannah type of *Trypanosoma congolense* Broden, 1904 (53% of positive cases) and the forest type of *T. congolense* (subgenus *Nannomonas* Hoare, 1964) were predominant. *Trypanosoma vivax* Ziemann, 1905 (subgenus *Duttonella* Chalmers, 1918) was only present in one animal and no trypanosome of the subgenus *Trypanozoon* Lühe, 1906 was found. Half of the positive cases were detected in Sine Saloum, where *T. congolense* savannah-type was predominant, and the other half in Basse Casamance, where *T. congolense* forest-type was predominant; no cases were found in Dakar or in the Kedougou region. A high risk of infection in dogs with *T. congolense* savannah-type was shown in Sine Saloum, requiring prevention and control of dogs in this area. The involvement of tsetse flies in the transmission of *T. congolense* in Sine Saloum and Basse Casamance is discussed.

Keywords: *Trypanosoma congolense*, dog, donkey, cattle, epidemiology

African animal trypanosomosis (AAT) is a serious economic constraint to livestock and agricultural development in sub-Saharan Africa (Swallow 1998, Itard et al. 2003). In Senegal, agriculture and animal production (cattle, donkeys, horses, small ruminants) are of great importance. Veterinary surveys conducted in Senegal between 1965 and 1970 indicated the presence of *Trypanosoma congolense* Broden, 1904, *Trypanosoma brucei* Plimmer et Bradford, 1899 and *Trypanosoma vivax* Ziemann, 1905, mainly in cattle, horses and donkeys (Toure 1976). Unfortunately, no more recent data on AAT in Senegal are available, except a survey conducted in 2007 on cattle in western Senegal confirming that AAT remains a major problem in the area (Seck et al. 2010).

Tsetse flies are vectors of AAT and have been described in Senegal for a long time (Toure 1971). In the Niayes area located along the Atlantic coast, *Glossina palpalis gambiense* Vanderplank was present and strongly impeded cat-

tle production (Seck et al. 2010). These tsetse flies are the target of an ongoing eradication programme using an area-wide integrated pest management approach to create a sustainable zone free from *G. palpalis gambiense* (see Vreysen et al. 2013). Such eradication can allow some 1500 breeders in this area to have a better standard of life raising animals for milk and meat production (M. Seck, I.S.R.A., Dakar, Senegal and J. Bouyer, C.I.R.A.D., France – pers. comm.). Based on this tsetse project in Senegal, it is clear that assessing the current situation of AAT in this country is sorely needed.

This paper presents the results of a veterinary survey, i.e. parasitological and serological data on AAT in dogs *Canis lupus familiaris* Linnaeus, donkeys *Equus asinus* Linnaeus, horses *Equus caballus* Linnaeus, sheep *Ovis aries* Linnaeus, goats *Capra hircus* Linnaeus and cattle *Bos taurus taurus* Linnaeus and *Bos taurus indicus* Linnaeus, from June 2011 to September 2012 in the four areas of Senegal.

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MATERIALS AND METHODS

Study area

Surveys were carried out in four areas in Senegal (see Fig. 1):

(1) Dakar, with four different locations: Dakar-Hann (14°42'01"N; 17°25'21"W) (watchdogs from a security company), Dakar Mbao (14°45'12"N; 17°20'51"W) (police dogs), Dakar Presidential guard (14°41'24"N; 17°26'40"W) (horses from the Presidential guard) and Dakar FA (14°43'13"N; 17°28'55"W) (horses from the French army);

(2) Sine Saloum in two villages: Dielmo (13°43'26"N; 16°24'38"W), 280 km south of Dakar and 10 km from the Gambia, located near the River Nema, and Ndiop (13°41'07"N; 16°23'01"W), 5 km from Dielmo;

(3) Kedougou region in eastern Senegal, with two villages, Ibel (12°30'42"N; 12°22'54"W) and Bandafassi (12°32'19"N; 12°18'38"W), about 8 km apart, close to the Guinean border;

(4) Basse Casamance, between Guinea-Bissau and the Gambia, with three villages located in the western part: Oussouye (12°29'14"N; 16°32'53"W), close to Basse Casamance National Park, Mlomp (12°33'326"N; 16°34'49"W), which gathers several villages North of Oussouye, and Elinkine (12°30'27"N; 16°39'43"W), a small fishing port located at the mouth of the River Casamance, 35 km west of Oussouye.

