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Molecular detection of *Trypanosoma congolense* and *Trypanosoma simiae* in small-scale pig farms of Kiasi-kolo area, Kongo-Central Province, Democratic Republic of Congo.

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ABSTRACT

The province of Kongo Central is the first pig meat production region in the country and is also known to be endemic for animal and human trypanosomiasis. However, pig farming system is still traditional and farmers cannot afford prophylactic measures for diseases control. Subsequently, pigs are exposed to several diseases such as pig trypanosomiasis for which little information is available in this region. The main objective of this study was to identify Trypanosoma species in pig populations in the village of Kiasi-Kolo. Fifty (50) pig blood samples were taken on filter papers and were analyzed by PCR (polymerase chain reaction). Four percent (2/50) of *T. congolense* and eight percent (4/50) of *T. simiae* were detected in these samples. No sample was found positive for *T. brucei gambiense*. These results showed that pigs are exposed to trypanosomiasis and may represent an obstacle to the development of pig farming, especially with the high pathogenic nature of *T. simiae*. Prophylactic measures such as vector control against tsetse in pig farms should be applied in the village and in all the other sites at risk in order to limit losses.

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INTRODUCTION

Trypanosomiasis is an infectious disease caused by flagellate protozoa in the genus Trypanosoma that affects domestic and wild animals, and humans. The pathogen is transmitted by Glossina spp. and/or other hematophagous insects such as Tabanus sp., Stomoxys sp. during their blood meal [CHARTIER et al, 2000].

Pig is susceptible to trypanosomiasis and can clinically develop the disease [MOLS *and* LENAERTS, 1950]. However, the evolution and extent of the disease depend on the nature of the parasite [CHARTIER et al, 2000]. Trypanosoma suis and Tryanosoma simiae are pig's most specific species. T. suis causes chronic infection in adult and acute infection in young pigs. T. simiae is the most virulent and causes an acute infection with high mortality [CHARTIER et al, 2000; ISSAC et al, 2016]. Pig is also susceptible to T. congolense that can cause a chronic disease in female pigs; T. brucei gambiense and T. brucei rhodesiense are responsible for Human African Trypanosomiasis (HAT). Pig is however refractory to T. vivax [CHARTIER et al, 2000; SIMO et al, 2006; HAMILL et al, 2013].

In the Democratic Republic of Congo, agricultural activities in general and pig farming in particular are largely motivated in recent decades by high demographic growth and economic crises. It is estimated that the Kongo Central Province has at least a quarter of one million pigs in the country. However, the farming system is still traditional and farmers cannot afford prophylactic measures against trypanosomiasis especially because of lack of veterinary framing [FAO, 2012]. The Kongo Central Province is also endemic for Human and Animal African Trypanosomiasis. There have been limited studies on pig trypanosomiasis in Kongo-Central Province. The first study was conducted by Greggio [1917] and focused on Trypanosoma in pigs in the Insiki valley. The more recent ones focused on pig as reservoir for T. b. gambiense in Kongo Central Province [KAGERUKA, 1987; MAKUMYAVIRI et al., 1989]. Nevertheless, these studies are old and used less sensitive diagnostic tools.

The main objective of the present study was to identify Trypanosoma species in pig farms in Kiasi-kolo, Kongo Central Province, Democratic Republic of Congo ,using modern diagnostic such as, molecular tests.

MATERIALS AND METHODS

Animals and study area

This study was conducted in pig farms in the village of Kiasi-kolo, Songololo territory, Kongo Central Province (**Figure 1**).

Pig farming is the main agricultural activity in the area [FELICIEN, 2005]. Local breeds and their crosses with Large White cross Pietrain constitute the pig breeds that are kept on diverse farming systems which include scavenging, housing and divagation cum housing. Generally, farmers keep between two to four animals without any form of prevention to enzootic diseases.

