Assessment in cattle under laboratory conditions of drug-resistance of *Trypanosoma congolense* field isolates from Zambêzia Province, Mozambique

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I Introduction

Animal African trypanosomosis is arguably the main disease affecting livestock in inter-tropical Africa. Control methods are based on either vector control, or chemotherapy. The latter mainly relies on two trypanocidal drugs, diminazene aceturate and isometamidium chloride, both introduced over sixty years ago, and the combination of inadequate dosage used by farmers and the presence of low-quality drugs on the African market, has resulted in resistance to trypanocides in many places.

The exact mechanism of drug resistance is still poorly understood, and thus no easy laboratory tests can be carried out to assess the resistance status of a given isolate. For this reason, in vivo experimentation is still the rule. Some field studies have been conducted, but the necessary follow-ups are difficult to perform in field conditions. *In vivo* assessment in laboratory conditions is thus often necessary to accurately measure the level of resistance. Although some tests have been developed using rodents, the murine model has limits and assessing resistance in the target species, ruminants, is often necessary.

Drug-resistance has long been reported in the Zambêzia Province of Central Mozambique. A survey of the area was carried out in 2014 by Mulandane et al.[1] as part of the TRYRAC project. Numerous cases of resistance were observed, to either one or both trypanocides, but the actual parasites were not isolated and corresponding strains were not characterized. Thus, whether strains are resistant to only one or both trypanocides and to what extent needs to be assessed in controlled conditions.

The aim of the study was to thoroughly assess the nature of the resistant strains. For this purpose, isolates were collected from field cattle that had reverted to parasitemia status following treatment. The isolates were used to infect experimental cattle under laboratory condition, and the animals were then treated and followed up.

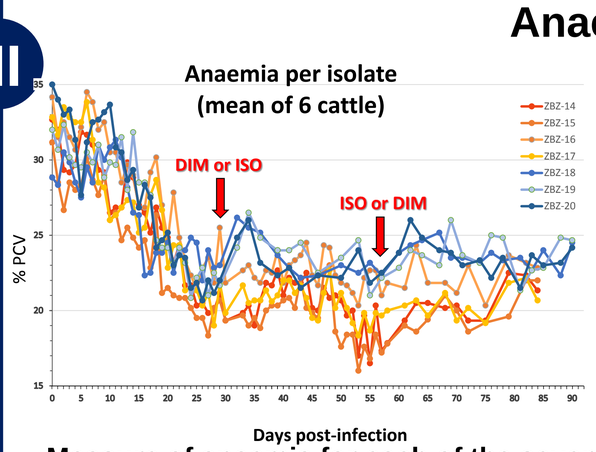
This study had two distinct goals. On one hand, we wanted to assess the extent of resistance of some of the circulating isolates in the area, as this has implications for control strategies. On the other hand, in the context of the quest for new trypanocides, well-characterized resistant field strains are an invaluable tool.

II Material and Methods

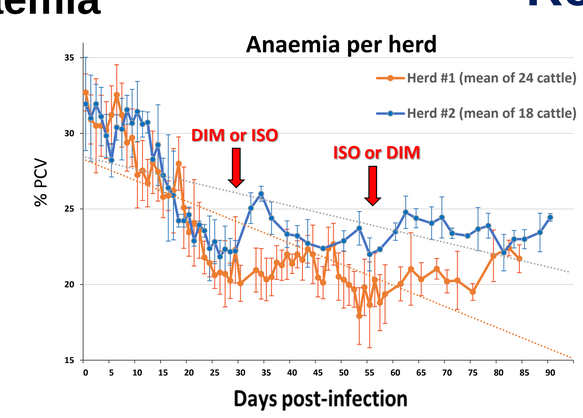
- Location: Nicoadala, Zambêzia, Mozambique
- Two cattle herds, one sampled in May 2016, the other in June 2016
- Cattle screened by Buffy-Coat technique in the field
- Positive animals treated with diminazene, resampled 7 days later
- Reverting animals treated with isometamidium, resampled after 7 days
- If still positive, blood shipped to Maputo. Seven isolates shipped
- Drugs used in the experiment courtesy of CEVA
- Shipped blood used to infect donor cattle at CB-UEM (one/isolate)
- Blood from each donor cattle used to infect 6 recipient cattle
- Cattle followed up daily for PCV, parasitaemia, temperature
- Weekly for weight gain, biweekly for clinical examination
- 3 cattle treated with diminazene, 3 with isometamidium
- If reversion, swapping of treatment
- Strict GvLP (Good veterinary Laboratory Practice)

