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The incrimination of three trypanosome species in clinically affected German shepherd dogs in Sudan

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Abstract Canine trypanosomosisis (CT) is a common disease caused by tsetse- and non-tsetse-transmitted trypanosomes worldwide. The severity of the disease varies from acute, sub-acute to chronic with non-specific clinical signs. Here, we attempt in a cross-sectional study to assess the current situation of CT and the role of dogs in transmitting trypanosomes to other domesticated animals. The study was carried out during July 2016 on 50 caged German shepherd dogs in Khartoum State to investigate the prevalence of dog trypanosomosis using both serological (*CATT/Trypanosoma evansi*) and molecular (KIN-PCR, RoTat1.2 VSG-PCR and *TviCatL*-PCR) tests to detect possible trypanosome infections. *CATT/T. evansi* detected antibodies against *T. evansi* in 15 (30%) dogs, while parasite DNA was detected in 17 (34%) dogs by RoTat1.2 PCR. In contrast, a KIN-PCR detected the subgenus *Trypanozoon*, *Trypanosoma congolense* savannah, *T. congolense* Kenya and *T. vivax* in 36 (72%), 3 (6%), 1 (2%), and 2 (4%) dogs, respectively. However, a species-specific PCR for *Trypanosoma vivax* was detected 7 (14%) positive cases. We concluded that CT was caused by at least three species of trypanosomes, namely *T. evansi*, *T. vivax* and *T. congolense*. *Trypanozoon* other than *T. evansi* could not be ruled out since other tsetse-transmitted trypanosomes have also been detected and species-specific PCRs were not used. This study illustrates that dogs play an important role in the transmission dynamic and the epidemiology of the abovementioned trypanosome species.

Keywords Dog · Sudan · *Trypanosoma evansi* · *Trypanosoma vivax* · *Trypanosoma congolense*

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Introduction

Trypanosomosis presents a serious economic constraint to livestock and agriculture development in sub-Saharan Africa, where it causes livestock deaths and reduced productivity. Animal African trypanosomosis, also known as "Nagana" in tropical Africa, is caused by hemoflagellated protozoa, primarily *Trypanosoma congolense*, *Trypanosoma brucei* subspecies *brucei* and *Trypanosoma vivax.* (Ilemobade 2009). Surra, another form of the disease, is caused by *Trypanosoma evansi* which is thought to be derived from *T. brucei* through the complete deletion of the maxicircle kinetoplastic DNA—a genetic material required for cyclical development in tsetse flies (Desquesnes et al. 2013).

Among the causative agents of Nagana, dogs are particularly susceptible to *Trypanosoma congolense*, which is transmitted cyclically by the *Glossina* species (Greene 2006). Although dogs pose a minimal risk for human infection, they seem to be an important sentinel for infection (Greene 2006). *T. evansi,* which is regularly found in African dogs, can affect a very large range of domestic and wild hosts, including dogs (Desquesnes et al. 2013). Thus, dogs may act as sentinels for *T. evansi* infection (Desquesnes et al. 2013). Transmission by the stable fly (*Stomoxys*) and Tabanids is also possible when dogs live in close contact with another infected animal (Singh et al. 1993). In Sudan, almost all *Trypanosoma* spp. have been reported in cattle, camels, equines, and small ruminants (Salim et al. 2011a; Salim et al. 2011b; Salim et al. 2014; Mossaad et al. 2017), but not in dogs despite the presence of stray, pet, hunting, and security dogs of both local and exogenous breeds in the country. Moreover, the role of dogs in the transmission of trypanosomosis has also not been studied. In this study, fifty German shepherd dogs, which were used as security dogs and kept in net-free cages in Khartoum State, Sudan, were screened for trypanosomosis. The aim of the study was to provide basic knowledge on trypanosome infection in dogs in Sudan and to shed light on the role of dogs in the transmission of trypanosomosis in livestock.

