Prevalence of different trypanosomes in livestock in Blue Nile and West Kordofan States, Sudan

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Abstract

African animal trypanosomosis, transmitted cyclically by tsetse flies or mechanically by other biting flies, causes serious health problems in livestock. Although tsetse infestations have been observed in Blue Nile State in Sudan, tsetse was eradicated in West Kordofan in 1962, and no further studies have been carried out. Accordingly, in this study, we investigated the prevalence of trypanosomosis in cattle, sheep, and goats in Blue Nile and West Kordofan States, Sudan. This cross-sectional study was conducted using 70 cattle, 62 sheep, and 116 goats, and the microhematocrit centrifugation technique was used as a parasitological test. KIN-multispecies polymerase chain reaction (PCR) was used to detect *Trypanozoon* sp., *Trypanosoma congolense*, and *T. vivax*; RoTat 1.2 variable surface glycoprotein-specific PCR was used to detect *T. evansi*; and TviCatL PCR was used to specifically detect *T. vivax*. The seroprevalence of trypanosomosis was assessed using card agglutination tests CATT/ *T. evansi*. The parasitological prevalence rates were 4% (3/70) in cattle, 2% (1/62) in sheep, and 4% (5/116) in goats. The molecular prevalence rates of *T. vivax*, the most prevalent parasite, were 99% (69/70) in cattle, 98% (61/62) in sheep, and 84% (98/116) in goats. *Trypanozoon* (*T. evansi* or *T. brucie*) rates were 30% (21/70) in cattle, 32% (20/62) in sheep, and 12% (14/116) in goats. Among *Trypanozoon*-positive isolates, *T. evansi* was confirmed in 24% (5/21) of cattle, 70% (14/20) of sheep, and 86% (12/14) of goats. Finally, *T. congolense* was recorded only in cattle in Blue Nile State, with a prevalence of 14% (10/70). The seroprevalence rates of CATT/*T. evansi* were 46% (32/70) in cattle, 45% (28/62) in sheep, and 14% (16/116) in goats. Thus, we confirmed molecularly, for the first time, the presence of *Trypanozoon*, particularly *T. evansi* and *T. vivax*, in sheep and goats in Sudan**.** Our results show that sheep and goats could be an important reservoir for trypanosomes, potentially leading to the spread of the disease to the northern parts of the country

following the movement of these animals. These findings provide important insights into the epidemiology of the disease and could affect the establishment of control strategies against trypanosomosis in Sudan.

Keywords: cattle; epidemiology; goats; sheep; Sudan; trypanosomosis

1. Introduction

Sudan has one of the largest livestock populations in Africa. Sudan official sources have estimated cattle, sheep, and goat numbers to be 41.653 million, 51.555 million, and 43.270 million, respectively (MARF, 2009). The livestock sector plays a critical role in the Sudanese economy and in the welfare of the whole population, resulting in a flow of essential food, large amounts of foreign exchange from export earnings, improved transport, draught power in support of crop production and processing, dung for fertilizers and fuels, and increased employment opportunities. Despite their importance to the national economy, livestock do not receive sufficient attention in terms of government policies and financing. Almost all animals are owned by small farmers or traditional pastoralists. Moreover, livestock are affected by a multitude of diseases, including trypanosomosis, but receive little veterinary care (Wilson, 2018).

Trypanosomes are flagellate protozoan parasites that inhabit the blood plasma, lymph, and various tissues of their hosts. They are the etiological agents of a group of parasitic diseases (trypanosomosis) that cause serious economic losses and impose a severe public health burden (Swallow, 2000; Simarro et al., 2010). Trypanosomosis has been recognized as an important disease of livestock in Sudan since the beginning of the last century. In the Sudan Veterinary Report (1907), trypanosomosis was described as one of the most serious veterinary problems in Sudan (Hall et al., 1983). A brief review of its history was reported by Elkarib (1961).

