



TRYRAC-Trypanosomosis Rational Chemotherapy
Mission Report
Training WP 4 activities in the Guraghe zone, Ethiopia
February 22- March 6, 2015



Training session in Wolkite, February 25, 2015, ©Hoppenheit

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List of Abbreviations

AAT	African Animal Trypanosomoses
ARD	Global Programme on Agricultural Research for Development
BCS	Body Condition Score
CAHWs	Community-based Animal Health Workers
CBOs	Community-Based Organizations
DA	Diminazene Aceturate
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FUB	Freie Universitaet Berlin
EpG	Eggs per Gram feces
EU	European Union
FECRT	Faecal egg count reduction test
<i>G.</i>	<i>Glossina</i>
ISM	Isometamidium Chloride
ITM	Institute of Tropical Medicine Antwerp
ITN	Insecticide Treated Nets
LUH	Leibniz Universitaet Hannover
NAHDIC	National Animal Health Diagnostics and Investigation Center
NARS	National Agricultural Research Systems
NICETT	National Institute for Control and Eradication of Tsetse and Trypanosomosis
PCV	Packed Cell Volume
RDU	Rational Drug Use
STEP	Southern Tsetse Eradication Project
<i>T.</i>	<i>Trypanosoma</i>
TRYRAC	Trypanosomosis Rational Chemotherapy
VSF	Vétérinaires sans Frontières Belgium
WP	Work Package

1. TRYRAC-project background and objective

Trypanosome Rational Chemotherapy, TRYRAC, is part of the EU-funded initiative “Global Programme on Agricultural Research for Development” (ARD) that supports agricultural research for development on a global basis. TRYRAC is an international cooperation of academic, governmental and non-governmental organizations and has the aim of optimizing African Animal Trypanosomosis (AAT) management in western, eastern and southern Africa- represented by Togo, Ethiopia and Mozambique. TRYRAC started in March 2012 with a total funding period of 5 years.

The major constraints to optimal AAT management are ineffective chemical treatments due to limited accessibility of quality trypanocides, gaps in smallholder knowledge and restrictions in the detection of trypanocide resistance to the only two available drugs isometamidium chloride (ISM) and diminazene aceturate (DA).

That is why the main objective of TRYRAC is supporting livelihoods of resource-poor livestock producers in smallholder production systems in sub-Saharan Africa through improving the efficacy of trypanocidal drugs for the control of human and animal trypanosomosis. Specific objectives are 1) detecting trypanosome resistance 2) support drug quality control in African laboratories 3) improving the efficacy of trypanocides through promotion of rational drug use and development of adapted disease control methods.

2. Best-bet strategies for Ethiopia

Best-bet strategies are optimizing trypanocidal efficiency and control drug resistance [1]. They comprise selection of effective veterinary products, the promotion of rational drug use, affordable vector control and improving animal health conditions in general, for instance by introducing strategic deworming. Supporting extension messages should be disseminated throughout the study area by national agricultural research systems (NARS) and private veterinarians. Towards the end of the project, a socio-economic impact assessment is planned to be conducted by the Leibniz Universität Hannover (LUH).

The most promising strategies for Ethiopia have been determined after an investigative fact finding mission that took place in November 2013 by FUB to before-identified AAT hotspot regions where trypanocidal resistance/s occurred. These strategies are summarized in Figure 1 and they are planned to be implemented and monitored during 24 months in the vicinity of 4 community-based organisations (CBO's): Misreta, Borer 4&5 and Wolaita.

