Journal of Veterinary and Biomedical Sciences
Vol. 1 (2). PP 152 - 160, February, 2019
ISSN: 2659 – 0743
DOI:10.36108/jvbs/8102.10.0261

Sensitivity of Trypanosome Isolates From Pigs In Enugu North Senatorial Zone of Enugu State To Diminazene Aceturate And Isometamidium Chloride

Omeje, J.N[1] and Akinbobols J.S[2]

[1]Department of Veterinary Medicine, University of Abuja, Abuja

[2]Veterinary Teaching Hospital, University of Abuja, Abuja

Accepted January, 2019 and Published February, 2019

ABSTRACT

The sensitivity of trypanosome isolates from naturally infected pigs in Enugu North Senatorial Zone was evaluated in mice at two dose levels each of diminazene aceturate (7 and 28 mg/kg body weight) and isometamidium chloride (0.25 and 2 mg/kg) using the infection and treatment methods. Multiple drug resistance was prevalent in the trypanosome isolates, as all 18 isolates (16 *T. brucei* and 2 *T. congolense*) tested were resistant to both diminazene aceturate (7 mg/kg b.w) and isometamidium chloride (0.25 mg/ kg b.w,), at the low dose levels tested. Sixteen of the isolates resisted the high dose levels of diminazene aceturate (28 mg/kg b.w), while six isolates were resistant to isometamidium chloride (2 mg/kg b.w). It was concluded that trypanosome isolates from pigs in the study area exhibited resistance to both diminazene aceturate and isometamidium chloride, the two most commonly used trypanocides in the area. This phenomenon constitutes serious threat to chemotherapeutic control of swine trypanosomosis in particular and animal trypanosomosis in general in Enugu North Senatorial Zone.

Key words: Diminazene aceturate, Isometamidium chloride, pigs, Sensitivity, trypanosomes

*Corresponding Author:
E Mail: omeje.nduka@uniabuja.edu.ng
Tel: +234705 443 4414

INTRODUCTION

The field control of animal trypanosomosis has, over the years, relied on two broad strategies: use of chemotherapeutic agents on infected animals and vector control [1]. At present chemotherapy and chemoprophylaxis are the only practical methods available for the control of animal trypanosomosis, but their effectiveness is being threatened by a number of factors, which include increasing parasite resistance [1], treatment failures and unacceptable toxicity [2,3,4,].

Drug control of animal trypanosomosis relies essentially on three drugs, namely: Diminazine aceturate (Berenil), Isometamidium chloride (Samorin, Trypamidium) and Homidium (Homidium chloride - Novidium; and Homidium bromide - Ethidium) [3]. Recently, however, Quinapyramine sulphate (Antrycide) has been reintroduced because of the need to especially combat camel trypanosomosis [5]

Diminazene aceturate and isometamidium chloride have, respectively, been used in the past as the best therapeutic and prophylactic trypanocides. The former was reputed as the only drug to which trypanosomes do not easily develop resistance because of its rapid elimination from the system when compared with the more persistent prophylactic drug isometamidium chloride [5]. However, recent finding has shown that isometamidium chloride is one of the best trypanocides [6]. Field and laboratory stocks of diminazene resistant trypanosomes have been reported, some field isolates requiring up to 45 mg/kg diminazene aceturate as the minimum required dose to achieve cure [7, 8]. Similarly, isometamidium chloride treatment failures and shortened prophylactic intervals have been attributed to infections with drug-resistant trypanosome species [9, 10]. Most of the trypanocides have been in use for over two decades and their long use and misuse has encouraged the emergence of parasites resistance to the drugs [11, 12, 13, 14]. The Increasing incidence and threat of drug resistance on trypanosomes on livestock production, therefore, highlights the need to conduct drug sensitivity test on trypanosomes field isolates. It is against this background that this study was designed to assess the occurrence and levels of resistance in trypanosome isolates from pigs in the study area to the two most commonly used trypanocidal drugs (diminazene aceturate and isometamidium chloride).