Animals

This study included 152 dogs, 23 donkeys, 63 horses, 43 sheep, 52 goats and 104 cattle distributed in the four sites. Data on age and sex of the positive animals only were recorded (see Table 1). All the dogs were domestic, living free in the villages or in kennels in Dakar. Cattle from Casamance were N'Dama whereas those in Sine Saloum were zebus and dutch cross-cattle. Sheep and goats were local breeds. None of the animals had received a trypanocidal drug before sampling.

Sample collection

Blood samples were collected from each animal in June 2011, August 2011, February 2012 or September 2012, on Whatman no. 4 filter papers kept at 4 °C until PCR processing. Serum was isolated either in June 2011 or September 2012 from animals and kept frozen until analyses.

Polymerase chain reaction

In the laboratory, each blood-impregnated Whatman paper was placed in 1.5-ml tube and 1 ml of a Chelex 100 (Bio-Rad, Foster City, CA, USA) 5% solution was added. The tubes were then heated to 56 °C for one h and to 95 °C for 30 min. After centrifugation at 14000 rpm for 10 min, the supernatants containing the DNA were diluted 1/10 before being used as template for PCR. The presence of trypanosomes was investigated using a nested pan-trypanosome PCR based on ITS1 primers (first round: GCAAATTGCCAATGTCG/GCTGCGTTCT-TCAACGAA; second round: CCTGCAGCTGGATCAT/ATCGCGACACGTTGTG). PCR cycling conditions were as follows: first round consists of initial denaturation of the samples at 94 °C for 3 min, followed by 30 cycles of 30 sec at 94 °C, 1 min at 51 °C and 2 min at 72 °C, with a final extension at 72 °C for 10 min; second round: initial denaturation of the samples at 94 °C for 3 min, followed by 35 cycles of 30 s at 94 °C, 30 s

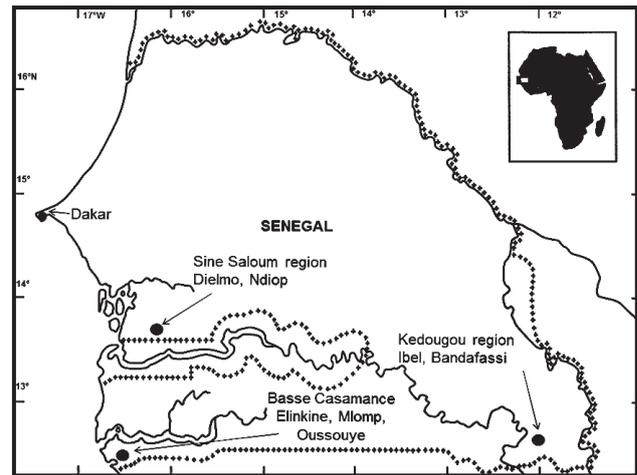


Fig. 1. Location of the four survey areas in Senegal.

at 47 °C and 1 min at 72 °C, with a final extension at 72 °C for 5 min.

Positive samples were then subjected to PCR using more specific primers for *T. congolense* forest-type (TCF1: GGACACGCCAGAAGGTACTT, TCF2: GTTCTCGCAC-CAAATCCAAC), *T. congolense* savannah-type (TCS1: CGAGCGAGAACGGGCAC, TCS2: GGGACAAACAAATC-CCGC) and *T. vivax* (TVW1 : CTGAGTGCTCCATGTGCCAC, TVW2 : CCACCAGAACACCAACCTGA), as previously described (Keck et al. 2009). PCR cycling conditions were as follows: initial denaturation of the samples at 95 °C for 3 min, followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C, with a final extension at 72 °C for 5 min.