Demonstration Herds		
study and control groups in 3 – 5 herds per CBO		
Rational Drug Use	Targeted Spraying	ITN
Identification of quality drugs	Establishment of crush pens and committees	Establishment of committees
Application to clinically diagnosed animals	Spraying beginning at the onset of the rainy season at bi-monthly intervals	Protection of selected kraals towards the second half of the rainy season
Longitudinal biological Monitoring: thoracic perimetre, FAMACHA test, EpG	Tick count pre-treatment and follow up 14 days post-treatment	Questionnaire surveys on perception of biting insects prior to and 4 weeks after protection
Block treatment study at the end of the intervention phase		

Figure 1. Outline of best-bet strategies (ITN in grey was excluded later)

CBO: Community Based Organisation, FAMACHA: Faffa Malon Chart [2,3], EpG: Eggs per Gram faeces

FUB, Vétérinaires sans Frontières Belgique (VSF) and the Ethiopian team (National Animal Health Diagnostics and Investigation centre=NAHDIC) invited stakeholders such as farmers, governmental/private vets and pharmacists to a meeting in Wolkite that took place in April 7/8 2014. There, outcomes of the so far conducted studies regarding drug quality tests, AAT prevalence, dissemination strategies and best-bet strategies were introduced to and discussed with the stakeholders. It was agreed to launch WP 4 activities in June 2014. Protocols for field interventions and monitoring were developed by the FUB team in close collaboration with ITM. Protocols and mission reports are available in the partner area of the TRYRAC website: <http://www.trypanocide.eu/>. After implementation failed in June 2015 (quote report), an extra Project Management Committee was held in October 2014 in order to decide on how activities were to be continued in Ethiopia [4]. It was decided to initialise rational drug use of trypanocides, anthelmintics and insecticides in the study villages as soon as possible. However, Livestock Protection fences were dropped from the agenda. Another training and implementation mission was planned for February 23 until March 06 2015 in order to treat, sample and analyse one or two study herds together with the NAHDIC/NICETT team to launch the best-bet protocol. NICETT, the National Institute for Control and Eradication of Tsetse and Trypanosomosis is replacing STEP (Southern Tsetse Eradication Project) as TRYRAC partners in Ethiopia.

3. Objective and schedule of the WP 4 implementation mission

- Introducing the best-bet protocol to the newly assigned technicians
- Training the use of mini-FLOTAC chambers [\[5\]](#) for fecal egg counts
- Training trypanosome diagnostics with the Woo-chamber
- Apply and train WP 4 protocol on 1 study herd (clinical inspection, fecal sampling, fecal egg counts, sedimentation, trypanocidal treatment)
- Transferring the Larval packet Test kit to the student and secure a laboratory with skilled personal for the conduction (Dr. Solomon Gebre, NAHDIC)
- Establish contacts between the Albendazole contact person: Dr. Eshetu and the Ethiopian TRYRAC team
- Visit the Ethiopian Drug Control Authority to facilitate the release of SMASH for animal usage
- Develop an obligatory schedule for the student until the end of the project

Table 1. Initial timetable for the second training and implementation mission in Ethiopia 23.02.-05.03.2015

Date	Time	Activity	Participants
Sunday, 22/02/2015	16:00	Berlin/Antwerp to Addis	
Monday, 23/02/2015	07:00	Arrival, resting, structural meeting ITM/FUB, visit officials	AH, VD
Tuesday, 24/02/2015	08:00-18:00	Meeting to plan the mission, travel to Wolkite	AH, VD, TC, TT
Wednesday, 25/02/2015	08:00-18:00	Set up the lab in Wolkite, test run of few blood and fecal samples to train examination, planning the field sampling of the next day, meet the farmers of Hudad and Borer 4 to prepare them for the samplings	AH, VD, TT, Technicians
Thursday, 26/02/2015	08:00-18:00	Blood and fecal sampling and recording of the health status of study animals 1-50; blank herd with DIM 7 mg/kg; analysing blood samples	AH, VD, TT, Technicians
Friday, 27/02/2015	08:00-18:00	Examine fecal samples of animals 1-30/training mini-FLOTAC technique of newly assigned technicians	AH, VD, TT, Technicians
Saturday, 28/02/2015	09:00-15:00	Finish fecal analysis, develop an action schedule for the PhD student; training in literature research and statistical analysis and planning Larval Packet Testing	AH, VD, TT,
Sunday, 01/03/2015		Resting	
Monday, 02/03/2015	08:00-18:00	Collecting blood and fecal samples; examination of blood samples of study animals 51-100	AH, VD, TT, Technicians
Tuesday, 03/03/2015	08:00-18:00	Examine fecal samples of animals 51-100 ; meet the radio manager of Wolkite 89.2	AH, VD, TT, Technicians
Wednesday, 04/03/2015	13:00	Travel back to Addis	AH, VD, TT
Thursday, 05/03/2015	variable	Appointment with the Ethiopian Veterinary Drugs and Feed Administration (and control authority); meet Dr. Eshetu, the Albendazole contact person of Vétoquinol	AH, VD, TC, TT, TC
Friday, 06/03/2015	01:20	Flight to Paris-(Berlin/Antwerp)	AH, VD