Materials and Methods

(I) Trypanocides: Diminazene aceturate (Veriben^{R} CEVA SANTE ANIMALE, CEDEX France) was reconstituted by dissolving 2.36 granules containing 1.05g of the drug in 15ml of sterile water to make 1% solution and serially diluted 10 times with sterile water to deliver 7mg and 28mg to the calculated mouse weight. Isometamidium chloride (Trypanidium

samorin[R] Merial, Lyon France) was prepared by dissolving 125granules in 12ml of sterile water to get 1% solution. It was serially diluted 10 times with sterile water to deliver 0.25mg and 2.0mg to the calculated mouse weight.

(ii) Sample collection

Blood samples were collected from the jugular vein of pigs slaughtered at the abattoir and from the marginal ear vein of pigs in farms during sample collection in the study area during the rainy and dry seasons respectively.

All the blood samples were collected in sample bottles containing EDTA and taken to the laboratory on an ice pack.

(iii) Blood examination for parasite identification

Blood examination for trypanosomes was done by wet blood film, hematocrit buffy coat examination method [15] and Giemsa stained thin film. Trypanosome species were identified by their morphological characteristics on Giemsa- stained thin blood film preparation [16].

(iv)Animal inoculation

Test on the isolates was done using 20 mice, which were injected intraperitoneal (IP) with freshly collected pig blood containing 10^5 trypanosomes diluted in 10ul of saline. The test was carried out as follows: Group 1 (4 mice used as control, and were not given any trypanosome isolate) ; Group 2 (8 mice treated with DA, 4 at a dose of 7mg/kg BW I.P and 4 at 28mg/kg BW I.P); Group 3 (8 mice were similarly divided but treated with IC at doses of 0.25 mg/kg BW and 2.0 mg/kg BW).

(v) Drug Sensitivity test

Treatment was administered on day 7 p.i and thereafter, the tail blood of the mouse was examined weekly as wet mount and using the buffy coat technique [17] until 60 days, post infection. If parasitaemia disappeared completely (i.e. no relapse), then the parasite was regarded as being susceptible, but if parasitaemia persisted, it was considered resistant to the test drugs. However, if after the treatment there was a relapse within the 60days observation period, the isolate was considered as not being susceptible to the trypanocides.

Result

Eighteen isolates comprising sixteen *T. brucei* and two *T. congolense* were tested. The prepatent period (PP) of infection in mice was 5 days for *T. brucei* and 7 days for the *T. congolense* species, respectively. All eight dry season isolates were resistant to diminazene aceturate at low dose of 7 mg/kg bodyweight (Table 1). At a higher dose of 28 mg/kg BW, relapses were observed in seven isolates, while one isolate was susceptible. For isometamidium chloride, the results were similar for the low dose of 0.25mg/kg where relapse occurred in all the eight isolates. Three

isolates resisted the high dose (2mg/kg) of isometamidium chloride while the remaining five were susceptible.

The drug sensitivity of rainy season trypanosome isolates is shown in table 2. All the ten isolates were not susceptible to the low dose of both diminazene aceturate and isometamidium chloride. Nine isolates were resistant to high dose of diminazene aceturate at 28mg/kg BW. Three of the isolates were resistant to 2mg/kg BW isometamidium chloride injection; whereas the remaining seven isolates did not show any relapse 60 days post treatment and were thus susceptible to the high dose of the drug.

Table 1: Trypanocidal sensitivity of trypanosome isolates from pigs in Enugu North Senatorial Zone, Enugu State in the dry season