Protocol for ELISA with *T. congolense*-savannah

The enzyme-linked immunosorbent assay (ELISA) procedure was the same as the one developed for *Trypanosoma evansi* Steel, 1885 in camels described in Desquesnes et al. (2009) except for the use of peroxidase-conjugated anti-bovine, -dog or -horse (all from Sigma-Aldrich Co., St. Louis, USA) instead of protein A-peroxidase conjugate and the use of *T. congolense*-savannah antigen. The optical density (OD) value of the blank well was deduced from the OD of each sample. The ELISA results were initially expressed by the mean OD of two duplicate wells.

Determination of the cut-off value and expression of ELISA results

For cattle: the cut-off value (COV) for *T. congolense*-savannah (mean \pm 2 SD) was determined using 40 serum samples collected from cattle that were presumably non-infected, sedentary and living in non-endemic sites in Kenya. The serum of *T. congolense*-savannah experimentally infected cattle in Kenya was used as positive control. The relative percentage of positivity (RPP) was determined as previously described (Desquesnes 1997) according to the following ratio:

$$\text{RPP of a sample} = \left[\frac{\text{mean OD of the sample} - \text{mean OD of the negative control}}{\text{mean OD of the positive control} - \text{mean OD of the negative control}} \right] \times 100$$

For dogs: the COV for *T. congolense*-savannah (mean \pm 2 SD) was determined based on a batch of 73 samples originating from presumably non-infected dogs from South-East France, a non-

Table 1. PCR-positive animals in the four areas of the survey in Senegal.

Site	Sampling date	Animal species	No. of animals sampled	No. of positive animals	Age and sex of the positive animals
Dielmo (Sine Saloum)	June 2011	Dog, <i>Canis lupus familiaris</i> Linnaeus	7	0	
Ndiop (Sine Saloum)	June 2011	Dog, <i>Canis lupus familiaris</i>	15	3 Tcs	Female 2 years old, female 1 year old, male 1 year old (these 3 dogs have died from 'infections' before 02.2012)
Ndiop (Sine Saloum)	February 2012	Dog, <i>Canis lupus familiaris</i>	4	2 Tcs	Two males 4 years old (negative in 2011)
Ibel (Kedougou)	June 2011	Dog, <i>Canis lupus familiaris</i>	9	0	
Bendafassi (Kedougou)	June 2011	Dog, <i>Canis lupus familiaris</i>	3	0	
Dakar Mbaou	August 2011	Dog, <i>Canis lupus familiaris</i>	14	0	
Dakar Hann	February 2012	Dog, <i>Canis lupus familiaris</i>	21	0	
Elinkine (Basse Casamance)	September 2012	Dog, <i>Canis lupus familiaris</i>	21	0	
Oussouye (Basse Casamance)	September 2012	Dog, <i>Canis lupus familiaris</i>	17	0	
Mlomp (Basse Casamance)	September 2012	Dog, <i>Canis lupus familiaris</i>	43	2 Tcf	Male 3 years old from Mlomp Djikomol and male 7 years old from Mlomp Cagnaout
Dielmo (Sine Saloum)	June 2011	Donkey, <i>Equus asinus</i> Linnaeus	8	1 Tcs and Tcf	Female 6 years old
Dielmo (Sine Saloum)	February 2012	Donkey, <i>Equus asinus</i>	3	1 Tcs, 1 Tcs and Tcf	Female 5 years old (negative in 2011) is Tcs+, Female 7 years old (the same as the one positive in 2011) is Tcs+Tcf+
Ndiop (Sine Saloum)	June 2011	Donkey, <i>Equus asinus</i>	10	0	
Elinkine (Basse Casamance)	September 2012	Donkey, <i>Equus asinus</i>	2	0	
Oussouye (Basse Casamance)	September 2012	Donkey, <i>Equus asinus</i>	1	1 Tcf	Male 4 years old from Oussouye Essinkine
Dakar GP	August 2011	Horse, <i>Equus caballus</i> Linnaeus	50	0	
Dakar EFS	August 2011	Horse, <i>Equus caballus</i>	13	0	
Dielmo/Ndiop (Sine Saloum)	February 2012	Sheep, <i>Ovis aries</i> Linnaeus	43	0	
Dielmo/Ndiop (Sine Saloum)	February 2012	Goat, <i>Capra hircus</i> Linnaeus	52	1 Tv	Female 3 years old from Dielmo
Dielmo/Ndiop (Sine Saloum)	February 2012	Cattle, <i>Bos taurus indicus</i> Linnaeus and <i>Bos taurus taurus</i> Linnaeus	56	0	
Elinkine (Basse Casamance)	September 2012	Cattle, <i>Bos taurus indicus</i> and <i>Bos taurus taurus</i>	12	2 Tcf	2 females
Oussouye (Basse Casamance)	September 2012	Cattle, <i>Bos taurus indicus</i> and <i>Bos taurus taurus</i>	12	0	
Mlomp (Basse Casamance)	September 2012	Cattle, <i>Bos taurus indicus</i> and <i>Bos taurus taurus</i>	24	1 Tcs, 1 Tcf	1 female is Tcs + and 1 female is Tcf+
Total			440	15	

