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PORCINE TRYPANOSOMIASIS IN SOUTHEASTERN UGANDA: PREVALENCE AND ASSESSMENT OF THERAPEUTIC EFFECTIVENESS

C. WAISWA

Department of Veterinary Medicine, Faculty of Veterinary Medicine,
Makerere University, Kampala, Uganda

Summary

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This study aimed at investigating the prevalence of trypanosomiasis and the usefulness of diminazene aceturate and isometamidium chloride in the treatment of pigs infected with *Trypanosoma brucei* sub-group.

Whole blood was collected from pigs kept in two disease endemic areas, with riverine and open savannah environments.

The prevalence of trypanosomiasis was recorded at 8.1% in the riverine environment as compared to the 2.1% in the open savannah environment and the infections in the former were significantly higher ($P < 0.001$).

All pigs that received a treatment of isometamidium chloride (Samorin®) at 1 mg/kg body weight did not show relapse when followed up to one month post treatment using microscopy. However, relapses were recorded among pigs treated with diminazene aceturate (Berenil®) at a dose rate of 7 mg/kg body weight and no relapses were recorded in those treated with 14 mg/kg body weight.

From this investigation, it is apparent that the trypanosome prevalence among pigs kept under the riverine environment is higher than those kept under the open savannah. In addition, 1 mg/kg and 14 mg/kg isometamidium chloride and diminazene aceturate respectively should be adopted for the treatment of trypanosome infections among the pigs in the trypanosomiasis endemic areas.

Key words: haemoglobin, PCV, pigs, therapy, trypanocidal agents, trypanosomiasis

INTRODUCTION

The impact of trypanosomiasis on the pig industry is still unknown in many tsetse infested areas with very little attention being paid on the significance of trypanosome infection among this animal species (personal observation). There is need to constantly design studies that will provide more information on trypanosomiasis infections and effectiveness of treatment using the available trypanocidal drugs used especially in the trypanosomiasis endemic areas.

Work by Waiswa *et al*. (2003a, 2003c) indicated a high prevalence of trypanosomiasis among pigs kept in some tsetse infested and trypanosomiasis endemic areas of Uganda. In addition, vector competence studies have indicated that *Glossina fuscipes fuscipes* (the predominant vector in this area) has the capacity to get blood meals from the available hosts (Waiswa *et al*., 2003b). Moreover, in one of the areas where this study was conducted, the pig leads as the prefered

host for tsetse and 30% of the tsetse get their bloodmeal from this species (unpublished). Since *G. f. fuscipes* is the predorminat vector for trypanosomiasis in many areas of eastern Uganda, the disease spread in pigs is thought to be high. In addition, observations have shown that pigs are kept in sheds close to the homesteads and this may be facilitating transmission of trypanosomiasis among the pigs since *G. f. fuscipes* has also been described as being peridomestic (Okoth, 1986; Okoth & Kapaata, 1986; Katabazi, 1983). Several studies (Ugandan example) have reported high trypanosome infections in pigs at different times (Okuna & Mayende, 1981; Okuna *et al*., 1986; Nowak *et al*., 1992; Katunguka-Rwakishaya, 1996). According to the available records, there has been no deliberate attempt by the Governments or any other authority to address the trypanosomiasis problem in pigs despite all the reports. In addition, recent steps to reduce the spread of sleeping sickness by treatment of cattle bought in the disease endemic areas before transportation (Wendo, 2002) ignores the fact that pigs are potential reservoirs for the human infective trypanosomes (Waiswa *et al.,* 2003a). The task to treat and control trypanosomiasis among pigs is entirely left to the local farmers who will react only when there is evidence of clinical disease. Since trypanosome infections among pigs (especially *T. brucei* sub-group) may not be associated with clinical disease (Waiswa *et al.,* 2003c), many pigs carrying infections miss out on any trypanocidal treatment available. This research sought to establish the prevalence of trypanosomiasis and the usefulness of the drugs currently used in the treatment of the disease in some disease endemic areas of Africa where pigs are increasingly being kept as a strategy by the farmers to increase on their house hold income.

