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Efficiency assessment of trypanocidal treatments in the research station of Avetonou in Togo

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ABSTRACT

In Togo as elsewhere, among the animal trypanosomosis control strategies, the chemotherapy constitutes the most used method. In order to assess the efficiency of two trypanocidal drugs (isometamidium chloride and diminazene) on cattle, a longitudinal study was conducted from September to November 2011 in the research station of Avetonou located in the Prefecture of Agou in Togo. A parasitological survey was carried out on two groups of 90 cattle each (for a total of 180 cattle) using the buffy coat technique at regular intervals of two weeks over a total period of 56 days. The animals of the first group were treated with isometamidium chloride (0.5 mg/kg) and the second group (untreated animals) was used as a control group. The comparison of the incidence of trypanosomosis between the two groups was performed using three statistical tests: the Chi-squared test, the test of risk Reduction, and the "Eisler ratio test". These analyses revealed an inefficiency of the preventive treatment with isometamidium chloride at the dose of 0.5 mg/kg bodyweight contrarily to the curative treatment that was effective. For diminazene, used at a dose of 3.5 mg/kg bodyweight, strains of *Trypanosoma vivax* and *Trypanosoma congolense* were not eliminated in 14.29% of cases.

Keyswords: Trypanocidal treatment, isometamidium, diminazene, Avétonou, Togo.

INTRODUCTION

Bovine trypanosomosis remains one of major constraint to agricultural development in sub-Saharan Africa. The diseases are caused by blood parasites called *Trypanosoma sp*. transmitted by tsetse flies, the main vectors. African animal trypanosomosis (AAT) are present in the best land of Africa. In cattle, control methods involve either tsetse control or treatment of animals with trypanocidal drugs (diminazene and isometamidium chloride) in a therapeutic or prophylactic approach. The two trypanocidal drugs are accessible to breeders and veterinarians for their relative cheap cost in the market and low toxicity. Unfortunately, their widespread use, especially prolonged and incorrect, leads to the emergence of strains of multi drug-resistant trypanosomes whose consequence is that many cases of trypanosome infections cannot be prevented or cured in a sustainable way (Eisler et al.,

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2000; Chaka and Abebe, 2003). In West Africa, trypanocidal drug resistance has been reported in several countries, including Burkina Faso (Pinder and Authié, 1984; Clausen et al., 1992; McDermott et al., 2000; Talaki et al., 2007), Ivory Coast, Nigeria (Peregrine, 1994, cited by Geerts and Holmes, 1998), Guinea, Mali (Talaki, 2011), Ghana (Allegye-Cudjoe et al., 2009) and Benin (Vitouley et al., 2013). In Togo, cases of resistance to trypanocides were reported in the north of the country, in the savannah region and more specifically in the prefecture of Oti (Boma, 2012 cited by Talaki et al., 2013). In all cases, this phenomenon is probably underestimated because the situation is unclear in many places that either investigations have not been conducted, or the results of the studies are not published. It is in this context that this study was conducted at the Research Station of Avétonou/ITRA-Togo, following numerous deaths recorded in spite of trypanocidal treatments performed against animal trypanosomosis.

MATERIALS AND METHODS

Study area

The study area (Research Station of Avétonou) is located in Agou prefecture (Plateau Region) (longitude: from 0° 40' to 0° 55' East, latitude: from 6° 40' to 6° 55' North) and covers about 650 ha. The area is covered by savannah mixed with gallery forests. Herbaceous stratum, consisting of *Andropogon sp.* and *Panicum sp.*, dominates the vegetation. On hydrography plan, the Zio River located approximately 0.8 km northeast of the station, is with its tributaries, the most important river system. The climate is sudano-guinean tropical type and can be divided into two rainy seasons (from March to June and September to October) and two dry seasons (from July to August and from November to February). The area has 1.000 -2.000 mm of rainfall per year with large inter-annual variations. The landscape of Agou prefecture is essentially marked by Agou Mountain with a peak at 986 m. The main economic activities are agriculture and breeding. The study area has a favourable biotope for tsetse-infestation.

Longitudinal survey

Cattle of Avetonou station were monitored from 22nd September to 17th November 2011 to assess the resistance of trypanosomes to isometamidium chloride.

Two groups of cattle were formed: a treated group (with isometamidium) and a control group (untreated animals). In each herd, the choice of individuals belonging to treated group or control group has been done randomly according to the order of animals through the contention lane. All animals in the test group were treated with isometamidium chloride at a dose of 0.5 mg/kg body weight while only cattle detected positive in the control group were treated with diminazene dose 3.5 mg/kg body weight (curative dose). The day of treatment with isometamidium was considered as day 0 (starting point, D_0). Animals were monitored every two weeks using parasitological examinations by buffy coat technique (Murray et al., 1977). Thus, animals were examined five times on the dates D_0, D_{14}, D_{28}, D_{42} and D_{56} in both groups (Table 1) and the study period covered eight weeks.