Tcf – *Trypanosoma congolense* forest-type; Tcs – *Trypanosoma congolense* savannah-type; Tv – *Trypanosoma vivax*.

endemic area. The serum of one dog from Sine Saloum that was positive by PCR and had a high OD value on the ELISA *T. congolense* (1.604) was selected as positive control, which allowed the determination of the RPP.

For donkeys: an anti-horse peroxidase-conjugate was used. The COV for *T. congolense* (mean \pm 2 SD) was determined based on horse samples from Thailand. The serum of one donkey from Basse Casamance that was positive by PCR and had a high OD value on the ELISA *T. congolense* (0.9) was selected as positive control, which allowed the determination of the RPP.

RESULTS

Out of 437 animals, 15 were positive for trypanosomes by PCR, in dogs, donkeys, goats and cattle (Table 1). One donkey from Dielmo and two dogs from Ndiop were sampled twice successively in June 2011 and February 2012. Seven dogs out of 152 (5%), three donkeys out of 23 (13%) and four cattle out of 104 (4%) were positive for *Trypanosoma congolense*, whereas only one goat out of 52 (2%) was positive for *T. vivax* (Dielmo area). Infected dogs were only present in Ndiop (5/26; 19% posi-

tive for *T. congolense* savannah-type) and in Basse Casamance (2/81; 2% positive for *T. congolense* forest-type).

Two positive donkeys infected with *T. congolense* savannah-type were identified in Dielmo; one of them had a mixed infection with *T. congolense* forest-type in June 2011, thereafter confirmed in February 2012. One donkey out of three was positive for *T. congolense* forest-type in Basse Casamance. Infected cattle were only found in Basse Casamance (4/48; 8%): one was positive for *T. congolense* savannah-type (in Mlomp) and three for *T. congolense* forest-type (two in Elinkine and one in Mlomp).

Serological analyses from 234 animals (109 dogs, 21 donkeys and 104 cattle) revealed that cattle from Sine Saloum (n = 56) and Basse Casamance (n = 48) were all negative for *T. congolense* savannah-type (all values were below the 30% COV). In contrast, 25% (7/28) of the dog samples from Sine Saloum and 23% (19/81) of the dog samples from Basse Casamance displayed an RPP value above the 70% COV for *T. congolense* savannah-type. In Sine Saloum, six donkeys out of 18 and in Basse Casamance two of three donkeys displayed an RPP value above the 30% COV for *T. congolense*.