Participants:

Antje Hoppenheit (AH), FUB; Vincent Delespeaux (VD), ITM; Tilahun Tekle (TT), NAHDIC; Thomas Cherenet (TC), NICETT; Technicians who will perform fecal analysis by mini-FLOTAC chambers and trypanosome diagnostics (Woo method), NAHDIC/others; drivers

4. Initiating best-bet-strategies

4.1. Synopsis

The connection flight from Paris to Addis Abeba was missed, so the arrival of Dr. Hoppenheit was postponed to Tuesday morning. Upon arrival, the mission and future activities were planned and the team left for Wolkite. A training session for blood and fecal sampling and analysis of the two newly assigned technicians Ayalew and Tesfaye took place in Wolkite on Wednesday. Further mini-FLOTAC and Woo-chamber training was assured by analysing fecal samples of the study animals. Two sampling sessions took place in order to sample 100 animals of Borer 4. Farmers were met before each sampling for ensuring good collaboration which was eagerly provided at all times. Examinations, treatment, sampling of blood and feces as well as diagnostic routines were conducted during two days per sampling unit of 50 animals.

Future activities of the Ethiopian team were planned for the period of March 2015 until July 2017. Several key people were met: an agreement was reached between Wolkite based radio station 89.2 and the TRYRAC team. A proposed program will be sent to the manager Mr. Wolde; he will then assign a journalist to record and finalize the program to be broadcasted.

In order to secure the access to insecticidal spraying formulations and liquid Albendazole, the Drug Administration and Control Authority and Dr. Eshetu's, the Vétuquinol import partner were contacted.

4.2 Insecticidal Spraying and deworming

Decatix by Cooper will not be imported in the near future due to complicated import procedures and because of the low quantity that is needed for the project. Thus, another Deltamethrin spraying formulation that is suitable for animals, SMASH, was organised with the support of Tagros chemicals of India Ltd. Currently, the shipping is held back at the airport and formalities have to be settled with the Drug Administration and Control Authority.

Since this training session is situated in the dry season, insecticidal spraying and anthelmintic treatment is not scheduled. However by June, when insecticidal spraying and anthelmintics will be crucial, the products should be available. Visits were paid to the Drug administration and Control Authority and to the Vétuquinol partner Dr. Eshetu in order to assure access to both drugs for the next rainy season.

4.3 Mini-FLOTAC and sedimentation training

Since new technicians were assigned to assist with fecal diagnostics, another training session was scheduled. Three animals were sampled at the veterinary office in Wolkite to train laboratory procedures.

The PhD student Tilahun Tekle and three technicians from the public veterinary service (Bogale), the Animal Drug and Feed and Residue Quality Control Laboratory (Ayalew) and NAHDIC (Tesfaye) were attending the training session.

One sample was prepared by Antje Hoppenheit for demonstration purposes while two following samples were prepared by the Ethiopian team under close supervision. Further, assembling, cleaning and maintaining the chambers was explained and shown to all of the present technicians. Two risible instruction sheets showing a) the mini-FLOTAC protocol and b) a diagnostic key with photographs of helminth eggs that can be found in bovines were used.

Six samples were pooled for demonstrating the sedimentation technique; the procedure was done with tap water in beakers and finally in petri dishes. Due to dry weather conditions during the sampling period, sedimentation was abandoned after the training.

Microscopic investigation of the mini-FLOTAC chambers and petri dishes was demonstrated by Antje Hoppenheit. All samples were then checked for helminth eggs by two veterinary diagnostic

technicians Tesfaye and Ayalew and, for further practice, the PhD student. The practice samples were found negative by all examiners.