Data statistical analysis

Data were entered in Microsoft Excel database. The resistance to isometamidium was evaluated by comparing the incidence of trypanosome infections in both groups (control and test group) using different statistical tests: Chi-squared test (Chi^2), Relative Risk Reduction (RRR) and "Eisler ratio test" (Eisler et al., 2000). The data were analysed according to the method of Talaki et al. (2007): (i) a numbering of new infections from D_{14}; (ii) a single entry of any animal tested positive during the corresponding monitoring period; (iii) any animal tested negative in previous controls and absent to the last control of the corresponding monitoring is not taken into account. It is then considered as a lost pet and therefore is subtracted from the effective monitoring; (iv) a consideration of any animal present on the last corresponding control monitoring even though it was irregular to previous checks.

Table 1: Longitudinal survey protocol.

Groups	D_o	D_{14}, D_{28}, D_{42} and D_{56}
Test group	- Parasitological examination by Buffy Coat Technique (Murray et al., 1977) - **Isometamidium** to all animals	- Parasitological examination by Buffy Coat Technique (Murray et al., 1977) - **Diminazene** only to positive animals
Control group	- Parasitological examination by Buffy Coat Technique (Murray et al., 1977) - **Diminazene** to positive animals	- Parasitological examination by Buffy Coat Technique (Murray et al., 1977) - **Diminazene** only to positive animals

RESULTS

Evaluation of treatment with isometamidium

Preventive effect

New infections were recorded in both groups between D_0 and D_{56}. In the test group (treated with isometamidium), the incidence was 32.58% (ie 29 new infections registered in 89 animals). In the control group, the incidence of trypanosome infections was 45.88% (ie 39 new infections on a really effective monitoring of 85 animals). The results of comparison between the two groups (over 56 days) using Chi^2 test are given in Table 2. The differences were not significant (Chi^2 value = 3.294) between the two groups (treated and untreated) reflecting the ineffectiveness of preventive treatment with isometamidium dose of 0.5 mg/kg body weight.

The results of "Eisler ratio test" are recorded in Table 3. The ratio of the risk of infection by dividing the risk in the control group and the one in the test group gave a value of 1.34. This showed the inefficiency of isometamidium at a dose of 0.5 mg/kg body weight, thus confirming the results of the previous analysis. Indeed, Eisler et al. (2000) have suggested that resistance to isometamidium can be suspected when this ratio is less than 2; which is equivalent to a reduction of the risk of infection in the test group of less than 50% compared to the control group.

Finally, the results of the Relative Risk Reduction (RRR) and its confidence interval at 95% are shown in Table 4. From the lower bound of the confidence interval (0.487), we determined the maximum rate of protection conferred by isometamidium (51.3%) and deducted the corresponding minimum failure rate (48.7%). The problem in this approach of analysis is the absence of a consensus threshold of failure rate from which we can conclude the presence of chemoresistance. Adopting 15% (Talaki et al., 2007) and even 25% (WHO, 2004) as the threshold value of failure rate from which we can conclude a resistance to trypanocidal drugs, it leads to inefficiency of preventive treatment with isometamidium at a dose of 0.5 mg/kg body weight.

Curative effect

The curative effect of isometamidium at a dose of 0.5 mg/kg body weight was evaluated by the rate of relapse on the fourteenth day post-treatment (D_{14}) in the test group. From a total of 20 positive animals treated on D_0, no cases of relapse were detected at D_{14}. In other words, the cure with isometamidium at a dose of 0.5 mg/kg bodyweight was effective.

Evaluation of treatment efficiency to diminazene

Only the animals in the control group were taken into account for this evaluation. Thus, positive animals, treated with diminazene (3.5 mg/kg body weight) on D_0 and having undergone a parasitological control the fourteenth day post-treatment were considered. Of the 21 animals treated at D_0,

only three have been tested positive on D_{14}, a rate of 14.29% post-treatment relapse. Taking 15% (Talaki et al., 2007) as threshold value, we cannot conclude with certainty ineffective therapy to diminazene since this failure rate of treatment (14.29%) is very close to the threshold value of 15%. With a threshold value of 25% (WHO, 2004) used for malaria control, we can conclude that the curative effect of diminazene was effective.

Table 2: Results of the Chi^2 test.

	Test group (ISMM)	**Control group**	**Value Chi^2**	**Conclusion (5% level)**
Number at D_0	90	90	3.294	Ineffective treatment (Difference is not significant)
Lost	1	5		
Number monitored	89	85		
Positive (new infections)	29	39		
Positive (%)	32.58	45.88		

Table 3: Results of "Eisler's Ratio Test ".