Materials and methods

Sampling

Fifty German shepherd dogs (2–7 years of age), which were kept together as security dogs, were sampled in July 2016 in Khartoum State, Sudan. The dogs were kept in separate net-free cages (one dog per cage), and received a special diet, training, regular vaccinations, and veterinary care. The dogs, which were originally imported from Germany and South Africa with health certificates in the early 1970s, had been bred for five decades. The dogs were used for security purposes and had been subjected to a special training program. The main complaint was that some dogs develop unjustifiable emaciation with non-specific symptoms, including anemia and intermittent fever.

Six milliliters of blood were drawn twice (3 ml each) from the cephalic vein into vacutainer tubes with EDTA (Terumo, Tokyo, Japan), for serology and molecular investigations. Serum samples were separated in 1.5-ml tubes and were kept at − 20 °C until use. Genomic DNA was extracted from whole blood after being loaded onto Whatman™ FTA™ Elute Cards (GE Healthcare, IL, USA), according to the manufacturer's instructions.

The card agglutination test for *T. evansi* (CATT/*T. evansi*)

The detection of *T. evansi*-specific antibodies in serum samples was performed using CATT/*T. evansi*, according to the manufacturer's instructions (Institute of Tropical Medicine, Antwerp, Belgium). Briefly, 25 μl of dog serum, diluted 1:4 in CATT-diluent, was dispensed onto the reaction zone of a plastic test card. After adding one drop (about 45 μl) of CATT reagent, the reaction mixture was spread using a stirring rod and was allowed to react on a CATT rotator (Institute of Tropical Medicine, Antwerp, Belgium) for 5 min at 70 rpm. A specimen was considered positive when blue agglutinates were visible (Bajyana and Hamers 1988; Verloo et al. 2000).

The PCR-based identification of the trypanosome species

Three different PCR techniques were employed to detect and identify trypanosome DNA in dogs, (Mossaad et al. 2017). These included the following: (i) a KIN-multi species-PCR, which amplifies internal transcribed spacer 1 (ITS-1) and which allows for the simultaneous detection of three major trypanosome species (*Trypanozoon*, *T. congolense*, and *T. vivax*) (Desquesnes et al. 2001); (ii) the RoTat 1.2 VSG-PCR (*T. evansi* type A-specific), which specifically amplifies the RoTat 1.2 VSG gene encoding the variable surface glycoprotein (VSG) of *T. evansi* (Urakawa et al. 2001); and (iii) the TviCatL-PCR, which amplifies the Cathepsin L-like gene, which is highly conserved among *T. vivax* isolates (Cortez et al. 2009). All of the primer sequences used in the PCRs are listed in Table 1. Trypanosomes were detected using single-step PCR methods with a total reaction volume of 10 μl, which included 1 μl of 10× reaction buffer, 0.3 μl of 50 mM magnesium chloride, 1 μl of 250 μM dNTPs, 0.1 μl of *Taq* DNA polymerase (Invitrogen™, Thermo Fisher Scientific Inc., MA, USA), 1 μl each of 10 mM forward and reverse primers, 4.6 μl of double-distilled water, and 1 μl of the DNA sample, which was added to the individual PCR mixtures. The PCRs were conducted using a Veriti™ Thermal cycler (Thermo Fisher Scientific). The PCR conditions that were used for the KIN-PCR, the TviCatL-PCR, and the RoTat 1.2 VSG-PCR were previously described (Mossaad et al. 2017). The PCR

Table 1 The primers used in the present study

PCR	Primer	Sequence (5′-3′)	*Trypanosoma* sp.	Target gene	Length	References
KIN	Kin1	GCGTTCAAAGATTGGGCAAT	*Trypanozoon* *T. vivax*	ITS-1	540 bp 300 bp	Desquesnes et al. (2001)
	Kin2	CGCCCGAAAGTTCACC	*T. congolense* (Savannah)		750 bp	
			T. congolense (Kenya)		680 bp	
RoTat 1.2	ILO7957 ILO8091	GCCACCACGGCGAAAGAC TAATCAGTGTGGTGTGC	*T. evansi*	RoTat 1.2 VSG	488 bp	Urakawa et al. (2001)
TviCatL	DTO 155	TTAAAGCTTCCACGAGTTCT TGATGATCCAGTA	*T. vivax*	Cathepsin L-like	200 bp	Cortez et al. (2009)
	TviCatL1	GCCATCGCCAAGTACCTCGC CGA				

products were electrophoresed in 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light (Fig. 1).