Further training was conducted by analysing study herd samples. The first 25 samples were double checked by both technicians, Tilahun and Dr. Hoppenheit with the following results:

Table 2. Results of the herd sampling for 25 animals

Sample ID	Animal ID	EpG result investigator Ethiopia	EpG result investigator FUB
1	1	A: GIN 190; Eimeria +	GIN 300; Eimeria +
2	3	T: GIN 40	GIN 35
3	4	A: GIN 250; Eimeria +	GIN 260; Eimeria +
4	5	T: GIN 15; Eimeria +	-
5	6	T: GIN 15; Eimeria +	GIN 35; Eimeria +
6	7	A: GIN 340	GIN 340
7	8	A: GIN 110; Eimeria +	GIN 100; Eimeria +
8	9	T: GIN 5; Eimeria +	GIN 5; Eimeria +
9	10	A: GIN 40; Eimeria +	GIN 40; Eimeria +
10	11	T: GIN 100	GIN 160
11	12	A: 0; Eimeria +	0; Eimeria +
12	13	T: GIN 10	GIN: 5
13	14	T: 0; Eimeria +	0; Eimeria+
14	15	A: GIN 0	GIN 0
15	16	A: GIN 160	GIN 150
16	18	A: GIN 45	GIN 45
17	19	T: GIN 30	GIN 30
18	20	Ti: GIN 15	GIN 250
19	21	A: GIN 130	GIN 130
20	22	A: GIN 210	GIN 200
21	23	A: GIN 20	GIN 20
22	24	T: GIN 10; Eimeria +	GIN 15; Eimeria +
23	25	T: GIN 35; Eimeria ++	GIN 65; Eimeria ++
24	26	A: GIN 0; Eimeria +	GIN 0; Eimeria +
25	27	A: GIN 90; Eimeria +	GIN 100; Eimeria +
26	29	A: GIN 50; Eimeria +	GIN 50; Eimeria +
27	30	T: GIN 40	GIN 40

Legend:

ID: Identification; EpG: Eggs per gram feces; FUB: Freie Universitaet Berlin; GIN: gastro intestinal nematode eggs; Investigators: A: Ayalew; T: Tesfaye; Ti: Tilahun

After the training sessions on Wednesday and Friday the technicians Ayalew and Tesfaye were found able to perform future fecal analysis by the mini-FLOTAC technique. The technicians Abraham and Bogale were found able to prepare and clean the mini-FLOTAC chambers. Remaining samples were analysed on Saturday in the presence of Dr. Hoppenheit.



Figure 2. Preparation and microscopic inspection of fecal samples using the mini-FLOTAC chamber

4.4 Training trypanocide diagnostics by the Woo-method

As one regular Woo examiner of the transversal study is not available for the implementation of the best-bet strategy, a new examiner was trained (Tesfaye). The training included: organisation of the blood samples for centrifugation, PCV reading, mounting centrifuged clean capillary tubes in the Woo chamber [6] and microscopic examination (microscope settings including adequate contrast and magnification, strategy for efficient parasite detection). Capillary duplicates are examined by the trainer and trainee until concordant diagnosis is reached.

4.5 Larval Packet (LPT) and FlyBox Tests

The material was transferred to the NICETT office in Addis and the sealed filter papers are being kept at 4°C until usage. Also, the adjusted LPT protocol was transferred to the student. Dr. Solomon Gebre, who was once trained at the FUB, will supervise the student for the LPT at the NAHDIC lab in Sebeta. Unfortunately, he could not be met but a visit to his laboratory is scheduled for the upcoming mission in June 2015. Then, extensive training of the student in tick identification and conduction of the LPT will take place. FlyBox tests will be conducted by Dr. Hoppenheit.

5. Herd sampling

When the farmers of Borer 4 were met, they expressed their enthusiasm about continuing TRYRAC activities. Part of the investigation team is assigned to the public veterinary service, so it was agreed that farmers could bring in sick animals to be treated by the service once the herd is sampled. After sampling in Borer, farmer leaders of Hudad were met in order to prepare them accordingly for the upcoming sampling period.