After a devastating outbreak of African Swine Fever which reduced pig population in some areas of Kongo Central Province, a systematic sampling of 50 pigs, aged between 9 and 36 months

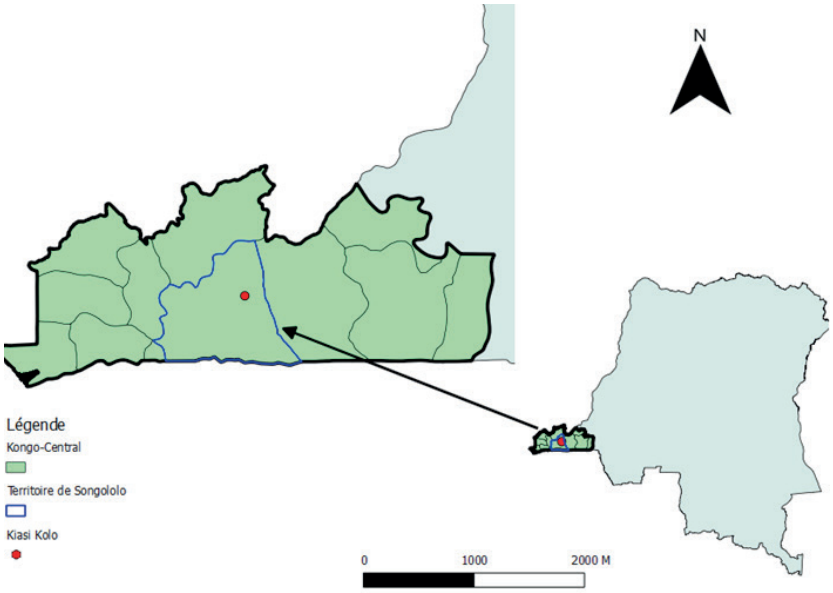

Figure 1| Map of Kongo Central Province showing Kiasi-kolo Village

(of which 68% females), was selected for blood collections.

Blood sampling

Animals were appropriately contained before sampling and blood was collected through venepuncture after disinfection with alcohol 70%. A drop of blood was deposited on Watman No 4 filter paper, identified by a code number, dried at ambient air, and kept in a clean envelope. The samples were kept in a fridge at 4oC until analyses.

Analyses

Extraction and DNA amplification

Confetti were obtained using a Harris micro punch and DNA extraction was done according to the procedure of Geysen et al. [2003]. The resultant DNA extract was stored at -20°C, before analyses.

PCR

Initially, a nested PCR using 3 primers targeting the genes 18ST: 18ST nF2 (CAA CGA TGA CAC CCA TGA ATT GGG GA), 18ST nR3 (TGC GCG ACC AAT AAT TGC AAT AC) and 18ST nR2 (GTC TCT TGT TCT CAC TGA CAT TCT AGT G) was performed according to the procedure of Geysen et al. [2003] in order to amplify all positive samples for trypanosomes, but without differentiating between species.

Thereafter, all the positive specimens were tested through a conventional PCR using two primers: Kin1: 3'-GCG TTC AAA GAT TGG GCA AT-5' and Kin2: 5'- CGCCCGAAAGTTCACC-3'. This allowed to differentiate Trypanosoma species that were present in the samples as described by Desquesnes et al.[(2001].

Finally, a PCR based on detection of T.b. gambiense by a specific glycoprotein was performed using two primers: Tgs GP-S: 5'-GCTGCTGTGTTCGGAGAGC-3' and Tgs GP-AS: 5'-GCC ATC GTG CTT GCC GCT C-3' following a procedure described by Radwanska et al. [2002].

The amplification was done through thermocycler Eppendorf ® (Germany) in the following conditions: An initial denaturation at 95 °C for 15 min followed by 45 cycles of denaturation at 95 °C for 1 min, hybridization at 63 °C for 45 sec, elongation at 72 °C for 45 sec, final extension at 10 minutes for 72 °C.

Samples migration was made on a 2 % agarose gel at 100 volts for 30 minutes and read on a UV table equipped with a photographic camera (Uvitec, Netherlands).

RESULTS AND DISCUSSION

The results showed that 12% (6/50) of the 50 animals sampled were positive according to the nested-PCR. Subsequent specific analyses indicated the presence of T. simiae (4/50) and T. congolense (2/50). No sample was found positive for T. b. gambiense.