Blue Nile State (southeastern Sudan) and West Kordofan State (southwestern Sudan) are considered rich states with large animal populations. Blue Nile State is a tsetse-infested state, with problem areas near the border of Ethiopia (Ahmed et al., 2016), whereas in West Kordofan State, *Glossina morsitans submorsitans* was reported to be successfully eradicated in 1961–1962

from Koalib Hills (Yagi et al., 1969). After this, tsetse control was neglected, and no updates with regard to the presence of the vector have been reported. Therefore, tsetse-transmitted trypanosomes are expected to be present in these areas. Salim and colleges identified four *Trypanosoma* species, i.e., *Trypanosoma vivax*, *T. congolense*, *T. simiae*, and *T. brucei*, in cattle in Blue Nile State (Salim et al. 2011). No data on cattle trypanosomosis in West Kordofan State are available. Unlike cattle, sheep and goats are kept in areas with varying agro-ecological zones, where they contribute considerably to the rural economies as sources of meat, milk, manure, and readily disposable income. Data describing the presence of trypanosomosis in sheep and goats in Sudan are limited.

Therefore, in this study, we aimed to provide basic epidemiological information on the presence of tsetse-transmitted and non-tsetse-transmitted trypanosomes in cattle, sheep, and goats in Blue Nile and West Kordofan States, Sudan.

2. Materials and Methods

2.1. Study area and sample collection

This study was conducted in two southern states of Sudan, namely, Blue Nile (11°16′ N 34°4′E) and West Kordofan (12°0′N 28°9′E; Fig. 1). The two states are known for their free-range breeding system for cattle, sheep, and goats. Sheep and goats were reared strictly within the semi-desert belt of Sudan, including these two states and were owned exclusively by nomadic tribes of Arab origin or others closely related to them in the region (Sulieman et al., 1990).

Overall, 248 animal blood samples from cattle, sheep, and goats were obtained after obtaining consent from the owners in Blue Nile State (n = 169; 57 cattle, 36 sheep, and 76 goats)

and West Kordofan State (n = 79; 13 cattle, 40 goats, and 26 sheep). In Blue Nile State, samples were collected from different villages around the town of Damazin. In West Kordofan State, samples were collected from five different villages around the town of Foola.

2.2. Parasitological examinations

All blood samples were examined *in situ* for the presence of trypanosome species using the microhematocrit centrifugation technique (MHCT), as described previously (Woo, 1970).

2.3. DNA extraction and PCR-based identification of the trypanosome species

DNA was extracted using the phenol-chloroform method from blood loaded on filter paper. The quality and quantity of DNA were verified using a NanoDrop 2000 instrument (Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA was stored at –30°C until further use.

Three different PCR assays were employed to detect and identify trypanosome DNA in the collected samples. These included: (i) a KIN-multispecies PCR assay, which amplified the internal transcribed spacer 1 (ITS1) and allowed for simultaneous detection of three major trypanosome species, i.e., subgenus *Trypanozoon* (*T. brucei* group), *T. congolense,* and *T. vivax* (Desquesnes et al., 2001); (ii) the RoTat 1.2 variable surface glycoprotein (VSG) PCR assay (*T. evansi* type A-specific), which specifically amplified the RoTat1.2 VSG gene encoding the VSG of *T. evansi* type A (Urakawa et al., 2001); and (iii) TviCatL PCR, which amplified the cathepsin L-like gene, a gene that is highly conserved among *T. vivax* isolates (Cortez et al., 2009). All primer sequences used in PCR are listed in Table 1. Trypanosomes were detected using single-step PCR 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 *Taq* DNA polymerase

(Invitrogen, Thermo Fisher Scientific Inc., MA, USA), 1 μL each of 10 mM forward and reverse primers, 4.6 μL double-distilled water, and 1 μL DNA sample. PCR was conducted on a Veriti Thermal Cycler (Thermo Fisher Scientific). The PCR conditions that were used for the KIN-PCR and TviCatL-PCR were described by Laohasinnarong et al. (2011), and the conditions for the RoTat 1.2 VSG-PCR were described by Urakawa and colleges (Urakawa et al. 2009). The PCR products were electrophoresed on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light.