III Results

Anaemia



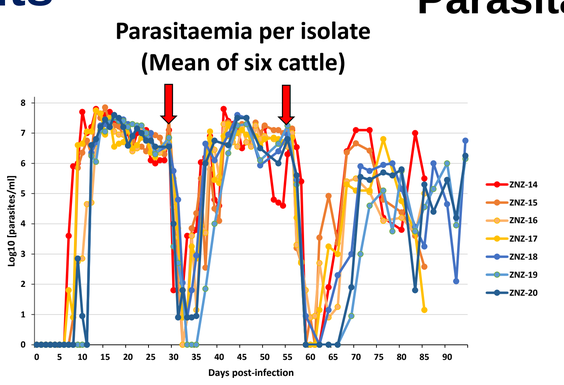
Measure of anaemia for each of the seven isolates, mean of 6 cows per isolate, irrespective of drug treatment.

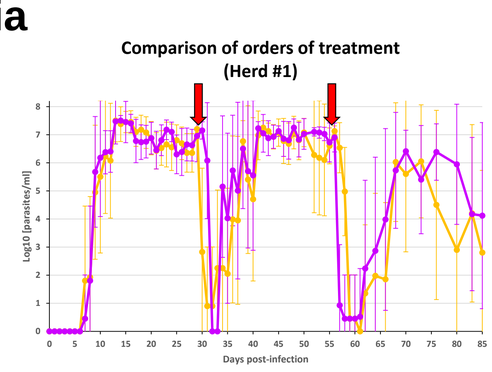


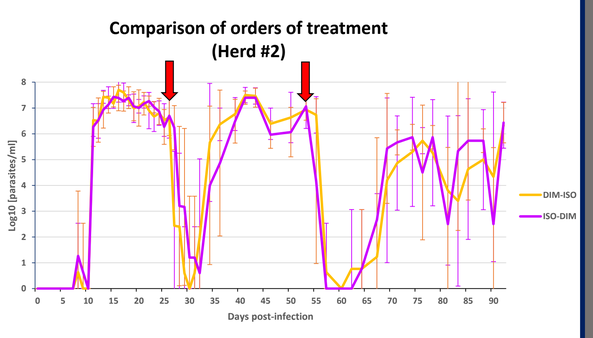
Measure of anaemia for isolates from two locations (herd #1 and #2) as mean PCV, irrespective of drug treatment.

Each of the 7 isolates behave as a classical, rather mildly pathogenic *T. congolense.* Isolates from the first herd/location appear somewhat more pathogenic than from the second herd/location. ZBZ-16 is somewhat intermediate. This argue for the presence of at least two, maybe three, genetically distinct strains. Treatments has no visible effect

Parasitaemia

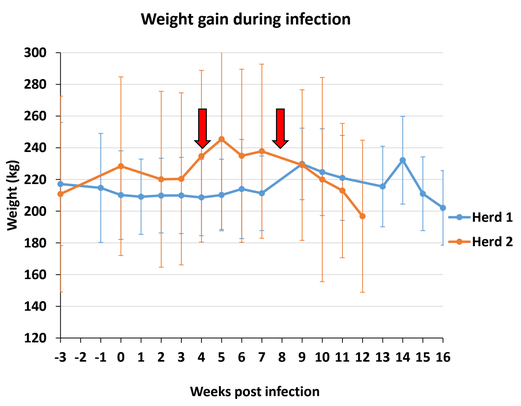






- **All stabilates behave in a similar way**
- **All are resistant to both drugs DIM & ISO**
- **Herd #1 isolates appear more resistant than those of herd #2 (quicker reversion)**
- **In most cases, but not always, animals become negative post-treatment**
- **Animals treated with DIM become aparasitaemic quicker and revert late than those treated with ISO**