Results

Antibodies against *T. evansi* infection were detected in 15 of the 50 (30%) samples by the CATT/*T. evansi*. Similarly, parasite DNA (~488 bp) was detected in 17 cases (34%) by the RoTat 1.2 PCR (Fig. 2). In contrast, the KIN-PCR amplified a (~540 bp) product, which indicated the amplification of all possible *Trypanozoon*, including *T. evansi*, in 36 of the 50 (72%) samples (Fig. 1).

A single KIN-PCR, detected *T. congolense* savannah (~750 bp) in 3 of the 50 (6%) samples and *T. congolense* Kenya coast (~680 bp) in 1 of the 50 (2%) samples (Fig. 1).

In the abovementioned KIN-PCR, 2 of the 50 (4%) cases were positive for *T. vivax* (~300 bp). However, the TviCatL-species-specific PCR, which amplified the 200-bp product of the *T. vivax* CatL-like gene, detected *T. vivax* in 7 of the 50 (14%) samples (Fig. 3).

Mixed infection with different *Trypanosoma* spp. was detected in 9 of the 50 (18%) samples using both the KIN-PCR and the TviCatL-PCR. One of the 50 (2%) samples was positive for *T. evansi*, *T. vivax* and *T. congolense* savannah, 5 (10%) were positive for both *T. evansi* and *T. vivax*, 2 (4%) was positive for *T. evansi* and *T. congolense* savannah and 1 (2%) was positive for *T. evansi* and *T. congolense* Kenya coast.

Discussion

Trypanosomosis causes serious economic losses and imposes a severe public health burden (Simarro et al. 2010). In Sudan, animal African trypanosomosis (AAT) is present in the tsetse-infested areas and beyond. Mechanical transmission by other biting flies and animal movement contribute to the spread of the disease in tsetse-free areas. However, following a referendum from South Sudan, Sudan is no longer considered endemic for sleeping sickness, the human form of the disease (Ahmed et al. 2016).

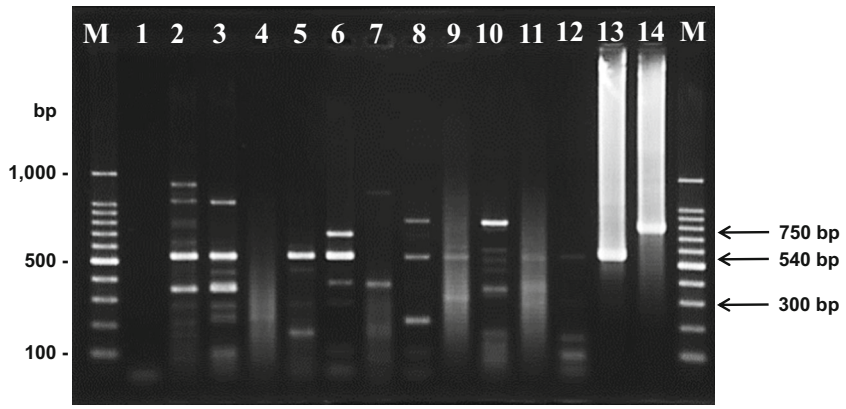

Fig. 1 Agarose gel electrophoresis (2%) with ethidium bromide staining of dog isolates of *Trypanozoon, T. vivax* and *T. congolense.* The DNA was amplified with a KIN-PCR. Lane M, 100-bp marker; lanes 2, 3, 5, 6, 8, 9 and 11 positives for *Trypanozoon* (540 bp); lanes 3 and 9 positives for *T. vivax* (300 bp); lanes 2, 8 and 10 positives for *T. congolense* savannah (750 bp); lane 6 positive for *T. congolense* Kenya Coast (680 bp); lanes 4, 7 and 12 were negative samples; lanes 2, 3, 6, 8, 9 and 10 were mixed infections; lane 1, negative control; lane 13, positive control (*T. evansi*); lane 14, positive control (*T. congolense* savannah); The extra bands were non-specific