2.4. Card agglutination test for *T. evansi* - CATT/*T. evansi*

All sera were subjected to antibody detection with CATT/*T. evansi*. CATT/*T. evansi* was performed according to the manufacturer's instructions (Institute of Tropical Medicine, Antwerp, Belgium). Briefly, 25 μL camel's serum was diluted (1:4) in CATT buffer and dispensed onto the reaction zone of a plastic test card. After adding one drop (approximately 45 μL) of CATT reagent, the reaction mixture was spread using a stirring rod and allowed to react on a CATT rotator for 5 min at 70 rpm. Samples were considered positive when blue agglutinates were visible (Bajyana Songa and Hamers, 1988; Verloo et al., 2000; Mossaad et al., 2019).

2.5. Statistical analysis

Statistical analysis was performed to evaluate the significance of differences in prevalence rates according to the sampling regions by chi-squared tests using VassarStats (http://vassarstats.net/). Results with a *P* value of less than 0.05 were considered statistically significant.

3. Results

Overall, 99% (69/70) of cattle, 98% (61/62) of sheep, and 84% (98/116) of goats in this study were found to be infected with at least one *Trypanosoma* spp. (Table 2). Analysis of the concentrations of trypanosomes by MHCT revealed trypanosome-positivity in 4% (3/70) of cattle, 2% (1/62) of sheep, and 4% (5/116) of goats (Tables 3–5). The prevalence rates estimated by this test in the two states did not differ significantly.

In contrast, KIN-PCR, which targets the ITS1 region conserved in all African trypanosomes, revealed that 30% (21/70) of cattle, 32% (20/62) of sheep, and 12% (14/116) of goats harbored a *Trypanozoon* parasite (Tables 3–5). There are three known genetically distinct types of *T. congolense* (savannah, forest, and Kenya coast). KIN-PCR detected bands of ~750 bp, indicating the amplification of *T. congolense* savannah in 14% (10/70) of cattle. However, *T. congolense* was not detected in sheep and goats. In all parasites detected by KIN-PCR, the prevalence estimated by this test in the two states did not differ significantly, except in the case of the prevalence of *Trypanozoon*, which was found to be significantly higher in goats in Blue Nile State than in goats in West Kordofan State ($P < 0.05$).

All KIN-PCR *Trypanozoon*-positive samples were further subjected to *T. evansi* RoTat1.2 VSG species-specific PCR. As a result, a ~488-bp PCR product, representing *T. evansi* type A, was detected in 24% (5/21) of cattle samples, 70% (14/20) of sheep samples, and 86% (12/14) of goat samples (Tables 3–5).

Two PCR approaches were performed to detect *T. vivax* in the current study. First, multispecies KIN-PCR, which is known to be less sensitive for detecting the parasite, detected *T. vivax* (~300 bp) in 7% (5/70) of cattle samples, 18% (11/62) of sheep samples, and 3% (4/116)

of goat samples (Tables 3–5). Second, TviCatL-specific PCR generated a ~200-bp amplicon of the *T. vivax* CatL-like gene, revealing higher prevalence rates of 99% (69/70) in cattle samples, 98% (61/62) in sheep samples, and 84% (98/116) in goat samples (Tables 3–5). The prevalence rates detected by the two PCR assays in the two areas did not show significant differences.

In addition to the parasitological and molecular techniques used in this study to detect the parasite and active infections, the seroprevalence rates of trypanosomosis in cattle, sheep, and goats were assessed using CATT/*T. evansi*. Antibodies were detected in 46% (32/70) of cattle sera, 45% (28/62) of sheep sera, and 14% (16/116) of goat sera (Table 6).

Mixed infections with at least two parasites were observed. In Blue Nile State, mixed infections with three parasites, i.e., *Trypanozoon*, *T. vivax*, and *T. congolense*, were recorded in 33% (17/57) of cattle samples. In West Kordofan State, mixed infections in cattle were not observed since only *T. vivax* was recorded (Table 3). Mixed infections with *Trypanozoon* and *T. vivax* were observed in 31% (19/62) and 9% (10/116) of sheep and goat samples, respectively (Tables 4 and 5).

4. Discussion

In Sudan, African animal trypanosomosis is present in tsetse-infested areas and beyond. Mechanical transmission by other biting flies and animal movement contributes to spreading the disease to tsetse-free areas, as observed for *T. vivax* in camels, and *T. vivax* and *T. congolenses* in dogs in northern Sudan (Mossaad et al., 2017a; Mossaad et al., 2017b).

