REPUBLIQUE DU SENEGAL

MINISTERE DE L'AGRICULTURE

INSTITUT SENEGALAIS DE RECHERCHES AGRICOLES (ISRA)

DIRECTION DES RECHERCHES SUR LES PRODUCTIONS ET LA SANTE ANIMALES

LABORATOIRE NATIONAL DE L'ELEVAGE ET DE RECHERCHES VETERINAIRES (L. N. E. R.V.) DAKAR-HANN

DETECTION AND CHARACTERISATION OF SUSPECTED DRUG RESISTANT TRYPANOSOME STRAINS IN SENEGAL USING BCT AND Ag-ELISA DIAGNOSTIC TECHNIQUES

by
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FIRST RESEARCH COORDINATION MEETING OF THE FAO/IAEA AFRICAN TRYPANOSOMIASIS NETWORK NAIROBI, KENYA, 7-11 FEBRUARY 1994
(I.A.E.A./I.S.R.A RESEARCH CONTRACT No 7596/RB)

RAPPORT N.002/PARHO. ANIM.
JANVIER 1994

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SUMMARY

Animal trypanosomiasis is a major livestock disease in the southern-central region of Senegal. *The* majority of the cattle *in* this area are of the *Diakme* breed (cross Zebu-Ndama). Because of their properties, these animals are widely used by the farming community for animal traction, but also for milk and meat production. Despite their reasonable level of trypanotolerance, many of these animals are affected by trypanosomiasis in the areas with significant tsetse challenge. For this reason, trypanocidal drugs such as Berenil and Samorin are being used on a large scale. Their use and particularly mis-use has lead to the occurence of drug resistant strains. In a previous study (1991-1992) carried out in this region, results were obtained to indicating this. The proposed study to be conducted in 1994, is to confirm these findings in greater details , using the BCT and Ag-ELISA, and to identify the most appropriate trypanocidal drug(s) to be used in the Sokone region. The project activities will involve the screening of approximately 1,000 *Diakme cattle in* the Sokone area, followed by the isolation of trypanosomes from infected cattle and their subinoculation into laboratory animals for subsequent chemotherapeutic trials with Berenil, Samorin and Ethidium. The results obtained will be provided to the Senegalese Ministry of Agriculture

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KEY WORDS

SENEGAL - BOVINE -TRYPANOSOMIASIS - DIAGNOSTIC - ELISA
TRYPANOCID - DRUG RESSISTANCE

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1. INTRODUCTION

The efforts to produce a vaccin against trypanosomiasis have failed so far, despite all subtantial funding and the high level of expertise inputs allocated to this for some decades throughout the world.

In view of this, the use of trypanocidal drugs remains the only alternative to treat trypanosomiasis affected animals. This has led to a wide spread use of trypanocidal drugs in those areas where the disease exists. However, the success of such chemotherapeutic treatment depends on specific criteria : identification of the causitive trypanosomes, correct determination of the animal's weight, correct use of effective drug. In field practice however, it is often difficult to meet those requirments. It is known that a frequently used suboptimal dosage (under-estimated weight, expired drug,etc.) may subsequently lead to the occurence of a resistance of the trypanosomes to the drug used. As a result, the ill-informed veterinary technicians will tend to increase the drug dosage with the risk of producing even more drug resistance and eventually even reaching the toxic level of the drug. The correct approach would be to abandon further use of that particular drug and to use an other effective sanative drug. Ifthis is not done, the occurence and subsequent distribution of such drug resistant trypanosome strains will eventually lead to heavy economic losses due to weight loss, loss in overall animal production and death. The range of trypanocidal drugs available to the veterinarian is very limited and the occurence of an eventual resistance to these few drugs will render all possible treatment in future ineffective.