MATERIALS AND METHODS

Study area and research design

The research was carried out in the trypanosomiasis endemic areas of Kamuli and Iganga districts, Southeastern Uganda (Fig. 1). The most recent records of the animal census in Iganga and Kamuli districts were used to select study sites.

A list of ten sub-counties with the highest pig population in the two districts was made and five sub-counties were randomly selected for the study. The area Veterinary Extension workers in the sub-county provided information of the villages keeping most of the pigs in the sub-county and four villages per sub-county were slected for the pig survey. Most of the pigs kept are local pigs with a few crosses (local breeds and Landrace or Large White) with a live body weight ranging between 40–80 kg at maturity. The pig production system in this area is mainly tethering of the pigs especially during the wet season to avoid them destroying the crops and moving freely after the crops are harvested (dry season).

Sampling and collection of pig blood samples

After approval of the study by the Uganda National Council for Science and Technology (UNCST), one day workshops were held in each village chosen for the pigs survey. The objectives of the research were explained to the farmers, local leaders plus the Agricultural Extension staff. A list of homes keeping pigs in each selected village was made and pig blood samples were taken from at least half of the homesteads keeping the pigs in the

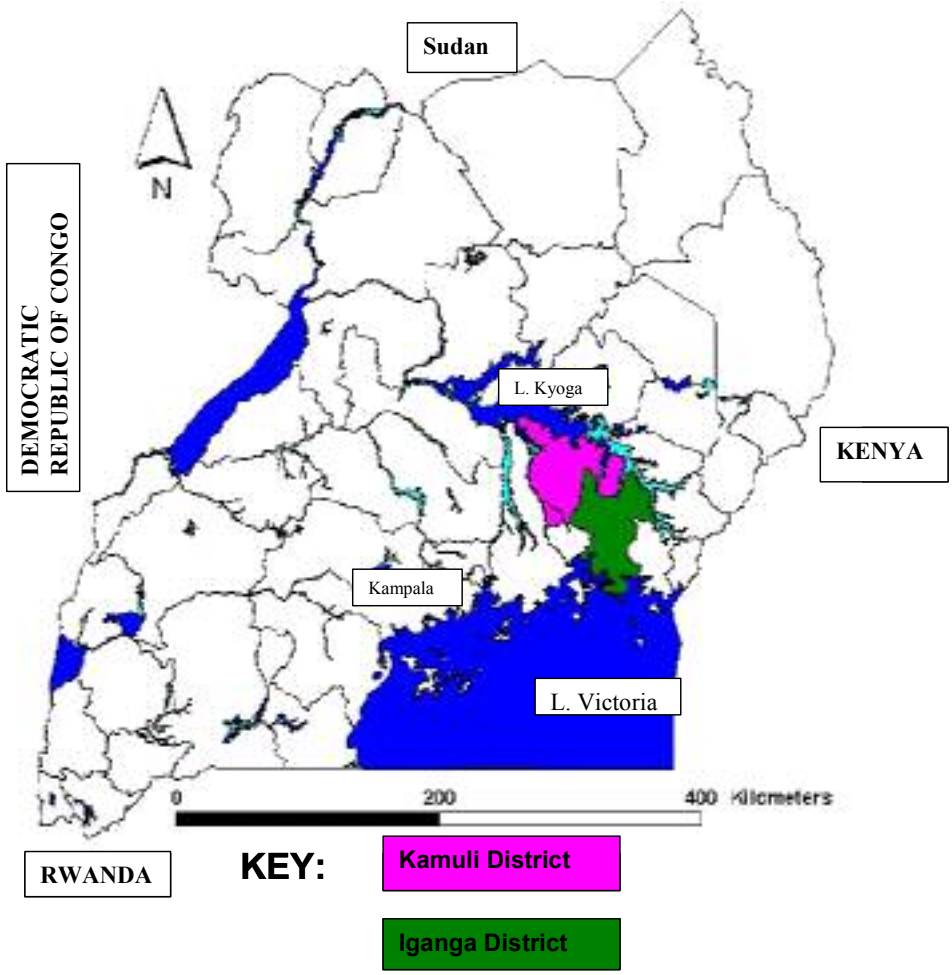

Fig. 1. Map of Uganda showing the study areas

village. The "home to home visit" sampling strategy was adopted during the survey.