Bovine trypanosomosis is one of the most economically important diseases affecting domesticated animals in Sudan. In one of the few economic studies conducted in Blue Nile State, Sudan, trypanosomosis was reported to be the cause of high calf mortality, high abortion rates, and reduced milk production (Ahmed et al., 2016). Since the first report of trypanosomosis in 1904 (Elkarib, 1961), the main trypanosome species reported in Sudan were *T. vivax*, *T. congolense*, *T. brucei*, *T. simiae*, and *T. evansi*, with the first three being the most widespread in cattle (Salim et al., 2011; Ahmed et al., 2016).

In this study, MHCT was found to be less sensitive as compared with PCR for detecting different trypanosomes, consistent with our previous study (Mossaad et al., 2017a).

Because KIN-PCR, which can detect different trypanosomes, is less sensitive for detection of *T. vivax*, we have adopted TviCatL PCR for specific amplification of the cathepsin L-like gene of *T. vivax*. In cattle, we report that *T. vivax* showed the highest prevalence among bovine-infective trypanosome species in the study area, with an overall prevalence of up to 99% (69/70), i.e., 100% (57/57) and 92% (12/13) in Blue Nile and West Kordofan State, respectively. This high prevalence of *T. vivax* was not surprising because these areas are known to be tsetse infested and because the parasite is known to be transmitted mechanically (Desquesnes et al., 2003; Luckins and Dwinger, 2004). Moreover, 100% prevalence of *T. vivax* in cattle has also

209 been reported previously in farms in Khartoum State, hundreds of kilometers away from tsetse-
210 infested areas (Ahmed at al., 2016). In cattle, we reported that *Trypanozoon* was the second most
211 prevalent trypanosome in the study area, with an overall prevalence of 30% (21/70), i.e., 37%
212 (21/57) in animals in Blue Nile State and 0% (0/13) in animals in West Kordofan State. Because
213 KIN-PCR cannot differentiate between *T. brucei* subspecies (*Trypanozoon*), *T. evansi* type A
214 was confirmed in 24% (5/21) by RoTat 1.2 PCR in *Trypanozoon*-positive samples, resulting in
215 higher prevalence of *T. evansi* type B or *T. brucie* in RoTat-negative samples. We report a lower
216 prevalence in cattle with *T. congolense* showing a prevalence of 14% (10/70), i.e., 17% (10/57)
217 Blue Nile State and 0% (0/13) in West Kordofan State. West Kordofan samples were infected
218 only with *T. vivax*. However, serologically, analyses using CATT/*T. evansi* showed that 46%
219 (32/70) of animals were infected with *T. evansi*, including 23% (3/13) of infected animals in
220 West Kordofan and 51% (29/57) of infected animals in Blue Nile State, confirming the
221 seroprevalence of the parasite in the two states. Notably, because CATT/*T. evansi* is not 100%
222 specific, false positive cases may also occur (Birhanu et al., 2015; Verloo et al., 1998). Data for
223 bovine trypanosomosis are comparable to data reported in previous studies, as reviewed by
224 Ahmed and colleges (2016), who reported that *T. vivax* infection is more prevalent.

225 Although investigations of animal trypanosomosis have been going on for decades, most
226 studies have been performed in cattle and camels, and relatively few studies have been
227 performed in sheep and goats. Moreover, very few reports on trypanosomosis in sheep and goats
228 are available in Sudan. Hall and colleges (1983) reported that the prevalence rates of *T.*
229 *congolense* and *T. vivax* were only 2% (2/90) and 2% (2/90), respectively, in goats in southern
230 Darfur, whereas no trypanosomes were detected in sheep. Serologically, antibodies against *T.*
231 *evansi* were detected in sheep and goats in eastern Sudan (Boid et al., 1981). Since then, sheep

and goats have not been screened for the disease in Sudan. Goats are thought to be highly resistant to trypanosomosis, with only few sporadic cases; moreover, this disease is of little economic consequence in goats (Griffin, 1978). However, current epidemiological information indicates that small ruminants can play an important role in the dissemination of the disease.