	Test group (ISMM)	**Control group**	**"Eisler's Ratio" value**	**Conclusion**
Number on D_0	90	90	1.34	Ineffective treatment
Lost	1	5		
Number monitored	89	85		
Positive (new infections)	29	39		
Positive (%)	32.58	45.88		

Table 4: Relative Risk Reduction (RRR).

	Test batch	**Control Batch**	**RRR**	**C.I**	**M.P (%)**	**Failure (%)**	**Conclusion**
Number Monitored	89	85	0.71	0.487-1.036	51.3	48.7	Ineffective treatment
Positive	29	39					
Positive (%)	32.58	45.88					

C.I: Confidence Interval M.P: Maximum Protection

DISCUSSION

Preventive effect of isometamidium

Drug resistance is defined as the heritable loss of susceptibility of a population of microorganisms previously sensitive to a molecule. The results of the three tests used (Chi^2 test, "Eisler's Ratio" and the calculation of the Relative Risk Reduction (RRR) and its confidence interval) showed that treated animals are also exposed to a high risk of infection. Hence, it appears that isometamidium chloride at a dose of 0.5 mg/kg body weight did not provide cross-protection of animals against trypanosomes for a period of three months. This ineffectiveness of preventive therapy to isometamidium had already been reported by some authors in other countries such as Burkina Faso, Guinea and Mali where isometamidium treatments at a dose of 1 mg/kg body weight was not sufficient to protect animals over a period of 56 days (Talaki, 2011). Indeed, prophylactic doses recommended for isometamidium chloride are ranging from 0.5-1 mg/kg body weight and the molecule has also shown remarkable activity in zebu cattle in East Africa (Trail et al., 1985; Moloo et al., 1987). Although significant changes in the duration of protection going from 2 to 22 weeks have been noted by many authors (Kirby, 1964; Pinder and Authié, 1984; Whitelaw et al., 1986; Peregrine et al., 1991), they seem not to depend upon the size of the risk of the disease, nor on the presence of infection nor on the time of treatment (Peregrine et al., 1988). In this study, could a treatment with 1 mg/kg body weight instead of 0.5 mg/kg provide protection for animals? The question remains open and deserves further investigation. Moreover, if the parasitological monitoring of animals by the method of Eisler et al. (2000) is the basic approach for evaluating the resistance of isometamidium on field, this method requires follow-up sessions with parasitological observations at regular intervals of 14 days, increasing the work time both for research teams and for farmers who have to spend a lot of time for control sessions. More the monitoring of animals is too long, less is the participation of animal's breeders and the number of dropouts during the experiments increases. When the dropouts become very important, data analysis will be complicated and sometimes it can lead to difficulties or impossibility to interpret results.

Curative effect of isometamidium and diminazene

The results of this study revealed an effective cure with isometamidium at a dose of 0.5 mg/kg bodyweight, unlike preventive at the same dose of the trypanocide treatment. Normally, diminazene is efficient at a dose of 3.5 mg/kg body weight on *Trypanosoma vivax* and *T. congolense* strains but, in the present study, we observed 14.29% of relapse. Indeed, for curative use, isometamidium chloride is used at the doses between 0.25 and 0.5 mg/kg body weight and was effective in the treatment of animal trypanosomosis (Petrovskii, 1974). For diminazene, the dose of 3.5 mg/kg body weight is sufficient to eliminate sensitive strains of *T. vivax* and *T. congolense* while *T. brucei* requires a higher dose up to 7 mg/kg body weight (Fussgänger and Bauer, 1958).

In this study, regarding the curative effect of the two trypanocidal drugs (isometamidium and diminazene), infections observed on D_{14} could be considered as relapses and those observed later as re-infections. The failure rates reported here were certainly underestimated because of the impossibility of taking into account the late relapses. Also, the technique of parasitological diagnosis and post-processing performed on the fourteenth day control (whether for isometamidium or diminazene), have certainly underestimated the failure rate considering the fact that the parasite diagnosis with BCT is not very sensitive. Further studies using PCR (*Polymerase Chain Reaction*) are needed because this technique is more sensitive than BCT and could increase parasitological

prevalence rate 2.5 times higher than the BCT method (Desquesnes et al., 1999).

Conclusion

Animal trypanosomosis is still a constraint to livestock development in the Avetonou station of the Institute of Agricultural Research of Togo. To control the disease in animals, two trypanocidal drugs are used: isometamidium chloride and diminazene. Results of this study showed ineffectiveness of prophylactic treatment with isometamidium chloride at a dose of 0.5 mg/kg b.w. Further study is needed to assess the prophylactic effect of isometamidium chloride with 1 mg/kg b.w. For curative effect, isometamidium showed an efficiency at a dose of 0.5 mg/kg b.w. For diminazene, the treatment failure rate recorded was in tolerable limits.

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