



Report NWO-ARF project “Healthy Cows – Healthy Food – Healthy Environment: Enhancing safety and quality of milk in Ethiopia with a focus on antibiotic residues”

**Laboratory skills training + NLF meetings
Wageningen, 29 October – 3 November 2018**



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1. Introduction

In the framework of the NWO ARF Project “Healthy cows - Healthy Food – Healthy Environment: Enhancing safety and quality of milk in Ethiopia with a focus on antibiotic residues” laboratory staff from VDFACA visited RIKILT a week for training on milk quality analysis. The training took place from 29 October – 3 November 2018. The project staff worked further on the project and made some visits in the Netherlands. This report describes in part 1 the content and the experiences of the laboratory training, and in part 2 the experiences from the project management team.

2. Part I RIKILT Training

RIKILT has organized a training in laboratory techniques for milk quality investigation, including somatic cell count, antibiotic screening and screening for aflatoxins. The training took place between October 28 - November 3, 2018.

The hosting unit was the RIKILT-Wageningen University & Research which is partner in the project; and the travel, accommodation, and associated costs were covered through RIKILT.

2.1 Set up and program

The training was set up and delivered by dr. Mariel Pikkemaat, supported by Hannie Vastenburger.

From Ethiopia the participants were:

- from the Ethiopian Institute of Agricultural Research (EIAR): Miss Adey Melesse Yalew
- from the Ministry of Agriculture and Livestock Resources (MoALR), Veterinary Drug and Feed Administration and Control Authority (VDFACA) : Dr Samuel Aytenfsu Desta, Livestock products and feed physio-chemical quality testing team leader;
- Dr Fanuel Fikiremaria Teferi, expert/technician, Livestock products and feed physio-chemical quality testing,
- Mr Tewahdo Teshome Amare, team leader, Livestock products and feed microbiological quality testing;
- Dr Misrak Netsere Haileyesus, expert/technician, Livestock products and feed microbiological quality testing;
- Dr Hussen Bedu Kewo, Technology testing expert; and
- Dr Belachew Tefera Zerihun, Quality control centre Director

Schedule training Ethiopia dairy project 29 Oct - 2 Nov 2018

	Morning 09:00 – 12:00 hr	Afternoon 13:00 – 17:00
Monday 29-Oct Room 0017	T: Introduction on RIKILT, introduction of the participants and trainers, scope and aims of the training, explanation of the project T: Milk quality testing in NL	T: Introduction Total Bacterial Count P: Total Bacterial Count
Tuesday 30-Oct Room 0017	T: Introduction Somatic Cell Count testing P: Preparation samples SCC	T: Introduction into antibiotic testing P: Calculation and preparation antibiotic control samples

Wednesday 31-Oct Room 0017	P: Antibiotic screening: DelvoTest (start) P: SCC analysis (discussion microscope)	P: Assessment and evaluation DelvoTest results P: Confirmatory antibiotic testing T: Introduction on aflatoxin analysis
Thursday 01-Nov Room 1042 (afternoon)	P: Start DelvoTest + confirmation with blind samples P: Evaluation TBC results T: Alternative methods	P: finish DelvoTest + confirmation with blind samples T: Antibiotic testing- legislation T: Confirmatory antibiotic analysis (incl. labtour)
Friday 02-Nov Room 1042	P: SSC analysis (individual counting) P/T: unfinished subjects	Summarizing/Evaluation the training, discussion on how to proceed
T: Theory - P: Practical		

2.2 Day 1 Training introduction

Mariel gave a presentation on milk quality control. In the following discussion the subjects below were discussed:

- Increased trend of selling of raw milk directly at the farm in NL; in Ethiopia majority of milk is sold as raw milk, risk mainly with Bovine Tuberculosis and Brucellosis
- NL exports 80% of milk production (mainly in form of cheese) – but at the same time also imports milk (e.g. organic milk and milk products from Germany) – due to risk management of dairy industries
- History of very strict AB controls in NL – due to cheese making (+ export pressure).
- In Ethiopia big challenge e.g. aflatoxin residues, which after the research results were made public has largely influenced consumer behaviour.
- Perception amongst public consumers that powdered milk are of high quality – Need to implement methods to identify adulterated powdered milk. More potential exists to convert liquid milk to powder, in Ethiopia due to fasting period. Important to include this. Also in-official import of powdered milk NIRS (near infra-red spectroscopy) combined with- PLS-DA model (Partial Least Squares Discriminant Analysis) to compare genuine vs adulterated milk powder)
- Laboratories for food quality control in Ethiopia: bringing different food quality labs together would be better under ministry of health rather than ministry of agriculture. Cattle feed quality control better under ministry of Agriculture? Better combined – One Health system?
- Long term: linking up with longer term collaboration on food quality control