In Senegal, the stock breeders in trypanosomiasis endernic areas regularly request the local veterinarians for trypanocidal treatrnent of their animals. The conditions under which these are often carried out form a permanent risk in the appearence of chemoresistant trypanosome s-trains. The majority of cattle in the Sokone region (southern-Central region) belong to the *Diakore* breed (cross Zebu-Ndama). These animals are widely used for both milk and meat production and animal traction. The region is almost entirely infested with ***Glassina morsitans submorsitans*** and G. ***palpalis gambiensis,*** vectors of *Trypanosoma brucei, T. congolense* and T. *vivax.* Since several years, the local veterinarians in the Sokone region have reported cases of ineffective trypanocidal treatments, in particular with Berenil, to a lesser extend with Samorin. The widespread use/mis-use of Berenil and Samorin in this region for many years could have resulted in creating resistant trypanosome strains for one or both of these drugs. Results of relevant studies carried out in the region during the period - 199 1-1992 indicated the possible presence of such trypanosome strains. These results will have to be confirmed by the isolation and identification of these strains following their subinoculation into laboratory animals and drug trials to determine the efficacy of the available trypanocidal drugs.

I.S.R.A./D.R.P.S.A./L.N.E.R.V., B.P. 2057, Dakar-Hann, Sénégal (on behalf of Dr. A DIAITE).

The proposed study will require the use of both specific and sensitive diagnostic techniques to enable the detection of possibly all infected animals. The combined use of the BCT and the Ag-ELISA proved to be satisfactory during ths 199 l-1992 study and will therefore be utilised again in this proposed study. In addition, sufficient funds shouid be available to carry out the proposed field visits required to verify the chemotherapeutic trials. Assistance will be requested from the FAO/IAEA's Joint Division of the IAEA to provide these fùnds for the study.

2. STUDY MATERIALS

- **Cattle :** 1000 from the Sokone area, from the villages Karang and Keur Aliou Guèye in particular, where suspected drugresistant strains were found in 199 l-92.

- **Mice :** 100 for subinoculation with T. *brucei* and *T. congolense* field strains.

- **Goats:** 10- 15 for subinoculation with T. *vivax* field strains.

- **Liquid Nitrogen: 2 X 20** litres for cryopreservation of trypanosome stabilates.

- **Diagnostics:** FAO/IAEA Ag-ELISA kit and materials for the serological diagnosis of *T. brucei, T. congolense* and T. *vivax.*

3. STUDY METHODS

The protocol used in 199 l-92 was the following:

- **First field visit (= Vi):** treatment with Berenil of all the study animals;

- **Visit** 2 **(V1 + 15 days):** subdivision of the animals into 3 groups:
  Group 1: Samorin treatment
  Group 2: Samorin treatment
  Group 3: No more treatment following V1

- **Visit 3 (V2 + 15 days):**
  Group 1: Ethidium treatment
  Group 2: No more treatment following V2
  Group 3 : No more treatment following V 1 and V2.

- **Follow up:**
  Regular visits at 1 month intervals for 5 months and recording of the parasitaemic or/and antigenaemic post-therapeutic persisting cases.
  Trial to obtain natural infection of 2 **sheep,** but finally cancelled due to lack of fùnds.

The above described study protocol was highly interesting, however, some problems remained to be investigated. The efficacy of Samorin and Ethidium could not be truely determined for the Berenil sensitive strains which were eliminated by the Berenil treatment

carried out during visit one. In addition, the use and properties of Berenil made it necessary to plan the second visit two weeks after the first. This period is too short to distinguish serologically between truly persisting infections and incompletely cleared parasite antigenaemia. It is for these reasons that the use of Samorin during the second visit, with a prophylactic period of approximately 3 months, would have been better.

The modified study protocol is therefore the following:

- A first study phase for the detection and isolation of suspected drugresistant strains.
- A second study phase involving the laboratory characterisation of the isolated strains, followed by field visits to treat the animals with the identified proper sanative drugs and verification of their efficacy.

3.1. Detection and isolation (See table 1)

- Visit 1: Screening of 1,000 cattle by BCT and Ag-ELISA in the villages where suspeded strains were found in 199 l-92. The serological and BCT results will identify the infected animals and the trypanosome species involved.