S/no	Isolates	Drugs		Relapse in mice	Relapse interval (days)	Remarks
		Name	Dose (mg/kg)			
1	*T. brucei*	DA	7.0	4/4	40	High multiple resistance
			28	2/4	52	
		IC	0.25	2/4	38	
			2.0	¼	49	
2	*T. brucei*	DA	7.0	4/4	47	High resistance to DA
			28	2/4	54	Low resistant to IC.
		IC	0.25	¾	42	
			2.0	0/4	A	
3	*T. brucei*	DA	7.0	¾	39	High Multiple resistance,
			28	¾	42	
		IC	0.25	¾	49	
			2.0	2/4	52	
4	*T. brucei*	DA	7.0	4/4	42	High resistance to DA
			28	2/4	49	Low resistance to IC.
		IC	0.25	¾	53	
			2.0	0/4	A	
5	*T. brucei*	DA	7.0	4/4	47	Low multiple resistance
			28	0/4	A	
		IC	0.25	2/4	45	
			2.0	0/4	A	
6	T. congolense	DA	7.0	4/4	35	High multiple resistance
			28	¾	56	
		IC	0.25	¾	45	
			2.0	¾	51	
7	*T. brucei*	DA	7.0	4/4	43	High resistance to DA
			28	2/4	49	Low resistance to IC.
		IC	0.25	2/4	52	
			2.0	0/4	A	
8	T. congolense	DA	7.0	4/4	47	High resistance to DA
			28	2/4	49	Low resistance to IC.
		IC	0.25	¾	51	
			2.0	0/4	A	

A= aparasitaemic; DA= diminazene aceturate; IC=isometamidium chloride

Table2: Trypanocidal sensitivity of trypanosome isolates from pigs in Enugu North Senatorial Zone, Enugu State in the rainy season

S/no	Isolates	Drugs		Relapse in mice	Relapse intervals (days)	Remarks
		Name	Dose (mg/kg)			
9	*T. brucei*	DA	7.0	4/4	49	Low multiple resistance
			28	¾	53	
		IC	0.25	4/4	49	
			2.0	0/4	A	
10	*T. brucei*	DA	7.0	4/4	38	Low multiple resistance
			28	¾	42	
		IC	0.25	¾	44	
			2.0	¾	53	
11	*T. brucei*	DA	7.0	2/4	56	High resistance to DA
			28	0/4	A	Low resistance to IC
		IC	0.25	2/4	49	
			2.0	0/4	A	
12	*T. brucei*	DA	7.0	4/4	45	High resistance to DA
			28	2/4	49	Low resistant to IC
		IC	0.25	2/4	45	
			2.0	0/4	A	
13	*T. brucei*	DA	7.0	¾	28	Low multiple resistance
			28	¾	42	
		IC	0.25	2/4	45	
			2.0	2/4	52	
14	*T. brucei*	DA	7.0	¾	48	High resistance to DA
			28	2/4	45	Low resistance to IC
		IC	0.25	2/4	42	
			2.0	0/4	A	

15	.*T. brucei*	DA	7.0	4/4	52	High resistance to DA
			28	4/4	52	Low resistance to IC
		IC	0.25	4/4	50	
			2.0	0/4	A	
16	*T. brucei*	DA	7.0	4/4	47	Low multiple resistance
			28	¾	53	
		IC	0.25	4/4	45	
			2.0	¾	53	
17	*T.* brucei	DA	7.0	4/4	43	High resistance to DA
			28	2/4	52	Low resistance to IC
		IC	0.25	4/4	49	
			2.0	0/4	A	
18	*T. brucei*	DA	7.0	4/4	42	High resistance to DA
			28	¾	51	
		IC	0.25	4/4	50	Low resistance to IC
			2.0	0/4	A	

Keys: A= aparasitaemic, DA= diminazene aceturate, IC= isometamidium chloride

Discussion

The finding from this study shows that in spite of the importance of trypanocidal drug treatment as the most practicable option for control of trypanosomosis in endemic areas of Africa [18], varying degrees of drug resistance were detected in trypanosomosis infected pigs in the study area involving diminazene aceturate and isometamidium chloride which are the two most commonly used trypanocides. This observation poses a serious challenge to the chemotherapeutic control of porcine trypanosomosis in particular and animal trypanosome in general in the study area.