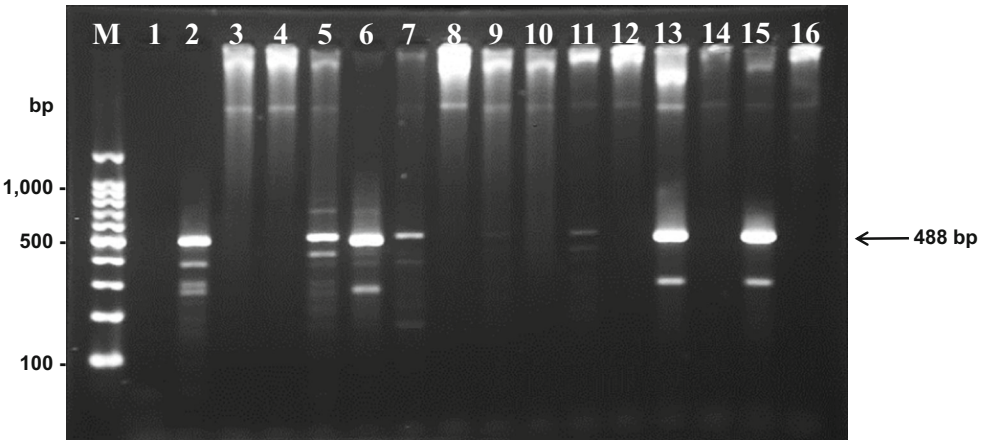

Fig. 2 Agarose gel electrophoresis (2%) with ethidium bromide staining of dog isolates of *T. evansi*. The DNA was amplified with a RoTat 1.2 VSG-PCR. Lane M, 100 bp marker; lane 1, negative control; lane 2, positive control (*T. evansi*); lanes 5, 6, 7, 11, 13, and 15, positive for *T. evansi* (488 bp); lanes 3, 4, 8, 9, 10, 14, and 16 were negative samples. The extra bands were non-specific

Agro-pastoralism is the dominant system of production in Sudan (Cecchi et al. 2010). Dogs are considered a main component of this system. Despite the fact that they are used as sentinels to guard livestock, they have been neglected in all studies on trypanosomosis in Sudan.

The serological screening with CATT/*T. evansi* revealed that 15 of the 50 (30%) dogs were infected with *T. evansi* type A. The DNA of almost all of the important *Trypanosoma* spp. was detected molecularly based on defined levels. *Trypanozoon* (brucei group) was detected by the KIN-PCR in 36 of the 50 (72%) of the tested dogs, with *T. evansi* type A infection confirmed by the RoTat 1.2 VSG-PCR in 17 of the 50 (34%) tested dogs. All of the samples that were found to be positive by the RoTat 1.2 VSG-PCR positive were also found to be positive by the KIN-PCR. It is possible that RoTat 1.2 VSG-negative *T. evansi* (type B) and/or other *Trypanozoon* including *T. brucei*, were present in the 19 of the 50 (38%) that were Rotat 1.2 VSG-PCR-negative. This could be because some of the security dogs in the study were introduced to the tsetse zone in a special mission as reported in the traveling records of the dogs. This was proved by the detection of *T. congolense* savannah— which are also tsetse-transmitted trypanosomes—in 3 of the 50 (6%) dogs and the detection of *T. congolense* Kenya coast in another (2%) dog. Another possibility is that dogs might have acquired the infection mechanically from AAT-infected animals elsewhere through other biting flies (Ahmed et al. 2016). Although the detection of other tsetse-transmitted trypanosomes was not expected, the detection of *T. vivax* in 7 of the 50 (14%) dogs was not a surprise since it was found even in sedentary cattle and camels located hundreds of kilometers outside of the tsetse belt (Ahmed et al. 2016; Mossaad et al. 2017). Although *T. vivax* is known to be common in ruminants, the detection of the parasite's DNA in the dogs in this study is in accord with results obtained by Nimpaye et al. (2011) who reported *T. vivax* in dogs and pigs in Cameroon. The fact that *T. vivax* is detected only by PCR-based methods and not by parasitological techniques could be explained by "transient" infections as previously discussed by Nimpaye et al. (2011). Moreover, the infectivity of the parasite to dogs remains to be studied.