Regional differences in the prevalence of caprine trypanosomosis, which can be high in some areas in Africa, exist (Kramer, 1986). In general, caprine trypanosomosis is more common in East Africa than in West Africa (Smith and Sherman, 1994). Sudan is an East African country wherein we report a very high prevalence of *T. vivax* (up to 98% and 84% in sheep and goats, respectively). This high prevalence may be related to the observation that *T. vivax*, the tsetse-transmitted parasite, can also be mechanically transmitted (Desquesnes et al., 2003; Luckins and Dwinger, 2004). *Trypanosoma congolense* was not detected in sheep and goats in this study. However, *Trypanozoon* was detected in 32% (20/62) and 12% (14/116) of sheep and goats, respectively. Among these, 70% and 86% were confirmed as T. evansi type A using the RoTat 1.2 PCR in sheep and goats, respectively; the remaining were unconfirmed *T. brucei* or *T. evansi* type B. Moreover, serologically, CATT/*T. evansi* was detected in 45% (28/62) and 14% (16/116) of sheep and goats, respectively. Mixed infections with at least two parasites were also observed in all animal species in this study. Therefore, careful screening is required. The high trypanosome prevalence found could be because sheep and goats are more tolerant to these parasites, which commonly results in subclinical infections with low but persistent parasitemia (Musinguzi et al., 2017). Consequently, sheep and goats may not receive treatment since they do not show clinical signs, resulting in a high prevalence. Our results show that sheep and goats could be an important reservoir for trypanosomes, potentially leading to the spread of the disease to the northern parts of the country following the movement of these animals. The controlled

255 treatment of sheep and goats could lead to a reduction in the incidence of trypanosomosis in
256 these animals.

257 **5. Conclusions**

258 In this study, we used PCR to show that cattle, sheep, and goats in Blue Nile and West Kordofan
259 States were generally infected with at least one trypanosome species (*Trypanozoon*, *T. vivax*, or
260 *T. congolense*), with *T. vivax* being the most prevalent parasite. As animals move into the
261 markets in northern part of the country, we suggest the need for stringent control policies and the
262 establishment of measures to help prevent the spread of these parasites. It is also important to
263 control the disease in Blue Nile and West Kordofan States as the most important animal
264 production areas in the country. Further studies are anticipated to confirm *T. brucei* and *T. evansi*
265 type B.

266 **Funding**

267 This study was financially supported by the Ministry of Higher Education and Scientific
268 Research, Republic of Sudan (grant no. SRI-VS-2015-71933). Additional funding was received
269 from the Japan Society for the Promotion of Science (JSPS), KAKENHI (grant no. 16K18793;
270 Grants-in-Aid for Young Scientists [B]), and the AMED/JICA SATREPS (grant no.
271 17jm0110006h0005).

272 **Ethics approval and consent to participate**

273 Permission was obtained according to the standards of animal experimentation at the Obihiro
274 University of Agriculture and Veterinary Medicine (Approval No. 28-46, 29-2, 18-18, 19-19).

Competing interests

The authors declare no conflicts of interest in association with the current study.

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Figure Legends

Fig. 1. Map of Sudan. The states in which blood samples were collected are indicated in brown. The biggest town in each state is indicated as a dot (Blue, Fula; Black, Damazin). The figure was generated and modified using GIMP 2.8.10 (https://www.gimp.org).

Highlights

- T*rypanosomes* were detected in cattle, sheep, and goats in Sudan using CATT/*T. evansi*.
- *Trypanoszoon* and *T. vivax* were detected in sheep and goats in Sudan.
- *Trypanoszoon*, *T. vivax*, and *T. congolense* were detected in cattle in Sudan.
- Sheep and goats may play important roles in the epidemiology of trypanosomosis.