Relapse infection after treatment is a reoccurring problem in the chemotherapy of trypanosomosis in animals [18]. Relapses are usually considered to indicate resistance to the drug under test at the dose rates employed [17]. Distinct reduction in the period between treatment and subsequent relapse of infection in treated animals is associated with expression of high level of resistance by the population (i.e the earlier the relapse, the greater the resistance [20, 21]. The development, with the spread of drug resistance is probably the greatest risk to the future use of the existing trypanocides [22]. The occurrence of multiple drug resistance to diminazene aceturate and isometamidium chloride in the study area is in agreement with reports in both experimental and field studies from East Africa including Zambia, Kenya and Somalia [23,6,24] and West Africa including Nigeria [25,26,27]. Some of the factors that may be responsible for the prevalence of resistance detected in this study include the long-term use of the same drugs, misuse of the drugs and the widely available counterfeit drugs in the local markets [14]. The occurrence of drug resistance and multiple resistances in trypanosome isolate of pigs in Enugu North Senatorial zone pose serious problem to piggery farming and livestock generally in the area.

Diminazene aceturate and isometamidium chloride are termed" sanative pair" [28] because trypanosomes are not usually resistant to both trypanocides [28]. Multiple drug resistance to both trypanocides will definitely render the application of the concept of the sanative pair using these drugs to control trypanosomosis in the area ineffective. There is yet no prospect of the development and use of vaccine because of the well-known phenomenon of antigenic variation. If chemotherapy is to be successful, the need for regular monitoring of the trypanosomosis risk cannot be overemphasized. It is essential to know at which point drug intervention would be appropriate, which species of trypanosome is prevalent and its drug sensitivities.

Because of the potential threat drug resistance in trypanosomes poses to pig production, there is an urgent need for continuous conduct of drug sensitivity test on trypanosome field isolates.

Acknowledgement

The effort of the entire staff of Veterinary Medicine laboratory, University of Nigeria (Nsukka) is appreciated for the laboratory support

REFERENCES

1. Afewerk, Y., Clausen, P. H., Abebe, G., Tilahun, G and Mehlitz, D (2000). Multiple drug resistant *Trypanosoma congolense* populations in village cattle of Metekel District, Northwest Ethiopia. *Acta Tropica*, 76: 231-238.

2. Akanji, M.A and Ngaha, E (1989). Effect of repeated administration of Berenil on urinary enzyme secretion with corresponding tissue patterns in rats. *Pharmacology and Toxicology*, 64: 272-275.

3. Onyekwelu, N.A (1999). Toxicity of existing trypanocides. A review: *West Africa Journal of Pharmacology and Drug Resistance*, 15: 1-6.

4. Triolo, N. (1990). A case of coma due to Arsobal in a newborn affected with congenital *Trypanosoma gambiense*. *Therapy of Infectious Disease*, 5: 165-166.

5. Aliu, Y.O., Odegaard, S and Sognen, E. (1984). Diminazene/Berenil: bioavailability and disposition in dairy goats. *Acta Veterinaria Scandinavica*, 25: 593-596.

6. Chitambo, H and Arakawa A. (1991). Therapeutic effect of Berenil and Samorin in mice infected with four trypanosome populations isolated from Zambian cattle. *Veterinary Parasitology*, 39 (1-2): 43-52.

7. Peregrine, A.S and Mamman M. (1993). Pharmacology of diminazene: A review. *Acta Tropica*, 54:185-203.

8. Chitambo, H. and Arakawa, A. (1991). Therapeutic effect of Berenil and Samorin in mice infected with four trypanosome populations isolated from Zambian cattle. *Veterinary Parasitology*, 39 (1-2): 43-52.

9. Sutherland, I.A., Peregrine, A.S., Lonsdale-Eccles, J.D and Holmes P.H. (1991). Reduced accumulation of isometamidium by drug-resistant *Trypanosoma congolense*. *Parasitology*, 103: 245-251.

10. Peregrine, A.S., Ogunyemi, O., Whitelaw, D. D., Holmes, P. H., Moloo, S. K., Hirumi, H, Urquhart, G. M and Murray, M.(1988). Factors influencing the duration of isometamidium chloride (Samorin) prophylaxis against experimental challenge with metacyclic forms of *Trypanosoma congolense*. *Veterinary Parasitology*, 28: 53-64.

