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Prevalence and Drug Sensitivity of Trypanosome Isolates from Slaughter Animals to Diminazene and Isometamidium in Sub Humid Tropical Zone of Southeastern Nigeria

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Abstract: A total of 465 animals comprising 116 goats, 155 pigs and 194 cattle presented for slaughter at Nsukka municipal abattoir were screened for trypanosomosis between March and June, 2011. Whole blood from the animals was examined by wet mount, stained smear and Buffy coat methods. The packed cell volume (PCV) was also determined. Samples positive for trypanosomes were inoculated into donor mice and mice for the drug sensitivity tests were infected. Twelve (12) mice were used for drug sensitivity test of each isolate. The mice were infected with 1 x 10^5 trypanosomes each and further divided into 4 groups of 3 each treated with discriminatory doses of 7.0 mg/kg, 28 mg/kg of diminazene aceturate (DA) and 1 mg/kg, 8 mg/kg of Isometamidium chloride (ISMC). Infected mice were treated 7 days post infection following parasitaemia. The results showed an overall prevalence of 19 (4.08%) comprising, 9 (5.81%) for pigs, 6 (3.09%) for cattle and 4 (3.44%) for goats. Infected animals had a significantly lower ($p<0.05$) PCV than uninfected ones. The isolates were predominantly *Trypanosoma brucei (T.brucei*) was78.9% especially in pigs. The *T. brucei* isolates showed sensitivity only to the high doses of diminazene aceturate and Isometamidium chloride. However, only 2 isolates were resistant to isometamidium at 1 mg/kg whereas all the 10 isolates showed variable degrees of resistance to diminazene aceturate at 7.0 mg/kg. The current study concluded that populations of trypanosomes resistant to diminazene aceturate exist and abound in our environment.

Key words: Trypanosomosis · Slaughter Animals · Sensitivity · Diminazene Aceturate · Isometamidium Chloride · Mice

INTRODUCTION

African trypanosomosis is an endemic disease in sub-Saharan Africa. It is responsible for the reduced productivity of livestock and prevents the development of sustainable and productive agricultural system [1]. In Nigeria, the disease constitutes a major hindrance to achieving food security [2, 3], in spite of attempts towards chemotherapeutic and tsetse control [4]. In the last decade, no conscious large scale surveillance and control programmes have been in place against the menace of trypanosomosis in Nigeria [5]. The control of trypanosomosis in Nigeria currently depends on the use of salts of isometamidium and diminazene. These drugs have been in use in the field for over 50 years [6] and their repeated use has led to resistance among trypanosome populations [7, 8].

Reports on the prevalence of trypanosomosis abound in Nigeria [2, 5, 9-11] and in the study area [12, 13]. However, there is need to investigate the presence, among the prevalent trypanosome populations in the country the distribution of drug sensitive or resistant trypanosome strains. The knowledge of this will inform control measures against trypanosomosis through an integrated approach encompassing both parasite and vector control strategies.

The aim of this study is therefore to investigate the sensitivity or resistance of prevalent trypanosome isolates from slaughter animals in Nsukka municipal abattoir to diminazene and isometamidium.

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MATERIALS AND METHODS

Study Area: The prevalence study was carried out in the Nsukka municipal abattoir between the months of April and June 2011 whereas the sensitivity of the trypanosome isolates was investigated in the laboratory of the Department of Veterinary Medicine, University of Nigeria, Nsukka.

Sample Collection: Whole blood samples were collected from animals from the jugular vein during slaughter into sample bottles containing EDTA. The samples were kept in ice boxes and transported to the laboratory for parasitological examination. The blood was examined using the wet mount and Buffy coat technique [14] and the packed cell volume (PCV) was determined using the haematocrit centrifugation technique [15]. Stained thin smear preparations were made for positive blood samples [16].

Parasite Species Identification: The parasites were identified using their motility under the microscope in wet mount and their morphological characteristics under stained smear [16].

Drug Sensitivity Tests: All parasites species positive for mice inoculation tests were then further subjected to sensitivity tests in mice.

Experimental Animals: 160 adult tropical outbred albino mice weighing between 22 and 30 g were used for the study. They were obtained from the laboratory animal unit of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. Each positive blood sample was inoculated into 2 donor mice and monitored for parasitaemia. Following parasitaemia, 12 mice were infected with1 x 10^5trypanosomes isolate suspended in 250 μl of PBS for each mouse for drug sensitivity test. The mice were further divided into 4 groups of 3 mice each and treated as follows:

- 7.0 mg/kg of diminazene aceturate (DA)
- 28 mg/kg of diminazene aceturate
- 1 mg/kg of isometamidium chloride
- 8 mg/kg of isometamidium chloride

Treatment commenced following parasitaemia on day 8 post infection (PI.) and was monitored daily for parasite clearance and then every 4th day for relapse. Treated groups were considered sensitive if no relapse occurs within 40 days post treatment.