- Visit 2 (= Vi + 21 days): All animals found positive following V1 (envisaged 250-300) will be re-sampled for BCT and Ag-ELISA and treated with Samorin. Prior to treatment however, a number of these positive animals will be selected (on basis of trypanosome species and animal location) and bled for subsequent subinoculation into 1 O-l 5 goats (*T.* v.) or 100 mice (*T.b. T.c.*).

- Visit 3 (= V2 + 30 days): Sampling of all V1 positive animals for BCT and Ag-ELISA to determine the effect of Samorin.

- Visit 4 (= V3 + 30 days): Sampling of all selected V1 positive animals for BCT and Ag-ELISA. Based on the overall (BCT and Ag-ELISA) results of V3, blood fi-om those animals with persisting infections will be collected for subsequent subinoculations.

- End of the first part of the field work

3.2. Characterisation (see table 2)

During the two months following V4, chemotherapeutic trials will be carried out at the laboratory. It is envisaged that this will provide essential information on the nature and extend of possible drugresistance of the isolated strains, and the identication of an effective sanative drug. During each stage of these investigations, trypanosome stabilates will be stored in liquid nitrogen.

Following the identification of ths effective sanative drug(s). two field visits will further be made; the first visit to treat the animals with that drug(s), the second visit one month later to verify the efficacy of that treatment.

3.3. *Negative reference sera*

In addition to the collection of sera from the tsetse-infested Sokone region, some 100 bovine sera will be collected in a tsetse and trypanosomiasis fi-ee region in northen Senegal for subsequent screening by Ag-ELISA. The sampled animals will then be treated with Samorin and sampled again after one month. This will provide additional information for the verification of the specificity of the assay.

These above described investigations are planned for the first year of the programme. The results of this study will first of all be used to formulate recommendations for the Government of Senegal to prohibit the use of determined non effective drug(s) in the study area. It is envisaged however, that the findings could be used on a larger scale within the Panafrican Rinderpest Campain (PARC) activities carried in Senegal.

Table 1. Detection and isolation of suspected drug rcsistsnt strains

Visit	Duration	Team	Action
1	8 days	M.SEYE, A. MANE	Screening of 1000 cattle by BCT and Ag-ELISA
2	5	M. SEYE	**Subinoculations from positives of visite 1** **Samorin treatment +ve animals: expected 300 cattle**
3	5	M. SEYE	Post therapeutic verification (BCT and Ag-ELISA)
4	5	M. SEYE	**Subinoculation from persisting cases** Second post **therapeutic** verification
End of Isolation phase: starting of laboratory trials for 2 months			
5	5	M. SEYE	3rd post therapeutic **verification** **Treatment with adopted sanative drug**
6	5	M. SEYE	**Verification** of the V5 treatments

Table 2. Characterisation of suspected drugresistant field strains

Phase 1: Nature of the eventual resistance

GROUP	TREATMENT (Day 0)	BCT EXAMINATION (Day 0 + 24-48 h.)	SIGNIFICATION
1	BERENIL:10.5mg/kg	***Positive Tryps.***	**Resistance for 3erenil**
		Negative Tryps.	Sensible for this dose
2	SAMORIN:1.0mg/kg	***Positive***	**Resistance for Samorin**
		Negative	Sensible for this dose
3	ETHIDIUM:1.5mg/kg	***Positive***	**Resistance for Ethidium**
		Negative	Sensible for this dose

Phase 2: Level of sensitivity (concerns the strains with post therapeutic negative results)

1	BERENIL : 7.0 mg/kg	***Positive***	**Resistance / usual dose**
		Negative	Normal sensitivity
2	***SAMORIN: 0.5*** mg/kg	***Positive***	**Resistauce / usual dose**
		Negative	Normal sensitivity
3	ETHIDIUM: 1.0 mg/kg	***Positive***	**Resistauce / usual dose**
		Negative	Normal sensitivity

In case of resistance to the normal dose of a drug the **sanative drug** for the **concerned** strain will be that showing a normal **efficacy.**
If such normally effective trypanocid is not **found,** the trials **will** be **continued using** other compounds.
For each phase, a non- treated control group (Gr. 4) will be constitued.