Results of the present study clearly demonstrate the presence of Trypanosoma in pig populations of Kongo Central Province. Previous studies by Kageruka [1987] reported prevalence of trypanosomiasis in pigs from commercial and traditional farms but the prevalence varied according to the farming system and the causative species was identified as T. congolense. Prevalence of Trypansoma spp. was also reported in two foci of sleeping sickness in the Bas-Congo (Now Kongo-Central Province) with T. congolense being predominant [MAKUMYAVIRI et al., 1989]. Furthermore, T. congolense has been reported in several regions of the country where tsetse fly is present [KAGERUKA, 1987] and elsewhere in Africa. For instance, in eastern of Zambia, T. congolense was identified from a total of 324 pigs, using PCR-RFLP [SIMUKOKO et al., 2007]. In Nigeria, the presence of T. congolense and T. brucei brucei was reported, respectively, at the rates of 4.7% (33/712) and 8.8 % (63/712) [KARSHIMA et al., 2016]. In endemic area of HAT in Cameroon, the presence of T. vivax (111/307) and T. congolense (61/307) was reported in pigs [NYMPAYE et al., 2011].

In addition to T. congolense, the present study has identified T. simiae in the village of Kiasi-kolo. Thus, these results corroborate previous report on disease outbreaks in 1965 in M'vela's industrial pig farms, Bas-Congo, which was attributed to T. simiae [KAGERUKA, 1987].

More recently, an Elisa-indirect serological-based study carried out in Kinshasa's pig farms reported 45% of T. simiae in analysed samples which indicates that T. simiae plays an important role in the transmission of trypanosomiasis in pig farms of Kinshasa [SUMBU et al., 2009]. In Tanzania, T. simiae was detected only in 3 out of 168 pigs [HAMILL et al., 2013]. However, in Cameroon, T. simiae was not detected in pigs but in a sheep and two goats sharing the same habitat with pigs [NYMPAYE et al., 2011].

Although the presence of T. b. gambiense was not observed in the Kongo Central Province in this study, it has been reported in pigs in some foci of HAT in Bas-Congo [KAGERUKA 1987; MAKUMYAVIRI et al., 1989]. Elsewhere, T. b. rhodesiense, also pathogenic to human, was reported in pigs around a focus of HAT in Tanzania [HAMILL et al., 2013]. Therefore, these results clearly indicate

that pigs can be considered as animal reservoir in the transmission of HAT.

CONCLUSION

The results of this study confirmed the presence of T. congolense and T. simiae in pigs in the village of Kiasi-kolo. This should alert the Animal Health officials to impose prophylactic measures such vector control against tsetse in the pig farms of Kiasi-kolo village and in all the sites at risk in order to limit losses. Moreover, pig could serve as animal reservoir in the transmission of HAT.

RÉSUMÉ:

La province du Kongo Central, premier centre de production de viande porcine du pays, est une province réputée endémique aux trypanosomiases animales et humaines. Cependant, l'élevage du porc n'y est pratiqué que de manière traditionnelle et les éleveurs ne sont pas capables d'intégrer des mesures prophylactiques appropriées pour contrôler les maladies, notamment la trypanosomiase porcine dont nous ne disposons pas d'assez d'informations. L'objectif principal de cette recherche était d'identifier les différentes espèces de trypanosomes chez les porcs dans le village de Kiasi-Kolo. Cinquante échantillons de sang de porcs prélevés sur papiers filtres ont été analysés par la technique de PCR (polymerase chain reaction). Quatre pourcent (2/50) de *T. congolense* et huit pourcent (4/50) de *T. simiae* ont été détectés dans ces échantillons. Aucun échantillon n'était détecté positif au *T. brucei. gambiense*. Ces résultats démontrent que les porcs restent exposés à la trypanosomiase qui peut constituer un frein pour l'élevage porcin, surtout à cause du caractère très pathogène de *T. simiae* pour le porc. Une bonne prophylaxie telle qu'une lutte vectorielle contre la mouche tsé-tsé doit être appliquée dans ces élevages mais aussi dans d'autres régions à risque afin de limiter les pertes économiques.

Mots clés: T. congolense, T. simiae, Porc, PCR, République Démocratique du Congo

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