Thus, on Thursday, it was scheduled to sample 100 animals in Borer 4. However, after initial difficulties with lacking material and a broken generator it was decided to reduce the sampling number to 50 and to conduct blood diagnostics in the laboratory.

In the afternoon, blood samples were processed until the centrifuge overheated after having four samples (negative). Trypanocidal diagnostics had to be abandoned and a new centrifuge was organised on Sunday. PCV's of the remaining 46 animals that were sampled on Thursday were recorded. Fecal analysis was trained and conducted on Friday and Saturday. Due to the time-consuming diagnostic routine it was decided to stick to 50 animals per day for future samplings.

On Monday the remaining 50 animals of Borer were inspected, ear-tagged and sampled. Diagnostics were conducted in the same manner as the previous batch.



Figure 3. Blood sampling in Borer 4

6. Future TRYRAC activities in Ethiopia

Another meeting took place in the NICETT office in order to plan and discuss the work schedule and other important issues:

Due to the additional techniques that are planned for the rainy season such as insecticidal spraying, Larval Packet and FlyBox testing, another training and implementation visit of Dr. Hoppenheit is planned within the timeframe of June 13 till 23, 2015. This visit will be joined with the annual PMC meeting in Wolkite. An exact date will be determined by Vincent Delespaux through doodle. Technical issues such as the over-heating centrifuge and a defect microscope were discussed. NICETT will replace the defect material.

Further, the training mission of Tilahun Tekle in Pretoria was planned and modalities discussed. It will take place between April and June 12, 2015. Shipping of the trypanosome positive DNA to the FUB will be organized between Pretoria, the ITM and FUB.

Ethiopian trypanosome stabilates will be transferred to the ITM for long-term cryo-preservation. Based on this discussion, a schedule was prepared for future activities of the student that comprise WP 4 (best-bet strategy) activities, a training of molecular techniques in Pretoria, South Africa and the annual PMC meeting 2015 in Addis Ababa (Figure 4).

Regarding WP 4 actions during the short rainy season it was discussed with the PhD student if deworming and insecticidal spraying should be scheduled: depending on weather conditions and egg counts and depending on the perceived fly pressure, a decision should be made before the mission of February 2016.

Year	2015							2016										
Month	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	
Season	dry			begin of rains				end of rains			dry		short rainy season		dry		begin of rains	
Implementation WP4	Finalize animal identification, inspection, FAMACHA score, blanking with 7 mg DIM/kg body weight, fecal and blood analysis with 5 hotspots and two control villages (100 animals each)	Ensure treatment and recording of sick animals during absence of PhD student	Study animals routine inspection, FAMACHA score, blood and fecal sampling and analysis, tick counts and insecticidal spraying, rational drug use: curative treatment 7 mg DIM/kg; deworming; <u>Extra</u> Egg count reduction test (return after 14 days for another fecal sampling and tick count and spraying); Larval Packet Test; FlyBox Test <u>Control animals</u> inspection and sampling as above; treatment of animals with a PCV below 24			Ensure treatment and recording of sick animals during absence of PhD student		Study animals routine inspection, FAMACHA score, fecal sampling/analysis, rational drug use: deworming; treating sick animals only with 7 mg DIM <u>Control animals</u> inspection and sampling as above; treatment of animals with a PCV below 24		Ensure treatment and recording of sick animals during absence of PhD student		Study animals inspection, FAMACHA score, rational drug use: (deworming), treating sick animals only with 7 mg DIM, (insecticidal spraying if necessary) <u>Control animals</u> inspection as above; treatment of animals with a PCV below 24	Ensure treatment and recording of sick animals during absence of PhD student		Study animals routine inspection, FAMACHA score, blood and fecal sampling and analysis, tick counts and insecticidal spraying, rational drug use: curative treatment 7 mg DIM/kg; deworming; <u>Extra</u> Final block treatment study, return after 14 days for another tick count and spraying and block treatment result and after 21 days for the final block treatment result <u>Control animals</u> inspection and sampling as above; treatment of animals with a PCV below 24			
Molecular Analysis Training						?												
PMC meeting 2015				beginning-mid June														

Figure 4. Schedule for TRY RAC activities of the PhD student

7. Visiting key people

Radio manager in Wolkite

For negotiating radio transmission as a means of extension messages, the manager of Wolkite 89.2, Mr. Ato Nigate Wolde, was met. He pointed out that there was no format of charging a broadcast regardless of the minutes on air. Additionally, he stated that there was no experience in developing programs or recording interviews.