DISCUSSION

The present study was conducted to assess the current situation of AAT in domestic animals in four different regions of Senegal. The villages where animals were sampled are villages where studies on human infectious diseases (especially malaria) have been conducted for several years. Data from Toure (1976) reported that *Trypanosoma congolense* and *T. brucei* were found in cattle, horses, donkeys and carnivores in the region where tsetse flies were present, whereas *T. vivax* was found mainly in cattle and horses throughout the country. Since this date, only data from the veterinary survey from Seck et al. (2010) conducted in western Senegal are available. The results of the present study revealed 3.4% (15/437) positive animals using PCR. Trypanosomes of the subgenus *Nannomonas* Hoare, 1964 are largely predominant because 53% of the infections were attributed to *T. congolense* savannah-type and 46% to *T. congolense* forest-type. Trypanosomes of the subgenus *Duttonella* Chalmers, 1918 were only present in one goat and no trypanosomes of the subgenus *Trypanozoon* Luhe, 1906 were found. For comparison, a study carried out from 2002 to 2004 in Cameroon revealed that 27% (237/875) of domestic animals was infected by at least one trypanosome species with *T. vivax* and *T. congolense* forest-type predominating (Nimpaye et al. 2011).

Whereas no animals were found positive for trypanosomes in Dakar (35 dogs and 63 horses) and in the Kedougou region (only 12 dogs examined), half of the positive cases were detected in Sine Saloum and the other half in Basse Casamance. In the Dakar area, given the existence of a tsetse eradication project (Bouyer et al. 2010, Solano et al. 2010), our samples might have been collected from the sites where tsetse flies are already under control. In the western parts of Sine Saloum and Basse Casamance, *Glossina palpalis gambiensis* and *Glossina morsitans submorsitans* Newstead are still present, especially in animal parks (Mediannikov et al. 2012; J. Bouyer, CIRAD, France – pers. comm.), which could explain the positive cases.

In our sample survey of dogs, 2% were positive in Basse Casamance (*T. congolense* forest-type only in Mlomp) and 19% in Ndiop, Sine Saloum (*T. congolense* savannah-type only) using PCR. Serological analysis revealed 23% positive dogs for *T. congolense* savannah-type in Basse Casamance and 25% in Sine Saloum. Because strong cross-reactions occur between species of different subgenera (Desquesnes et al. 2001), it is highly probable that stronger cross-reactions can occur between *T. congolense* savannah-type and *T. congolense* forest-type, especially in dogs, which proved to be sensitive to both savannah and forest types of *T. congolense* (see Museux et al. 2011, Desquesnes et al. 2012). This could explain positive serologies of dogs from Basse Casamance where PCR identified only *T. congolense* forest-type.

Out of the five positive dogs in Ndiop, the three diagnosed in June 2011 died eight months later. This is in accordance with the report that *T. congolense* savannah-type produces rapidly fatal infections in dogs (Gow et al. 2007, Museux et al. 2011). Moreover, the two dogs diagnosed in February 2012 were negative eight months before, indi-

cating an active transmission of *T. congolense* savannah-type in this location. Surprisingly, no trypanosome cases were detected in Dielmo, although it is located only five kilometre from Ndiop. The occurrence of *T. congolense* forest-type in dogs from Basse Casamance has also been recently reported in France, in a dog returning from a one-month stay in Senegal, Cap Skiring, Basse Casamance (Desquesnes et al. 2012). Forest-type has also been described in several indigenous and non-indigenous dogs in Ivory Coast, with prevalence up to 30% (Keck et al. 2009, Watier-Grillot et al. 2013).