2.3 Laboratory training day 2-5

Focus of the training was primarily on milk quality parameters such as:

- Total bacterial count (TMC) – according to ISO 4833
- Somatic cell count (SCC) – according to ISO 13366
- Antibiotic residues – using Delvotest, and follow up with lateral flow

Basic parameters – Lactostar and Aflatoxin M1 – lateral flow were only briefly dealt with, because Lactostar is essentially operational in the laboratory already and Afla M1 test is similar to the antibiotics lateral flow test and the Charm method currently running in the laboratory for feed analysis.

Fresh milk samples taken from 5 individual cows, kindly supplied by an acquainted farmer, were used throughout the training. Based on prior recordings, two out of five samples were expected to have increased somatic cell count. Samples for SCC were stabilized using 1:1000 of 20% bronopol preservative.

Total Bacterial Count (TBC)

First a theoretical background and explanation of total bacterial or microbial count was given. The parameter should be considered as a hygiene indicator, and can be effectively influenced by improvement of management practices. The ISO was explained, stressing to apply it in a pragmatic approach. TBC is performed according to ISO 4833 pour plate method, a simplified protocol was introduced. In brief, the analysis requires plate count agar (PCA) supplemented with skimmed milk powder, which was on this occasion prepared from PCMA dehydrated culture medium, but can also be composed from individual ingredients. The agar is sterilized and cooled down to 47°C. Serial dilutions of the samples are prepared by the participants, of which 1 ml is mixed with ~15 ml of the agar in a petri dish and left to solidify.

The analysis requires 72 hours of incubation at 37°C, so the results were assessed by the participants on Thursday morning. According to the ISO 4833 at least two duplicates of two subsequent dilutions (countable, < 300 CFU) should be included in the data processing. Results showed this is not always feasible (overgrowth by swarming bacilli or yeasts) and alternatives were discussed (partial plate counting). The results showed a partial coincidence of high TBC with the (expected) high SCC.

Interestingly, the one sample containing bronopol that was included in the sample set, still showed high TBC, but was devoid of the interfering overgrowth. Bronopol is added to prevent bacteria from multiplying, but seems hardly to affect the viability. Since field trial logistics (cooling, time to analysis) may have to be compromised upon, the possibility of using bronopol preserved samples for TBC should be investigated. Finally, some alternative (high-end) methods for enumeration and identification of microorganisms were presented: TEMPO (automated most probable number method) and MALDI-TOF (microbial identification).

Somatic Cell Count (SCC)

On Tuesday Somatic Cell Count was introduced with a presentation on the relevance of this parameter in relation to udder health and mastitis. SCC is performed microscopically, according to ISO 13366 – Enumeration of somatic cells. The ISO was explained: milk is spread over a predefined surface on a

slide to form a smear, which is dried and stained. Subsequently, stained cells are counted and multiplied by a working factor yielding the number of cells per millilitre. It was particularly emphasised that data acquisition and processing is dependent on microscope specification and slide lay-out.