A total of 1147 pigs were examined and 707 of these were from the riverine environments of Iganga district and 440 from the open savannah environments of Kamuli district. Two mL of blood were collected from the anterior vena cava of the pig using a 5 mL syringe and 1.5–2.5" x 19G needles. The blood was immediately transfered to vacutainers containing heparin and was examined for presence of trypanosomes on the same day using the haematocrit centrifugation technique, wet blood film and thin smears.

Trypanosoma detection and identification

This was carried out in the field immediately following blood sample col-

lection and examination by the haematocrit centrifugation technique (HCT) (Woo, 1970), and microscopic examination of wet and stained blood smears. For the haematocrit centrifugation technique, two capillary tubes (for each blood sample), were filled by capillary attraction up to 3/4 way. Each capillary tube was sealed at one end using placiticin. After centrifugation at 10,000 revolutions per minute (1000 g) for 5 min, the capillary tubes were examined for the presence of trypanosomes using a microscope with x40 objective.

Wet smears, each made of sandwiching a drop of whole blood between a glass slide and a coverslip, were examined at x400. The size and motility of any trypanosomes seen in the wet smear and the morphology of the parasites in the stained smears were used to differentiate between the *Trypanozoon*, *Nannomonas*, *Duttonella* and *Megatrypanum* sub-genera (Hoare, 1966).

Estimation of the packed cell volume (PCV) and haemoglobin content

Packed cell volumes (PCV) and haemoglobin concentrations were some of the parameters recorded as indicators of the health status and success of therapy. The centrifuged haematocrit tubes were used following the method desribed by Woo (1970) and a microhaematocrit tube reader was used to estimate the PCV (as a % of the blood volume) of each animal investigated. The haemoglobin estimation was carried out using the Sahli's method for determination of haemoglobin and the results recorded as g/dL.

Treatment of all the pigs positive for trypanosomiasis and follow up

The pigs found positive for trypanosomiasis as described above were randomly given numbers starting with one and treated with either of the two drugs: isometamidium chloride (Samorin®, Rhône Mérieux, Lyon, France) at the dose of 1 mg/kg body weight (for the pigs allocated odd numbers) or diminazene aceturate (Berenil®, Intervet International GmbH, Unterschleissheim, Germany) at the dose of 7 mg/kg (for the pigs given even numbers). Both drugs were administered using a 1.5" x 19G needle to give a deep intramuscular injection. The initial dosages were the standard dosages used by the Veterinary Extension staff for the treatment of trypanosomiasis in cattle and pigs (information provided by the Veterinary Extension staff in charge of the sub-counties). The treated pigs were monitored for a period of sixty days, for purposes of detecting any infection by taking blood samples from them twice in the first week after treatement and thereafter once a week for the following two months. Following detection of infection in some of the dimininazene treated pigs, seven pigs with infection were transferred to a fly proof stable at the Faculty of Veterinary Medicine, Makerere University (to rule out any possibility of acquiring new infections and to enable effective monitoring for any relapse infections). Three of the pigs were subjected to treatment using 7 mg/kg (dose given during the field treatment) and the other four were given 14 mg/kg diminazene aceturate. The treated pigs were then monitored for the development of parasitaemia and recovery of the packed cell volume and haemoglobin content for a period of four weeks post treatment. During the monitoring period in the field and in the animal house, blood was taken from the ear vein and examined by HCT, wet smear, thin blood smear using a microscope with x40 objective.

Statistical analysis

Compilation of the results (trypanosome prevalence, packed cell volume, haemoglobin content) was entered in the Microsoft Excel worksheets and graphs were ploted using the Microsoft Excel chart Wizard. Statistical comparisons by means of the Chi-square test were carried out using Minitab 13 (Minitab, State College, PA) software packages.

RESULTS

Prevalence of trypanosomiasis among pigs

The parasitological prevalence of trypanosomiasis was the highest (9.4%) in samples picked from pigs kept in the riverine areas of Bukooma sub-county, Iganga district and the lowest (2.0%) among pigs kept in the open savannah environment of Namwendwa sub-county, Kamuli district. The prevalence of trypanosomiasis among pigs in the different study areas is given in Table 1.