In donkeys, used as draught animals in Senegal, all trypanosome infections were found in the same areas and were also attributed to *T. congolense*, with prevalence of 13%. In Dielmo, out of ten animals examined, one was positive for savannah-type and one displayed mixed infection with the savannah and forest types. The latter animal survived eight months after the first PCR diagnosis even though it remained co-infected. On the other hand, the ten donkeys tested in Ndiop, the second village visited in Sine Saloum, were all negative for trypanosomes. In these two villages, it is estimated that the number of donkeys included in the study accounts for about 20% of the total number of donkeys. In The Gambia, not far from Sine Saloum, successive studies revealed 9.2% positive donkeys in 1990–1991 (Mattioli et al. 1994) and 6.2% in 1997–1998 (Faye et al. 2001) using microscopic examination of the buffy coat, with *T. congolense* being the most frequently observed species. In 2006, it has been reported approximately 30% positive donkeys for *T. congolense* savannah-type but no animals positive for the forest-type were found by PCR (Pinchbeck et al. 2008). In Basse Casamance, it is usual to see low numbers of donkeys because they are known locally to “die from AAT” (B.D. – pers. comm.). Indeed, one donkey was positive for *T. congolense* forest-type out of only three donkeys examined. Surprisingly, serological analysis revealed higher positive rate than PCR. The determination of the COV for donkeys, based on a different species (horse) combined to the use of an anti-horse peroxidase conjugate (because donkey conjugate is not available), could account for the discrepancy between PCR and ELISA data. Furthermore, during infection with trypanosomes, the antibodies level can stay high even few months despite no parasite DNA can be detected with PCR technique (Bossard et al. 2010).

In cattle, PCR analysis revealed that 8% of the animals were positive for *T. congolense* in Basse Casamance (mainly due to the *T. congolense* forest-type – 3/4) and did not detect the infection in Sine Saloum, whereas the serologies were nil in both regions, which was surprising. Although cross-reactions between the ELISA *T. congolense* savannah-type assay and antibodies directed against *T. congolense* forest-type are expected (Desquesnes et al. 2001), because of the low pathogenicity of the forest type in cattle (Bengaly et al. 2002), antibodies directed against *T. congolense* forest-type might not be strong enough to detect the *T. congolense* forest-type infections with the ELISA *T. congolense* savannah-type assay used in this study. Additionally, ELISA might still be negative in early infections

that PCR may detect. For comparison, in a recent veterinary survey conducted in Senegal, in the Niayes area and La Petite Côte prior to the beginning of a tsetse eradication campaign, a mean prevalence of 2.4% for trypanosomes by direct examination of the Buffy-coat was observed at the herd level, whereas a prevalence of 4.4% by serological analysis was obtained for *T. congolense* savannah-type (Seck et al. 2010).

Trypanosoma vivax was only detected in one goat while the 63 horses and 43 sheep analysed were negative for all trypanosome species assayed.

In Sine Saloum, whatever the animal species, *T. congolense* savannah-type was the predominant type whereas *T. congolense* forest-type predominated in Basse Casamance. This could be related to differences in the ecological conditions of these two areas, salt backwaters in the mouth of the River Saloum and dense forests in Basse Casamance (Toure 1971) in relation to affinity relationships between *T. congolense* type and tsetse species and subspecies (McNamara and Snow 1991, Reifenberg et al. 1997, Solano et al. 2001). Nevertheless, the concomitant presence of the two *T. congolense* types in the same species of tsetse fly has been largely described (McNamara et al. 1995, Solano et al. 2001, Jamonneau et al. 2004).

Discrepancy between data obtained using PCR and ELISA in the three animal species analysed may have been caused by the fact that the two methods provide different

information: PCR highlights the presence of the parasite (via its DNA) whereas ELISA detects the presence of antibodies directed against the parasite. In other words, PCR is able to detect an active infection whereas ELISA can detect active or past infection because of the persistence of antibodies (Bossard et al. 2010).

Because of the limited number of animals sampled in the AAT survey conducted here, it is not possible to compare the trypanosome prevalence between the four sites where different host species were sampled, nor to end definitively on the absence of trypanosomes in the negative sites. The present survey, however enabled us to assess the relative importance of the different trypanosome species in the host species sampled. It indicated that species of the subgenus *Nannomonas* dominated in Sine Saloum and Basse Casamance, two regions where tsetse flies were present, in dogs, donkeys and cattle (Basse Casamance only). The highly pathogenic *T. congolense* savannah-type was mainly present in Sine Saloum, whereas the *T. congolense* forest-type was predominant in Basse Casamance. Risks of infection in dogs by trypanosomes in these areas of Senegal are therefore high and prevention and control are thus strongly recommended.

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