Lab training in progress



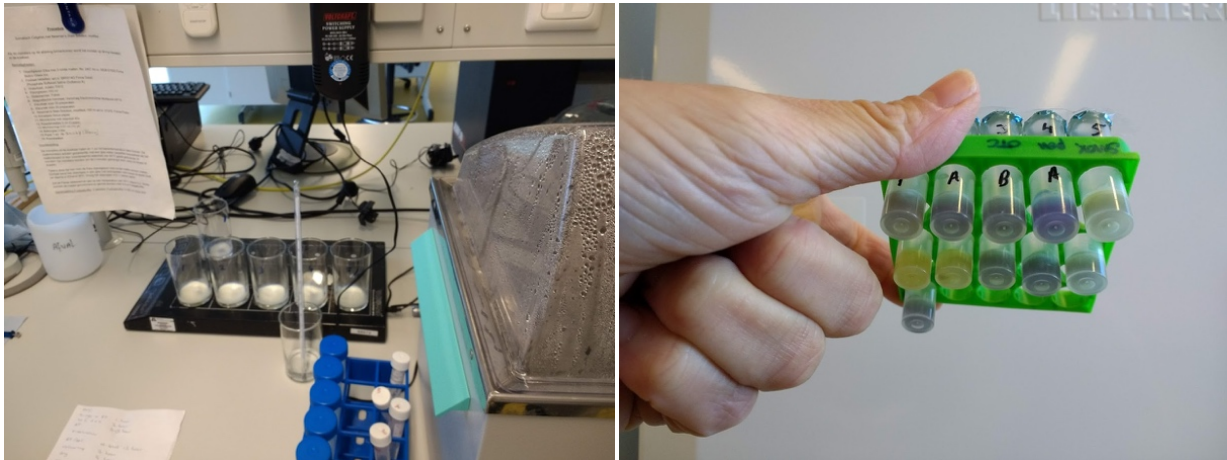
The visual aspects of stained somatic cells were introduced. A simplified protocol was provided for sample preparation and counting. Sample prep itself was started with help of Ms Hannie Vastenbureg. Preparation of the so called “smear” involves staining, washing and several drying steps, yielding countable samples only the next day. Each of the participants prepared their own glass slide with “smear”, to learn how to evenly distribute the milk sample in the predefined area. The actual assessment of the smears prepared by the participants and some additionally prepared slides was performed on two separate occasions, on Wednesday using a microscope coupled to a video screen and Friday using a discussion microscope, allowing for parallel analysis and judgement of the results. Emphasis was put on how to distinguish different type of somatic cells from other cells (bacteria, yeasts) and staining debris.

To support this, participants were introduced to a pdf library with a broad range of visual examples, collected collaboratively by RIKILT and ANSES. The library is included in the electronic supportive material made available by the end of the training. Data processing was performed according to the custom made excel used by RIKILT, and the absolute requirement to adjust it to local specifications was elaborated upon. It was agreed upon that once local specifications are established, RIKILT will provide an adapted excel for SCC calculation. Participants expressed additional interest in microscopic feed composition analysis, for which RIKILT has a lot of expertise and an extensive reference library of feed ingredient materials.

Antibiotic residues monitoring

Antibiotic residue monitoring was introduced with a presentation on veterinary use of antibiotics and its consequences, touching upon associated legislation, registration procedures, monitoring, RASFF etc. The possibilities for detection were introduced, essentially distinguishable in microbial, biochemical and analytical chemical approaches. Pros and cons in terms of detection spectrum, sample throughput and costs of the different methods were discussed. The monitoring strategy to be used in

the project, a two-tier approach starting with Delvotest, followed by a group confirmatory lateral flow test, was discussed. It was explained this is not a fully comprehensive approach in terms of detection spectrum, but was chosen as the most feasible approach given the limited budget and time available for implementation.



Milk control training

Specific attention was given to proper calculation of control samples to secure the performance of the applied methods. Participants performed some theoretical exercises, which were put in practice when actual control samples were prepared. These samples were successfully used for a first round of practical antibiotic screening on Wednesday. The experiment effectively illustrated the importance of closely monitoring the actual length of the incubation of the Delvotest, as extension of the incubation period may result in failure to identify positive samples that cause only delay in bacterial growth. The lateral flow test used (Twinsensor) is capable of detecting beta-lactam antibiotics and tetracyclines, this will be expanded with sulfonamides (Trisensor) when analysing the actual field samples in the project.

The next day the participants were provided with a set of blind samples and were asked to judge these samples using the techniques introduced to on the previous day. Two additional confirmatory “tricks” were introduced, allowing for distinction of beta-lactam and sulfonamide from non-beta-lactam and non-sulfonamide in the Delvotest. The results clearly illustrated the limitations of the chosen approach (limited detection of aminoglycosides, quinolones and colistine) and the required scrutiny with respect to (claimed) detection limits. A major limitation of the use of a microbial growth inhibition method that should be kept in mind when analysing the project samples is that this test may also be vulnerable for preservatives and disinfectants, as was shown with the bronopol preserved sample which yielded a ‘suspect’ analysis result with Delvotest.