There was a significant difference ($P < 0.001$, df=1, $x^2 = 18.105$) in the prevalence of infection with pigs kept in the riverine environment carrying more infections than those in the open savannah. Similarly, there was a significant difference in trypanosome infections in the different study sub-counties ($P < 0.001$, df = 4, $x^2 = 24.095$), while there was no difference ($P = 0.569$, df = 1, $x^2 = 0.324$) when infections among pigs kept in two different sub-counties (e.g. Bukooma and Nawandala) in a riverine environment were compared. There was also a significant difference ($P < 0.001$, df = 1, $x^2 = 12.846$) when the number of infected pigs from a sub-county with a typical riverine environment (e.g. Bukooma) were compared to those from a sub-county with a predominant open savannah environment (e.g. Nawanyago).

Trypanosome species among pigs kept in the two areas

66.7% of the trypanosome species were classified as *Trypanosoma brucei* sub-group with no *Duttonella* group infections. 33.3 % of all the infections were classified as mixed infections as given in Table 2.

Packed cell volumes

Table 3 gives a summary of the number and percentage of pigs with PCV values

Table 1. Trypanosoma prevalence among pigs in the different sub-counties

District	Sub-county	Number examined	Number negative	Number positive	Trypanosoma prevalence
Iganga (Riverine environment)	Bukooma	383	347	36	9.4%
	Nawandala	224	206	18	8.0%
	Bulongo	100	97	3	3.0%
	District totals	707	650	57	8.1%
Kamuli (Open savannah)	Nawanyago	240	235	5	2.1%
	Namwendwa	200	196	4	2.0%
	District totals	440	431	9	2.1%
Total		1147		66	5.8%

Table 2. Trypanosoma species in the different study areas

District	Sub-county	Number Tryps +ve	Trypanosoma species		
			T. b.	T.c./T.s.	T.b./T.c./T.s.
Iganga	Bukooma	36	27 (75%)	0	9 (25%)
	Nawandala	18	13 (72.2%)	1 (5.6%)	4 (22.3%)
	Bulongo	3	1 (33.3%)	0	2 (66.6%)
Kamuli	Nawanyago	5	1 (20%)	0	2 (80%)
	Namwendwa	4	2 (50%)	1 (25%)	1 (25%)
Total		66	44 (66.7%)	2 (3%)	20 (30.3%)

Tryps = Trypanosoma; Tryps +ve = Trypanosome positive ; T.b. = *Trypanosoma brucei* sub-group; T.c./T.s. = *Trypanosoma congolense/T. simiae.*

Table 3. Packed cell volumes of pigs in the different study areas

District	Sub-county	Number of pigs	Number with PCV < 32	Number with PCV ≥ 32
Iganga	Bukooma	383	171 (44.7%)	212 (55.3%)
	Nawandala	224	78 (34.8%)	146 (65.2%)
	Bulongo	100	58 (58%)	42 (42%)
	District totals	707	307 (43.4%)	400 (56.6%)
Kamuli	Nawanyago	240	36 (15%)	204 (85%)
	Namwendwa	200	24 (12%)	176 (88%)
	District totals	440	60 (13.6%)	380 (86.4%)
Total		1147	367 (32%)	780 (68%)

Table 4. Packed cell volumes and haemoglobin content of parasitologically Trypanosoma-positive pigs

Indices	Number with PCV < 32%	Number with PCV ≥ 32%	Number with Hb content <10 g/dL	Number with Hb content ≥10 g/dL
Number	46	20	38	28
Percentage	69.7%	30.3%	57.6%	42.4%

less than the normal minimum value of 32% in each sub-county and for the two study environments. More pigs (43.4%) had their PCV lower than normal (32%) under the riverine environment (Iganga district) as compared to the 13.6% of those kept in the open savannah environment (Kamuli district).

Packed cell volumes and haemoglobin content in infected pigs

The average PCV for all trypanosome-positive pigs was 27.9% and the average haemoglobin content was 9.6 g/dL all below the lowest reference limits for the porcine species (32% and 10 g/dL respec-

tively). Table 4 gives the numbers of infected pigs with PCV and haemoglobin contents below and above the lowest normal limits.