11. Stevenson, S., Sones, K. R., Gicheru, M and Nwangi E.K (1995). Comparison of Isometamidium chloride and Homidium Bromide as prophylactic drug for Trypanosomiasis in cattle at Nguruman, Kenya. *Acta Tropica*, 59 (2): 77-84.

12. Schrevel, J., Millieriouz, V., Sinor, V., Frappier, F., Santus, S and Grither, P. (1996). New trends in chemotherapy of Human and Animal Blood Parasites. *Parasitology Research*, 82 (93): 283-254.

13. Anene, B.M., Onah, D. N and Nawa, Y (2001). Drug resistance in pathogenic African trypanosomes; what hopes for the future? *Veterinary Parasitology*, 96: 83-100.

14. Geerts, S., Holmes P. H., Diall O and Eisler M. C. (2001). African Bovine trypanosomiasis. The problem of drug resistance. *Trends in Parasitology*, 17: 25–28.

15. Murray, M., Trail J. C., Turner, D. A and Wissocq, Y (1983): Livestock Productivity and Trypano-tolerance: Network Training Manual: International Livestock Center for Africa (Ireland) Vol. 6, Pp159-165.

16. Soulsby, E.J.L (1986). Helminths, Arthropods and Protozoa of domestic animals, 8th Edition, Balliere Tindall, London, 203-206.

17. Williamson, J and Stephen I. E (1960): A test for drug resistant trypanosomes in experimental Tsetse fly challenge of cattle *Annals of Tropical Medicine and Parasitology,* 54: 366-370.

18. Peregrine, A.S and Mamman, M. (1993). Pharmacology of diminazene: a review. *Acta Trop.* 54,185-203.

19. Ezeh, I.O., Agbo, L.I., Emehelu, C.O., Nweze, N.E; Ezeokonkwo, R.C and Onah. D.N. (2009) Berenil resistant *Trypanosoma brucei bruei* infection in a dog in Nsukka area of Enugu State, Nigeria. *Nigerian Veterinary Journal* (29): 34-42

20. Stephen, L.E (1986): Trypanosomiasis: A veterinary perspective. Oxford, New York, etc, : Pergamon Press, 1986.450Pp

21. Peregrine, A.S., Moloo, S.K and Whitelaw, D.D. (1991). Differences in sensitivity of Kenyan *Trypanosoma vivax* populations to the prophylactic and therapeutic actions of isometamidium chloride in Boran cattle. *Tropical Animal Health and Production.* 23: 29-38.

22. Uilenberg G (1998). Chapter 3: Diagnosis. In: *A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis*. FAO, Rome, 59- 86.

23. Rottcher, D and Schillinger, D. (1985). Multiple drug resistance in Trypanosoma multiple drug resistance in *Trypanosoma vivax* in the Tana River District of Kenya. *Veterinary Record*, 117:557-558.

24. Ainanshe, O.A., Jennings, F. W and Holmes P. H (1992). Isolation of drug resistant strains of *Trypanosoma congolense* from the lower Shambelle region of southern Somalia. *Tropical Animal Health and Production*, 24: 65-73.

25. Authie, E. (1984). Mise en evidenced`ure resistance aux

trypanocides parmi des souches de trypanosome congolense recemment isolees au Burkina. *Revue d'elevage et de medecine veterinaire des pays tropicaux,* 37: 219-235.

26. Pinder, M and Authie E. (1984). The appearance of isometamidium resistant *Trypanosoma congolense* in West Africa. *Acta Tropica,* 41: 247-252.

27. Anene, B.M., Ogbuanya, C. E., Mbah, E. S and Ezeokonkwo R. C. (1999). Preliminary efficacy trial of cymelarsan in dogs and mice artificially infected with *Trypanosoma brucei* from dogs in Nigeria. *Revue d'elevage et de medecine veterinaire des pays tropicaux,* 52 (2): 123- 128.

28. Whiteside, E. F (1960): Recent work in Kenya on the control of drug-resistant cattle trypanosomiasis. In: Proceedings of the Eighth Meeting of the International scientific Council for Trypanosomiasis Research. CCTA publication No.62, Jos, Nigeria, pp. 141-153.