The RIKILT online training on antibiotic residue detection was demonstrated (accessible through www.scantest.selamat.net) and discussed. Finally, some alternative (high-end) methods were presented and discussed. All presentation materials, protocols, SCC pictures library and calculation sheet, and other supporting information were provided in electronic format on a flash drive at the end of the training.

2.4 Agreement on way forward with VDFACA

On day 5 the whole group discussed with Mariel how to follow up.

1. **Sampling** – procedure to be established by RIKLIT in collaboration with VDFACA. Plan for sampling procedure will be made for approval. Check: needs to be compatible with the analysis procedure.
 - o Procedure to be followed as used at VDFACA
 - o Who: Adey has experience with sampling.
 - o Take sample on-farm: from total milk of each farm – every 6 months (note Getachew: Sampling interval of six months can be revisited. I think we need to do sampling during the dry period and the wet period in the year. The exact time in the dry season and wet season can be recommended by VDFACA)
 - o Equipment for sampling – from the VDFACA laboratory. In case items like cold boxes and accessories e.g. car charger for the cold box, RIKILT through the project can provide. VDFACA to make inventory of sampling equipment and indicate what they have and what they need soonest.
 - o Transport to laboratory – milk should not be frozen
 - o Laboratory: should be ready to receive the samples
 - o If changes: all needs to be noted down
2. **Shipment of materials**
 - o VDFACA to make complete list of materials required
 - o What is available and what needs to be sent from outside
 - o Requirements for the shipment – how to arrange this?
3. **Responsible person VDFACA: Belachew**
 - o Make plan and inventory, list of materials required
 - o Importing will be easy because VDFACA has own staff operating at the port of entry -Addis Airport
4. **In-kind contribution VDFACA: Belachew**
 - o Proof of time spent and materials used for co-funding
5. **Other interest – other training needs identified**
 - o Mycotoxin confirmatory methods – in cattle feed, meat and honey: through SNV project or directly with embassy, in NL through NVWA – Ministry
 - o NIRS : infra-red spectroscopy – feed composition analysis available. Possible to use/adapt it for milk powder analysis – as well as other foods. Belachew to send specifications of the equipment
 - o Microscopic feed composition analysis – mixing of different oil seeds, adulteration of rape seed in cattle feed
 - o Meat residue analysis – antibiotics, hormones etc
 - o In time: herbal product quality analysis

2.5 Evaluation of the week

- o Mariel: positive the way the group is learning and interacting
- o Samuel: training as expected, only physical chemical part was not included, confirmation methods. Appreciate motivation of Mariel, Maria, Katrien
- o Tewahado: happy with the training – interesting, practical experience. Mariel positive in all the training. Thanks to Maria for support in Frankfurt delay. Thanks for ESAP also.
- o Hussein: Thank for the training, captured many knowledge from RIKILT, in Ethiopia use it now.
- o Fanuel: Motivated to accomplish more with the knowledge, we will work hard
- o Misrak: training interesting
- o Adey: well organized, have accomplished much in short period, very intensive – would be nice to have more time for sight-seeing, creating network with VDFACA
- o Belachew: clear picture of project background, milk quality control techniques, Dutch dairy development, friendship approach – we will make realise this project, everything is clear to use, we will make our contribution
- o Getachew: there has been commitment and discipline, happy to be able to come to one of the best labs of EU – important milestone in the project. Young people now trained will be moving up and mentoring others. Ministry has selected the right people for this training, would have loved to have more women
- o Daniel: we worked on project planning, reported to our donors with positive feedback – influence and make change. Encouraged with the NLF network – like work in Uganda.

3. Part II ARF project + NLF meetings

This part describes the work of the project team dr. Getachew Gebru, dr. Daniel Temesgen (ESAP), drs. Katrien van 't Hooft (DFE) and dr. Maria Groot (RIKILT Wageningen University & Research) during the week that the VDFACA staff was enjoying the laboratory training on milk quality.