Response to treatment with isometamidium chloride (Samorin®)

There was no relapse up to 60 days post-treatment of pigs given isometamidium chloride (Samorin®) at 1 mg/kg.

Recovery of packed cell volumes and haemoglobin content in the different dose levels of diminazene aceturate

Following the relapse of infection of the seven pigs at 7 mg/kg diminazene aceturate, three were again treated with 7 mg/kg while the other four were treated with 14 mg/kg body weight. The recovery of the packed cell volume and haemoglobin content in both groups is shown on Fig. 2 and 3, respectively.

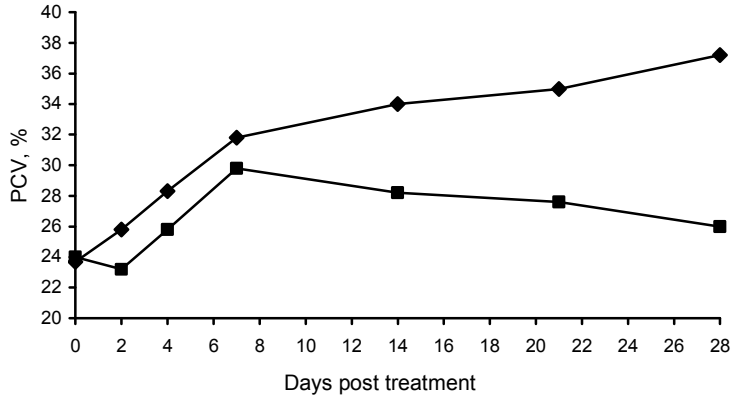

Fig. 2. Average packed cell volume (PCV, %) in pigs treated at 7 mg/kg (–■–) and at 14 mg/kg (–◆–) diminazene aceturate.

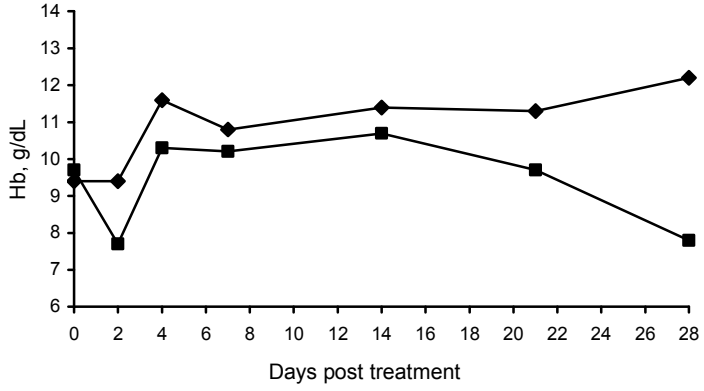

Fig. 3. Average haemoglobin content (Hb, g/dL) in pigs treated at 7 mg/kg (–■–) and at 14 mg/kg (–◆–) diminazene aceturate.

When the PCV was used to assess the effectiveness of therapy and estimate the average performance of the treated pigs, the recovery of both PCV and haemoglobin was better among those treated with 14 mg/kg body weight vs those treated with 7 mg/kg body weight. The group of pigs treated with diminazene aceturate at 14 mg/kg attained an average PCV >32% within 2 weeks compared to the group that received 7 mg/kg which did not attain this average even 4 weeks after treatment. Similarly, the average haemoglobin in both groups recovered to normal levels within the first week. However, it dropped to below 10 g/dL in the group treated with 7 mg/kg diminazene aceturate and all the pigs in this group had a relapse of infection.