Conclusively, it was agreed upon sending him a proposal draft of extension messages for his estimation of the work time a journalist has to spend for finalizing the spots. Accordingly, a per diem will be calculated for an appointed journalist who will travel with the team to record the messages in the field. Messages and questions will be prepared by FUB and VSF and send to the Ethiopian team in order to translate it into Amharic and Guraghe before sending the proposal to Mr. Wolde.

The Drug Administration and Control Authority

Dr. Terzu was contacted through Thomas Cherenet in order to secure access to the spray formulation as soon as possible.

Ethiopharma

The Vétoquinol contact person in Addis and manager of Ethiopharma, Dr. Eshetu, was met. It was discussed how liquid Albendazole could be made accessible to the TRYRAC team first and then, in the long run, to the farmers. He stated that a decade ago it was attempted to import liquid Albendazole but that the acceptance amongst farmers was perceived as low. So the import was abandoned and obtaining another permit may take up to a year.

It was then decided to ask for an initial import of a low quantity for research purposes. This product will at least be included in the egg count reduction protocol. If results are promising, further import could be enabled.

The Dr. Hoppenheit will estimate the amount needed by the project and then issue an official document in collaboration with Dr. Delespaux. Further, Mutu Ngalamulune (Vétoquinol, Paris) will be contacted for free samples of liquid Albendazole.

8. Summarizing conclusions for future missions

Best-bet strategies could be launched in one of the study herds, new techniques were transferred and key personal were identified and trained for future activities. Acceptance of the project work amongst farmers greatly improved and the mission was well prepared. Further, a deltamethrin spray formulation and liquid Albendazole are in the pipeline for the upcoming rainy season when they will be needed. Therefore, contacts need remain active until the products are at hands. Collaboration with radio Wolkite 89.2 was renewed. As soon as possible radio messages have to be prepared by FUB and VSF in order to establish a contract for the journalist involved in broadcasting. All in all, this mission was conducted successfully. The next field visit for implementing insecticidal spraying and strategic deworming in rainy season is scheduled for mid-June 2015.

9. Acknowledgements

We would like to thank everyone who facilitated this mission. First of all we are grateful for the help of Thomas Cherenet and Tilahun Tekle who organized the mission at field level and who were able to animate the farmers for a fruitful collaboration. We also like to thank the technicians Ayalew, Tesfaye, Terekegn, Abraham and Bogale for providing their skills and for the quick uptake of new techniques. Also, we are grateful to the district veterinarian of Wolkite for offering his outpost for the conducted laboratory analyses. After all, this mission would not have been possible without the eager support of the village managers and farmers of Borer and Hudad.

We were shocked to hear that Solomon, the village manager of Borer 4, had died in a motorbike accident. This report is dedicated to him.

8. References

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9. Annex

List of people attending the mission and other important contacts

Name	Affiliation	Charge	Contact
Vincent Delespaux	ITM	TRYRAC-coordinator	vdelespaux@itg.be
Antje Hoppenheit	FUB	Post-Doc WP 4 (best-bet strategies)	antje.hoppenheit@fu-berlin.de
Thomas Cherenet	NICETT	TRYRAC country co-ordinator	
Tilahun Tekle	NAHDIC	PhD student	
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Legend

ITM: Institute of Tropical Medicine; TRYRAC: Trypanosomosis Rational Chemotherapy; WP: work package; NICETT: National Investigation Center for the Eradication of Tsetse and Trypanosomosis; NAHDIC: National Animal Health Diagnostic and Investigation Center