Experimental Drugs: Diminazene aceturate (Ceva Sante Animale, Spain) given at a single discriminatory dose of 7.0 mg/kgand 28 mg/kg body weight intraperitoneally was used. The sachet contained 2.36 g of powder dissolved in 15 ml of injection water before use. Isometamidium chloride (Merial, France), on the other hand was given at a single discriminatory dose of 1 mg/kg and 8 mg/kg body weight intraperitoneally. 125 mg of the powder was dissolved in 12 ml of injection water to obtain a 1% w/v solution before administration at the given dosage.

Statistical Analysis: Data generated were computed into means and standard error of means and the difference in mean PCV between infected and non-infected animals was determined using the student's t-test. The prevalence of the infection among slaughter animals was based on the mean PCV of the animals for the period of study. Probability value of $P < 0.05$ was considered significant.

RESULTS

Prevalence results of trypanosome species in sampled animals (Table 1).

Of the 116 goats, 155 pigs and 194 cattle sampled; 4, 9 and 6 positive cases were recorded representing 3.44%, 5.81% and 3.09% respectively. Thus, the highest prevalence rate of 5.81% was recorded in pigs. *T.brucei* accounted for 75% of all positive cases and was predominant in pigs whereas 15.7% of the cases were due to *T.vivax* predominantly in cattle. However, a mixed infection of *T. brucei* and *T. congolense* was recorded (0.22%) in a goat.

The mean packed cell volume of infected animals was 25.16 ± 1.5 and this was significantly lower ($p < 0.05$) than the uninfected 35.47 ± 0.4 (Table 2). The mean PCV of the infected animals species were also significantly lower ($p < 0.05$) than the uninfected animals.

A total of 218 male and 247 female animals were sampled representing 46.88% and 53.12% of the sample population respectively (Table 3). However, a prevalence rate of 5.05% was observed in males while 3.24% was observed in females.

The results of the sensitivity tests conducted showed that, out of the ten isolates tested, nine were resistant to diminazene aceturate at the dose of 7.0 mg/kg out of which four were slightly resistant whereas all the isolates were sensitive to DA at 28 mg/kg (Table 4). On the other hand, only two isolates were resistant to isometamidium chloride (ISMC) at the dose of 1 mg/kg out of which one was slightly resistant. All isolates were

Table 1: Prevalence of trypanosome species in sampled animals

Animal sampled (No)	Positive samples	% Positive	*T. brucei*	*T. congolense*+ *T. brucei*	*T. vivax*
Goat (116)	4	3.44%	3 (75%)	1 (25%)	0
Pig (155)	9	5.81%	9 (100%)	0	0
Cattle (194)	6	3.09%	3 (50%)	0	3 (50%)
Total (465)	19	4.08%	15 (78.9%)	1 (0.22%)	3 (15.7%)

Table 2: Mean PCV ± SE (%) of infected and uninfected animals

No of animals	Mean PCV ± SE	Mean PCV of infected ± SE	Mean PCV of uninfected ± SE
Goat (116)	33.81±0.59	20.75 ± 3.2	34.28 ± 0.6
Pig (155)	38.65 ± 0.91	25.67 ± 1.9	39.46 ± 0.91
Cattle (194)	32.89 ± 0.41	27.33 ± 2.8	33.07 ± 0.41
Total (465)	35.04 ± 0.39	25.16 ± 1.5	35.47 ± 0.4

Table 3: Sex prevalence among infected and uninfected animal species

Animal species	Sex	Infected (%)	Uninfected
Goat	Male (12)	1(8.33)	11
	Female (104)	3(2.88)	101
Pig	Male (70)	4 (5.71)	66
	Female (85)	5 (5.88)	80
Cattle	Male (136)	6(4.41)	130
	Female (58)	0(0)	58
Total	Male (218)	11(5.05)	207
	Female (247)	8(3.24)	239

Table 4: Sensitivity profile of the trypanosome isolates to low and high doses of diminazene and Isometamidium