3.1 Day 1 General discussion on project

This day was filled with a general discussion on the project by the whole group, after a summarizing presentation from Katrien. Main conclusions were:

- 90% milk produced in Ethiopia from local breeds
- Antibiotics residues hardly in local breeds/antibiotic residues mainly in crossbreeds, practically 0 in pastoral systems
- Resistance against antibiotics (AMR) is a major issue, in next project? This is not covered in the current project
- VDFACA: has sample from milk in market, packed milk processed from local farmers value chain (already included into project). This is of interest to the ministry and consumers (preventing situation of aflatoxin)
- Local breeds – need to measure antibiotic residues and AMR
- FAO: national AMR program – Getachew is to get in touch with the contact person
- Project addresses solutions for antibiotic residues, though this is not linked (yet!) to regulations of Ethiopian government
- Urban areas: common use of medicinal plants in dairy farming
- SNV collaboration: bentonite trial, medicinal plants? To be discussed.
- Discussion on cattle feed: feed quality needs to be a central focus but also feed production needs to increase.
 - o Production of oil cakes has changed. There is an increasing trend of exporting intact oil seeds, thus causing a drop in availability of oil cakes and the availability of these to local farmers is very low
 - o Importation of oil has also become a disincentive for local oil processors, and local seed oil processing plants are closing, and this adds to the scarcity of oil cakes
 - o Salesmen buy up crop residues and sell at higher price to farmers
 - o Livestock Master Plan clearly shows projected deficit in cattle feed availability by 2030 in good, bad or average rainfall years. Feed needs attention.
 - o Linking producers with sugar cane processing plants so as to make best use of the sugar processing by-products including molasse, bagasse, and sugar cane tops)
- Discussion on breeding – most producers are using local bulls for breeding. With insemination service from the government there is no risk of inbreeding, but risk of uncontrolled crossbreeding. No choice of breed type for farmers for insemination. This could be changed.

3.2 Day 2 - Visit to Hanneke Jellema-Hansma

Goal of the meeting: share information on activities of Hanneke in Ethiopia and possible links with ARF project (also present veterinarian Jan vd Zee).

Outcomes – agreements:

- a. Hanneke is involved in various dairy projects and training activities in Ethiopia, e.g. linked to Dairy Campus – and has done sampling and analysis of milk for antibiotic residues in rural communities for 3 years, but needs funds to continue this. She does not want to share the outcomes at this moment.

- b. Hanneke aims to do her PhD – Getachew/Daniel can be co-supervisors and offer to support Hanneke while in Ethiopia. Maria can try support Hanneke in finding professor for PhD – though difficulty due to major costs involved.
- c. Bring on board Hanneke for training on animal management to reduce calf mortality, to be conducted in February 2019 so it coincides with Hanneke’s planned travel to Ethiopia. Maria to follow up on this arrangement, talk to Hanneke and we plan from there.
- d. Role Jan: can be linked to herd health monitoring plans of Minister. Jan/Katrien to develop concept note on this to be sent to GG and Daniel



Visit to Hanneke

3.3 Day 2 - Visit to farmer Sjoerd and Janna Miedema

In this meeting were also present farmers Ton Spijkerman, Klaas de Lange and Hanneke Jellema-Hansma. Goal of the meeting was to review the effects of ‘export of Dutch knowledge’ in international dairy development, and to identify joint actions to include experiences related to natural livestock farming.

Outcomes – agreements:

- a. Our main goal: Increase animal health – and by doing so reducing the use of antibiotics and other chemicals, leading to improved environment as well as quality of animal products for human consumption.
- b. **Focus:**
 - Challenging dominant influence of western/Dutch conventional experiences, knowledge and products on international dairy development
 - Sharing lessons learnt in Dutch dairy farming at international level
 - Awareness raising – more trust in and support of local production systems, including
 - o Local farm and cattle management
 - o Medicinal plants – ethnoveterinary medicine
 - o Local breeds and crossbreeding strategies
 - o Local marketing
- c. **Possible activities** can be (to be further developed by Janna-Katrien):

- Exchange related
- o Continue the veterinarian-farmer exchanges – important to listen, look and learn from each other
- o Important to find the right people to talk to and connect to big organizations
- o Organize ourselves as a group and exchange experiences
- PR related
- o Make film – video
- o Strategic conferences
- o Newsletters in local languages
- o Interviews with famers in NL – Storytelling as a tool

- d. **Internal platform influencing** – each within own network
- o Ton: talking to Agriterra, LTO, GD
- o Hanneke: just start with young farmers between NL and Ethiopia
- o Getachew and Daniel: during ESAP yearly meeting
- o Maria/Katrien: with ARF project in Ethiopia and SNV project Uganda

- e. **Scientific proof**
- o Sjoerd – to meet up with scientists – to have data for scientific proof

3.4 Day 4 - Visit NWO-ARF office – the Hague

Meeting with Sonja Döpp and Wiebe Smit (ARF), Getachew, Daniel, Maria, Katrien project team.