DISCUSSION

Trypanosomiasis is a major impediment to livestock production and economic development in those areas of Africa where it is endemic and chemotherapy, chemoprophylaxis and vector control have been largely employed in the control of the disease in some areas. Use of trypanocides is still the most widespread strategy against human and animal trypanosomiasis. However, trypanosomosis among pigs has not been extensively investigated. The presence of Nannomanas group infections (33.3%, single and mixed infections combined) among the pigs in the tsetse infested areas of Uganda is of great concern due to the high pathogenicity of *T. simiae* that can lead to death of pigs. This study indicated that *T. brucei* sub-group infections in some parts of Southeastern Uganda are higher than any other species. As stated by earlier research findings (Katunguka-Rwakishaya, 1996, Waiswa *et al.,* 2003c), the presence of *T. brucei* infections among pigs is of great public health significance as some of them end up being human serum resistant and therefore potentially human infective (Waiswa *et al*., 2003a). Despite the intensive tsetse and trypanosomosis control efforts over the past three years by the European Union funded project – (Farming in tsetse controlled areas (FITCA) – Uganda), the parasitological prevalence of porcine trypanosomiasis was significantly high in pigs kept in some areas, especially those with a riverine environment. The highest trypanosome point prevalence of 9.4% was recorded in the riverine areas of Bukooma sub-county, Iganga district and the lowest (2.0%) in open savannah areas of Namwendwa sub-county, Kamuli district. Moreover, these prevalences have been recorded after using the parasitological techniques which could have missed some infections as it is known that these techniques usually miss the chronic *T. brucei* infections (Killick-Kendrick, 1968). Similar to earlier findings (Waiswa *et al.,* 2003a), about 70% of the trypanosome species were classified as *T. brucei* with no *Duttonella* group infections. Although the trypanosome prevalence during this investigation was lower than that recorded by Waiswa *et al*., (2003a), the importance of chemoprophylaxis in the control of trypanosomiasis, especially *T. brucei* infections among pigs needs further investigation as the prevalence could be higher than those recorded if more sensitive diagnostic technologies are used.

For this study, diminazene aceturate given at 14 mg/kg body weight was recorded as the curative dose in the treatment of pigs and relapses were recorded in those that received 7 mg/kg body weight. Basing on this observation, failure to clear trypanosomes when 7 mg/kg di-

minazene aceturate is used and negligence of pigs during the livestock therapeutic and prophylactic treatments (personal observation) seem to explain the high prevalence of infection in some areas despite the control efforts. The results obtained show that the prevalence of the disease in some areas is above the 5% point prevalence which is the point at which the disease is considered to be under control by the Coordinating Council for the control of tsetse and trypanosomiasis in Uganda. Meanwhile, the relapses could be an indicator of the development of trypanosome resistance to diminazene aceturate and this should be investigated further since there are existing reports of resistance to this drug (Mwambu & Mayende, 1971; Peregrine & Mamman, 1993 ; Codjia *et al*., 1993).

On the basis of packed cell volume, it is apparent that pigs kept in the open savannah environment where trypanosome prevalence is low are healthier since the majority of pigs (86.4%) had their PCV values $\geq$32% as compared to 56.6% of those kept under the riverine environment. Trypanosomiasis as a possible cause of anaemia among pigs in this area is supported by the fact that 66.7% of all the infected pigs had their PCV values below 32% plus the average PCV of all the trypanosome infected pigs being 27.9%. Our results imply that 7 mg/kg diminazene aceturate was not effective in the treatment of some trypanosome infections in pigs. From this study, both PCV and haemoglobin content have been recorded as useful parameters and are complementary if used in monitoring the success of trypanocidal therapy among pigs.

The results showed that infections in pigs mainly occurred in those kept in the riverine areas as evidenced by the survey in Iganga which was carried out in such areas compared to the Kamuli results where samples were collected from pigs kept within a savannah environment. Therefore, the inland pigs acquired fewer infections than those kept in the riverine (predominated by swampy areas) suggesting a predominance of the role by the riverine type of tsetse in the transmission of the disease. This is supported by earlier findings (Katabazi, 1983; Okoth, 1986), that *G. f. fuscipes* is a major vector in this area and pigs could be the major source of blood for this vector.

Finally, in the control of trypanosomiasis among pigs, there should be deliberate efforts to give more emphasis to the pigs kept in the riverine environments and isometamidium chloride is recommended for prophylactic treatments in such areas.

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Correspondence:
Dr. Charles Waiswa PhD, Senior Lecturer,
Department of Veterinary Medicine,
Faculty of Veterinary Medicine,
Makerere University,
P.O Box 7062, Kampala, Uganda.
Tel. +256 77 501274; Fax: +256 41 554685
E-mail: cwaiswa@vetmed.mak.ac.ug;
cwaiswa@yahoo.co.uk