Table 1. Primers used in the current study

Parasite	PCR method	Primer	Sequence	Target gene	Length	Source
T. evansi	KIN	Kin1	5′-GCGTTCAAAGATTGGGCAAT-3′	*ITS-1*	540 bp	Desquesnes et al., 2001
		Kin2	5′-CGCCCGAAAGTTCACC-3′			
T. evansi	RoTat 1.2	ILO7957	5´-GCCACCACGGCGAAAGAC-3´	RoTat 1.2 VSG	488 bp	Urakawa et al., 2001
		ILO8091	5´-TAATCAGTGTGGTGTGC-3´			
T. congolense savannah	KIN	Kin1	5′-GCGTTCAAAGATTGGGCAAT-3′	*ITS-1*	750 bp	Desquesnes et al., 2001
		Kin2	5′-CGCCCGAAAGTTCACC-3′			
T. vivax	KIN	Kin1	5′-GCGTTCAAAGATTGGGCAAT-3′	*ITS-1*	300 bp	Desquesnes et al., 2001
		Kin2	5′-CGCCCGAAAGTTCACC-3′			
T. vivax	TviCatL	DTO 155	5′-TTAAAGCTTCCACGAGTTCTTGATGATCCAGTA-3′	cathepsin L-like	200 bp	Cortez et al., 2009
		TviCatL1	5′-GCCATCGCCAAGTACCTCGCCGA-3′			

Table 2. Overall prevalence of different trypanosomes in cattle, sheep, and goats in Blue Nile and West Kordofan States, Sudan

	Cattle	Sheep	Goats
Blue Nile	100% (57/57)	100% (36/36)	87% (66/76)
West Kordofan	92% (12/13)	96% (25/26)	80% (32/40)
Total	99% (69/70)	98% (61/62)	84% (98/116)

Table 3. Prevalence of different trypanosomes in cattle in Blue Nile and West Kordofan States using PCR and MHCT

Area	MHCT	KIN-PCR			RoTat-1.2	TviCatL-PCR	
		Trypanozoon	*T. vivax*	*T. congolense*	*T. evansi*	*T. vivax*	*Mixed*
Blue Nile	4% (2/57)	37% (21/57)	9% (5/57)	17% (10/57)	24% (5/21)	100% (57/57)	33% (17/57)
West Kordofan	8% (1/13)	0% (0/13)	0% (0/13)	0% (0/13)	NA	92% (12/13)	NA
Total	4% (3/70)	30% (21/70)	7% (5/70)	14% (10/70)	24% (5/21)	99% (69/70)	33% (17/57)

Table 4. Prevalence of different trypanosomes in sheep in Blue Nile and West Kordofan States using PCR and MHCT

Area	MHCT	KIN-PCR			RoTat-1.2	TviCatL-PCR	
		Trypanozoon	*T. vivax*	*T. congolense*	*T. evansi*	*T. vivax*	Mixed
Blue Nile	0% (0/36)	17% (6/36)	19% (7/36)	0% (0/36)	83% (5/6)	100% (36/36)	17% (6/36)
West Kordofan	3% (1/26)	54% (14/26)	15% (4/26)	0% (0/26)	64% (9/14)	96% (25/26)	50% (13/26)
Total	2% (1/62)	32% (20/62)	18% (11/62)	0% (0/62)	70% (14/20)	98% (61/62)	31% (19/62)

Table 5. Prevalence of different trypanosomes in goats in Blue Nile and West Kordofan States using PCR and MHCT

Area	MHCT	KIN-PCR			RoTat-1.2	TviCatL-PCR	
		Trypanozoon	*T. vivax*	*T. congolense*	*T. evansi*	*T. vivax*	Mixed
Blue Nile	3% (2/76)	18% (14/76)*	3% (2/76)	0% (0/76)	86% (12/14)	87% (66/76)	13% (10/76)
West Kordofan	8% (3/40)	0% (0/40)	5% (2/40)	0% (0/40)	NA	80% (32/40)	0% (0/40)
Total	4% (5/116)	12% (14/116)	3% (4/116)	0% (0/116)	86% (12/14)	84% (98/116)	9% (10/116)

* indicates statistically significant difference ($P < 0.05$).