Some conclusions:

1. The rapidly changing (political) situation in Ethiopia has led to several adjustments in financial control, budgeting and start of activities. Getachew acknowledges the ability of ARF to agree to these necessary adjustments.
2. There is no room for no-cost extension of the project beyond Oct. 1 2020, due to the fact that ARF has to close the entire ARF project by Jan. 2021.
3. We can adapt the budget within the budget lines according to the changing situation. Only in case of changes between the budget lines the approval of ARF (Soja Dopp). Maria to send her adapted budget.
4. Sonja and Wiebe are impressed with recent developments within the project, especially:
 - a. The pressing need and urgency of milk quality as a topic
 - b. The possibility to include the topic of aflatoxin residues (besides antibiotic residues) - in training of lab staff and possibly in collaboration with SNV in Bentonite trial at community level
 - c. The link with SNV to collaborate already started, through donations on milk cans, and possibly other activities in the near future
 - d. The possibility to include the NLF experiences in Uganda on herbal tick control
 - e. The direct link with the Minister of Agriculture, which will facilitate the upscaling of outcomes of the project
5. Important to keep posting project results and messages on both ARF site and site of Business and Knowledge Platform

3.5 Day 5 - Meeting on way forward

NLF Meeting agreement on way forward, the following items were discussed.

1. Budget

Maria will indicate budget adaptations to Sonja for approval

2. Procedures

- *Questionnaire* – to be done in November – formalised by the end of the year
A publication can be made on basis of that
- Katrien: Simple poster of 5-layered approach – explaining the five layers in English, which can be translated. To be printed in Addis both in English and 3 Local languages (Amharic, Oromifa, Tigrigna)
- Planning animal health interventions: January 2019
- Katrien: Prepare list of possible interventions that are going to be used to measure impact, and that can be able to produce results during the project period. Also Katrien will develop the indicators for each interventions that we can measure at the start and end of the project.
- Bring on board Hanneke for training to prevent calf mortality, to be conducted in February 2019 so it coincides with Hanneke's planned travel to Ethiopia. Maria to follow up on this arrangement, talk to Hanneke and we plan from there.

3. Base line analysis milk quality:

- Prepare procedure milk sampling – discuss with Adey, make a list of what is required
- Talk to the State Ministry on how best to be sending research materials to Ethiopia. Best would be sending materials needed for the milk sampling and analysis directly to VDFACA.
- On package indicate research material or donation – get suggestions from Ministry/VDFACA
- Explore ways of aligning milk sampling with that of Hanneke's milk sampling procedure done at farm level, but outside the project areas.
- Hanneke: involved in calf management – 1 field visit to carry out farmer training in February 2019, timing to fit her planned travel to Ethiopia for other activities

4. Workshop SNV in Uganda, NLF meeting and workshop herbal medicine

These will be combined in February 2019

- Communities: Combine with Hanneke calf management training
- Communities: Nair/Punniamurthy – visit medicinal garden, review

5. ESAP mid-term conference: organized on the theme of awareness raising and new approaches to antibiotic resistance and milk quality

- Katrien/Elizabeth/Robert Uganda NLF experience on herbal tick control
- Maria: antibiotic resistance problem
- Include Bentonite and ask Taffese to present
- Gondar University participation (Student of Punniamurthy who is an assistant professor in Gonder University)
- Nair/Punniamurthy – on herbal medicine

6. Communication

- App call every first Wednesday of every month – 10.30 NL time, 12.30 Eth time

7. Publications

- o Papers
- i. Training report milk quality training – additional training on meat quality control for export ?
- ii. community survey animal health
- iii. Interventions 5-layers:
- iv. milk quality – end of the project
- v. workshop proceedings – febr 2019

8. SNV – proposal for joint activities:

- o Bentonite trial to reduce aflatoxin effects
- o Herbal gardens – trials



Enjoying the visit to the Netherlands: Daniel, Getachew, Adey and Katrien