		Drugs			
		Diminazene aceturate		Isometamidium chloride	
Animal	Isolate sp	7.0 mg/kg	28 mg/kg	1 mg/kg	8 mg/kg
Pig	*T. brucei*	S (0/3)	S (0/3)	S (0/3)	S (0/3)
Pig	*T. brucei*	R (2/3)	S (0/3)	S (0/3)	S (0/3)
Pig	*T. brucei*	R (2/3)	S (0/3)	S (0/3)	S (0/3)
Goat	*T. brucei/T. congolense*	SR (1/3)	S (0/3)	S (0/3)	S (0/3)
Pig	*T. brucei*	R (2/3)	S (0/3)	S (0/3)	S (0/3)
Pig	*T. brucei*	R (2/3)	S (0/3)	S (0/3)	S (0/3)
Pig	*T. brucei*	SR (1/3)	S (0/3)	SR (1/3)	S (0/3)
Pig	*T. brucei*	SR (1/3)	S (0/3)	S (0/3)	S (0/3)
Pig	*T. brucei*	R (2/3)	S (0/3)	R (2/3)	S (0/3)
Pig	*T. brucei*	SR (1/3)	S (0/3)	S (0/3)	S (0/3)

R = resistance; S = sensitive; SR = slightly sensitive; 0/3 = all were sensitive; 1/3 = two were sensitive; 2/3 = only one was sensitive.

sensitive to ISMC at the dose of 8 mg/kg. One of the *T. brucei* isolate tested was cross resistant to both drugs at the low doses.

DISCUSSION

The overall prevalence rate of 4.08% recorded in this study from the various animal species of which 5.81%, 3.09% and 3.44% were observed in pigs, cattle and goats respectively did not differ with other reports by Ameen *et al*. [10] in Kaduna State, Ohaeri [11] in Abia State and Samdi *et al*. [17] in Ogbomosho, Oyo State where prevalence rates of 4.1%, 1.9% and 2.2% respectively were reported. Although the previous surveys represented prevalence of trypanosomosis in cattle, sheep and goats, pigs have been known to harbour symptomless trypanosomosis infections and serve as potential sources of the infection to other domestic livestock as carriers. The prevalence observed in pigs in this study agrees with the earlier studies by Onah [12] and Omeke [13] who conducted surveys on pigs in the same area as the present study. Interestingly, inSouth-eastern Nigeria, pork is widely consumed and its production is not constrained by religion as occurs in some areas in Northern Nigeria, therefore its production has greatly increased in recent times. Integrating government policies in control

programmes against porcine trypanosomosis is necessary to stem the possible reduction in pig production on account of trypanosomosis bearing in mind the attendant implications to animal protein availability. Conversely, cattle production in the study area is restricted to only the indigenous breeds (Muturu) which are trypanotolerant and less productive than their northern breeds (Zebu). However, the northern breeds of cattle are mostly slaughtered in this abattoir and these cattle are predominantly from nomadic herdsmen who travel several distances before they arrive at their destinations which expose them to the risk of trypanosomosis [11]. In the present study, high prevalence was recorded in males than females

In the current study, we did not use the method as described by Eisler *et al*. [18] for testing trypanosome drug resistance. We however simulated the infections as natural infection. Hence, the mice were allowed to show parasitaemia before treatment was instituted. Consequently, the low and high dose groups in both drugs were used to ensure that dose related resistance to the drugs were ruled out [19].Therefore, the high incidence of resistant trypanosome isolates to the dose of 7.0 mg/kg of diminazene aceturate implies that the drug was not effective at this dose against the infection in mice. However, at high dose (28 mg/kg), diminazene aceturate was shown to be effective. It therefore implies that treatment of trypanosomosis which is resistant to diminazene at the usual dose may be effective at a higher dose. On the other hand, isometamidium chloride which was effective at both the low (1 mg/kg) and high (8 mg/kg) doses against trypanosomosis implies that the drug irrespective of the dose was effective. Trypanosome drug resistance has been attributed to the abuse of anti-trypanosomal drugs, presence of fake and adulterated drugs and low drug dosage and use by unqualified persons [20]. Diminazene aceturate is a common trypanocides among pig farmers in the study area and most of the drug resistant parasites were isolated in pigs. It is therefore possible that any of the above reasons may have caused the resistance observed with this drug. Furthermore, the ability of isometamidium to cure most of the infection is an indication that the principle of sanative pairs [16] is still effective.

In conclusion, the high incidence of porcine trypanosomosis is of great economic and epidemiological importance. Since domestic pigs acts as potential carriers of infection especially with *Trypanosoma brucei gambiense* which causes human trypanosomosis (sleeping sickness) in west Africa [21, 22], campaign for improved pig husbandry practices consisting of netting of farms (vector control) as a strategic control for porcine trypanosomosis is advocated. Similarly, proper use of chemotherapeutic and chemoprophylactic agents against this disease should be monitored. This will promote a better performance of pigs and encourage large scale and profitable pig farming in the study area.

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