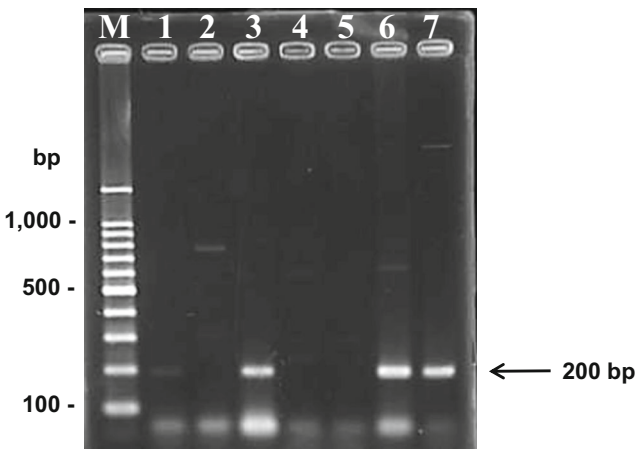

Fig. 3 Agarose gel electrophoresis (2%) with ethidium bromide staining of dog isolates of *T. vivax*. The DNA was amplified with a TviCatL-PCR (DTO155 and TviCatL1 primers). Lanes M, 100-bp marker; lanes 1, 3 and 7 positives for *T. vivax* (200-bp); lanes 2 and 4 were negative samples; lane 5, negative control; lane 6, positive control (*T. vivax*). The extra bands were non-specific

In general, dogs might play an important role in the transmission of the disease since the infected dogs do not receive appropriate treatment. Furthermore, no attention is paid to diagnosing infected dogs, which makes them a potential source of infection even where livestock herds are uninfected. The detection of the causative agents of AAT and Surra in the dogs in this study should alert veterinary services and authorized bodies to take actions to prevent infection in dogs by considering them in all of their adopted control strategies.

The German shepherd breed is exogenous; however, the group of dogs in this study was well adapted to the environment of Sudan, where they had been bred since the early 1970s. It is clear that all of the infected dogs had acquired the infection in Sudan since the grandparents were brought from Germany and South Africa after being certified as

trypanosome-free. It is also important to screen local breeds, strays, pets, and pastoralists' dogs to further clarify the role of dogs in the transmission of the disease and to improve animal welfare.

Trypanosomosis (AAT and Surra) is a common protozoan infection in livestock in Sudan. The prevalence of *T. evansi* and *T. vivax* in camels was 37 and 31%, respectively (Mossaad et al. 2017). Furthermore, the prevalence of trypanosomosis in horses and donkeys was estimated to be 12.7 and 3.4%, respectively (Salim et al. 2014). In an outbreak in Blue Nile State, the prevalence of the disease (with different trypanosomes) in cattle was reported to be 56.7% (Salim et al. 2011a). In this study, we reported, for the first time, the high prevalence of trypanosomosis in German shepherd dogs that were kept together in Khartoum State, Sudan.

It has been reported that a German shepherd dog with *T. congolense* infection was found in France after a 6-month stay in Senegal (Deschamps et al. 2016), while a German wire-haired dog was also infected with *T. congolense* in France after traveling to Senegal (Museux et al. 2011). These cases and the results of the present study suggest that caution should be taken when transporting dogs to and from Sudan and that transported dogs should be subjected to strict quarantine measures.

Conclusion

We concluded that canine trypanosomosis in the study area was caused by at least three species of trypanosomes, namely *T. evansi*, *T. vivax*, and *T. congolense*. In addition, the disease is highly prevalent in the study area, which strengthens the need to change control policies and underscores the importance of including dogs in all control measures against the parasite.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval Permission for this study was obtained according to the standards of animal experimentation at Obihiro University of Agriculture and Veterinary Medicine (Approval No. 28-46).

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