Weight gain



- **Male *Nguni*, 8 - 18 months, 120 to 280 kg**
- **No drastic weight loss, strains of low pathogenicity**
- **No effect of treatments on weight**
- **One out of the 48 experimental animals died**

Drug-resistance

	Isolates	Prepatent period (**days**)	Number of **days post-treatment** before **negativation** (3 animals per group)				Number of **days post-treatment** before **repositivation** (3 animals per group)			
			DIMINAZENE 1st treatment (= group 1)	DIMINAZENE 2nd treatment (= group 2)	ISOMETAMID. 1st treatment (= group 1)	ISOMETAMID. 2nd treatment (= group 2)	DIMINAZENE 1st treatment (= group 1)	DIMINAZENE 2nd treatment (= group 2)	ISOMETAMID. 1st treatment (= group 1)	ISOMETAMID. 2nd treatment (= group 2)
Herd #1	ZBZ-14	7 to 8	1-Never-1	1-3-1	3-3-3	3-3-3	7-always-4	12-10-11	7-5-5	12-10-8
	ZBZ-15	8 to 9	2-2-1	1-1-1	3-3-2	3-3-2	4-7-9	7-12-Dead	5-5-5	4-6-5
	ZBZ-16	9 to 13	1-2-1	1-1-1	3-3-3	3-3-4	4-9-8	6-14-3	5-5-5	12-12-12
	ZBZ-17	7 to 9	Never-2-1	1-1-3	3-3-3	3-3-3	Always-7-7	7-9-8	5-5-7	10-12-14
Herd #2	ZBZ-18	12 for all	3-2-3	3-1-3	4-3-Never	3-3-6	8-6-5	10-15-15	8-8-always	13-12-14
	ZBZ-19	12 for all	1-1-1	3-1-3	4-2-3	3-3-3	13-13-10	15-17-13	13-13-10	15-22-17
	ZBZ-20	9 to 12	3-1-1	1-3-3	2-2-2	3-3-3	8-5-3	15-13-13	8-8-8	15-17-13

- **Herd #1 isolates have a longer prepatent period than those of Herd #2 (ZBZ-16 is again an exception)**
- **Animals clears their parasitaemia more rapidly with DIM than with ISO, from Herd #1 earlier than Herd #2**
- **Three animals, infected by distinct strains, never became negative, 2 after DIM treatment, 1 after ISO treatment**
- **All animals reverted post-treatment, but QUICKER:**
  - **1/ post-DIM treatment than post-ISO treatment**
  - **2/ if infected with isolates from Herd #1 (ZBZ-14 to ZBZ-17) than from Herd #2 (ZBZ-18 to ZBZ-20)**
  - **3/ after first treatment than after the secong, for both drugs**

IV Conclusion and Perspectives

We reported here the systematic characterization under laboratory conditions of seven drug-resistant *T. congolense* field isolates from Zambêzia Province, Mozambique. This work furthers a recent survey of the area, long considered as a hotspot for drug resistance. All isolates proved highly resistant to both the most commonly used drugs available on the market. Of particular interest is the finding that parasites actually disappear from the bloodstream for up to two weeks post-treatment, giving the false impression of cure, but in all cases came back in full force.

These isolates show rather mild virulence and pathogenicity, and proved unable to readily infect rodents, arguing for a trade-off between the mechanisms of infectivity and resistance. This may explain why livestock rearing continues is this endemic area despite the presence of seemingly an incurable disease.

In the current context of novel trypanocide drug discovery, these isolates could certainly prove instrumental in new molecule testing.

In the meantime, there is no doubt these findings raise a challenge in terms of disease control strategy in the area, and beyond. An integrated approach combining public awareness about rational use of trypanocides with vector control should be implemented. Still, the area of origin of these isolates is rather circumscribed, and while it is unlikely to be an isolated case, a wider survey should be considered. Likewise, the genetic relationship between these geographically connected isolates must be investigated.

V Reference

1. Resistance to trypanocidal drugs in cattle populations of Zambezia Province, Mozambique (2018). Mulandane FC, Fafetine J, Van Den Abbeele J, Clausen PH, Hoppenheit A, Cecchi G, Oosthuizen M, Delespaux V, Neves L.. Parasitol Res. 2018 Feb;117(2):429-436. doi: 10.1007/s00436-017-5718-1.