Table 6. Seroprevalence of trypanosomosis using CATT/*T. evansi*

	CATT/*T. evansi*		
	Cattle	Sheep	Goats
Blue Nile	51% (29/57)	31% (11 /36)	12% (9/76)
West Kordofan	23% (3/13)	27% (7/26)	18% (7/40)
Total	46% (32/70)	45% (28/62)	14% (16/116)

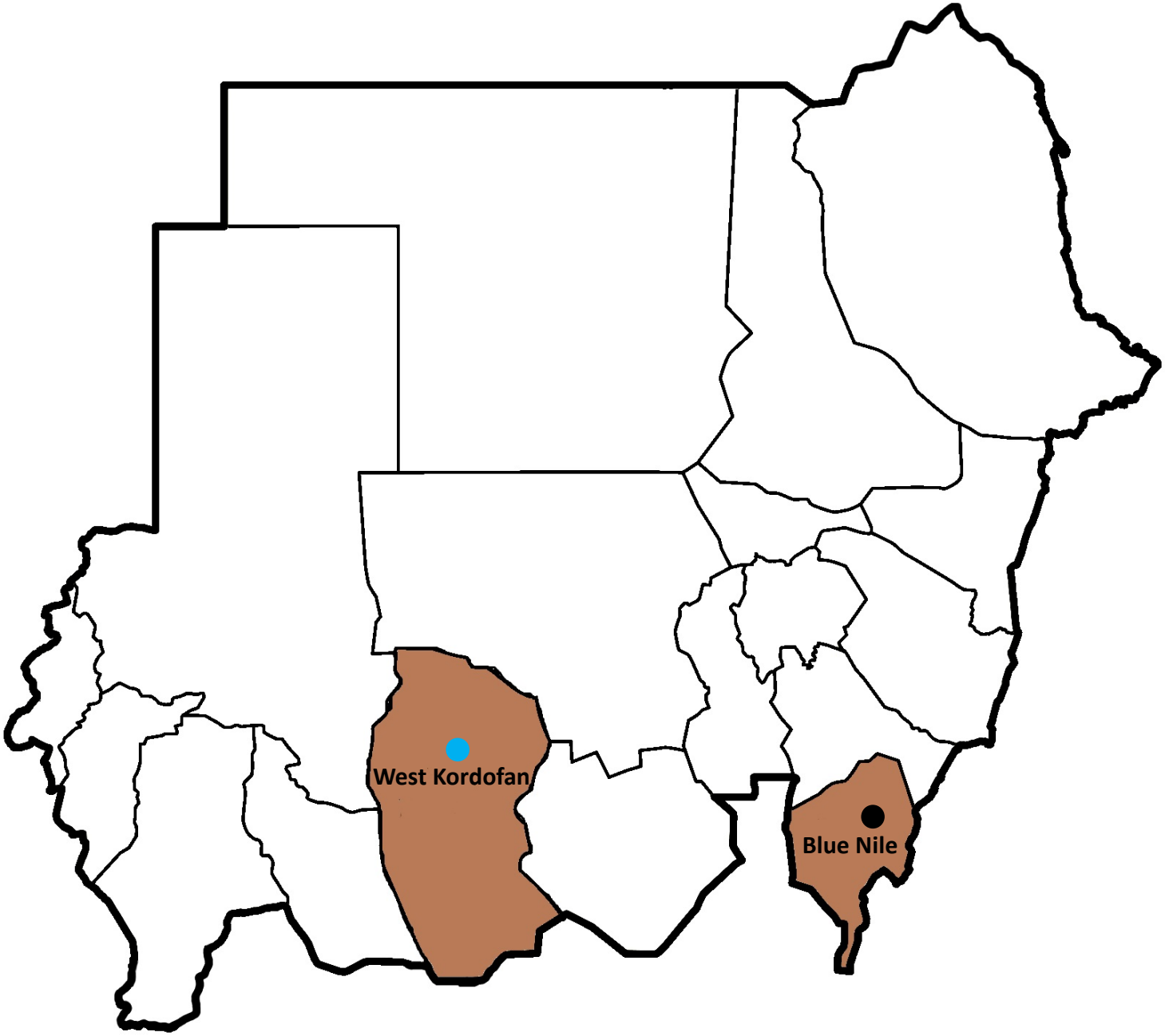

Figure 1