Veterinary extension on sampling techniques related to heartwater research

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ABSTRACT

Heartwater, a tick-borne disease caused by *Ehrlichia ruminantium,* is considered to be a significant cause of mortality amongst domestic and wild ruminants in South Africa. The main vector is *Amblyomma hebraeum* and although previous epidemiological studies have outlined endemic areas based on mortalities, these have been limited by diagnostic methods which relied mainly on positive brain smears. The indirect fluorescent antibody test (IFA) has a low specificity for heartwater organisms as it cross-reacts with some other species. Since the advent of biotechnology and genomics, molecular epidemiology has evolved using the methodology of traditional epidemiology coupled with the new molecular techniques. A new quantitative real-time polymerase chain reaction (qPCR) test has been developed for rapid and accurate diagnosis of heartwater in the live animal. This method can also be used to survey populations of *A. hebraeum* ticks for heartwater. Sampling whole blood and ticks for this qPCR differs from routine serum sampling, which is used for many serological tests. Veterinary field staff, particularly animal health technicians, are involved in surveillance and monitoring of controlled and other diseases of animals in South Africa. However, it was found that the sampling of whole blood was not done correctly, probably because it is a new sampling technique specific for new technology, where the heartwater organism is much more labile than the serum antibodies required for other tests. This qPCR technique is highly sensitive and can diagnose heartwater in the living animal within 2 hours, in time to treat it. Poor sampling techniques that decrease the sensitivity of the test will, however, result in a false negative diagnosis. This paper describes the development of a skills training programme for para-veterinary field staff, to facilitate research into the molecular epidemiology of heartwater in ruminants and eliminate any sampling bias due to collection errors. Humane handling techniques were also included in the training, in line with the current focus on improved livestock welfare.

Keywords: *Ehrlichia ruminantium,* livestock welfare, molecular epidemiology, sampling precision, veterinary extension.

Steyn H C, McCrindle C M E, Du Toit D **Veterinary extension on sampling techniques related to heartwater research**. *Journal of the South African Veterinary Association* (2010) 81(3): 160–165 (En.). Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa

INTRODUCTION

The rickettsial agent *Ehrlichia ruminantium* is the causative agent of the disease known as heartwater and is transmitted to domestic and wild ruminants by the tick *Amblyomma hebraeum*[7,8]. *E. ruminantium* is an obligate intracellular parasite that infects endothelial cells[7]. Traditionally, extensive cattle, sheep and goat farming systems have been the central focus of livestock production in South Africa. Because of exposure to *A. hebraeum* ticks in such grazing systems, heartwater is regarded as one of the most important diseases in the region[1,17,24].

In South Africa, veterinary extension has changed since 1994 from a dualistic service to a single amalgamated service to facilitate the control of animal diseases and thus increase productivity of previously disadvantaged, small-scale livestock farmers. State veterinary services cooperate with researchers, private veterinarians, agricultural extension services, farmers and farmers' organisations[9,19,21].

Previously the diagnosis of heartwater depended mainly on the detection of clinical signs and a *post mortem* brain smear. The IFA and enzyme-linked immunosorbent assay (ELISA) tests, used on serum from live animals, have a low specificity for *E. ruminantium* organisms, as cross-reactions with antibodies against *Anaplasma* and other *Ehrlichia* spp. can occur, resulting in false positives[1]. These techniques allowed for the diagnosis of outbreaks and determination of endemic areas, but were insufficiently sensitive to allow for epidemiological studies. A new diagnostic assay (qPCR) developed to detect the organism in the blood of infected animals and ticks, together with the *in vitro* culture of new genotypes, facilitate molecular epidemiological studies which should contribute to better control of heartwater and the development of a more effective vaccine[22]. It is recognised, however, that the sensitivity of any diagnostic test can be considerably reduced by poor sampling techniques[23].

Most animal disease surveillance and monitoring was previously based on serological tests and the antibodies required for diagnosis are relatively stable. However, *E. ruminantium* is an extremely fragile organism; it dies 6 hours after an animal has died and will lose its infectivity when exposed to the sun for only 5 minutes, to a depth of 1 cm, in a blood collection tube[4]. Although the DNA may still be identifiable using the new qPCR, *in vitro* culture of the genotype to test its virulence and cross-protection capacity will not be possible.

Animal health technicians (AHTs) work under the supervision of the state veterinarian and are well-trained professionals who constitute an integral part of the veterinary team to produce high-quality animal health care and public education. They are responsible for restraining and handling animals (cattle, sheep and goats), collecting blood samples, tick identification, vaccination of animals and are involved in dipping programmes for the control of ticks and tick-borne diseases (Veterinary and Para-veterinary Act of 2000). A pilot study, however, showed that AHTs lacked the knowledge and skills to collect blood and tick samples correctly for a research study on the epidemiology of heartwater based on identification of the organism. A possible solution for this problem was specific skills training of the AHTs. Existing Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI) extension material was found to be suitable for training farmers in the recognition of heartwater and understanding of its importance. The ARC-OVI also has preserved samples and illustrations available

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Received: May 2010. Accepted: July 2010.

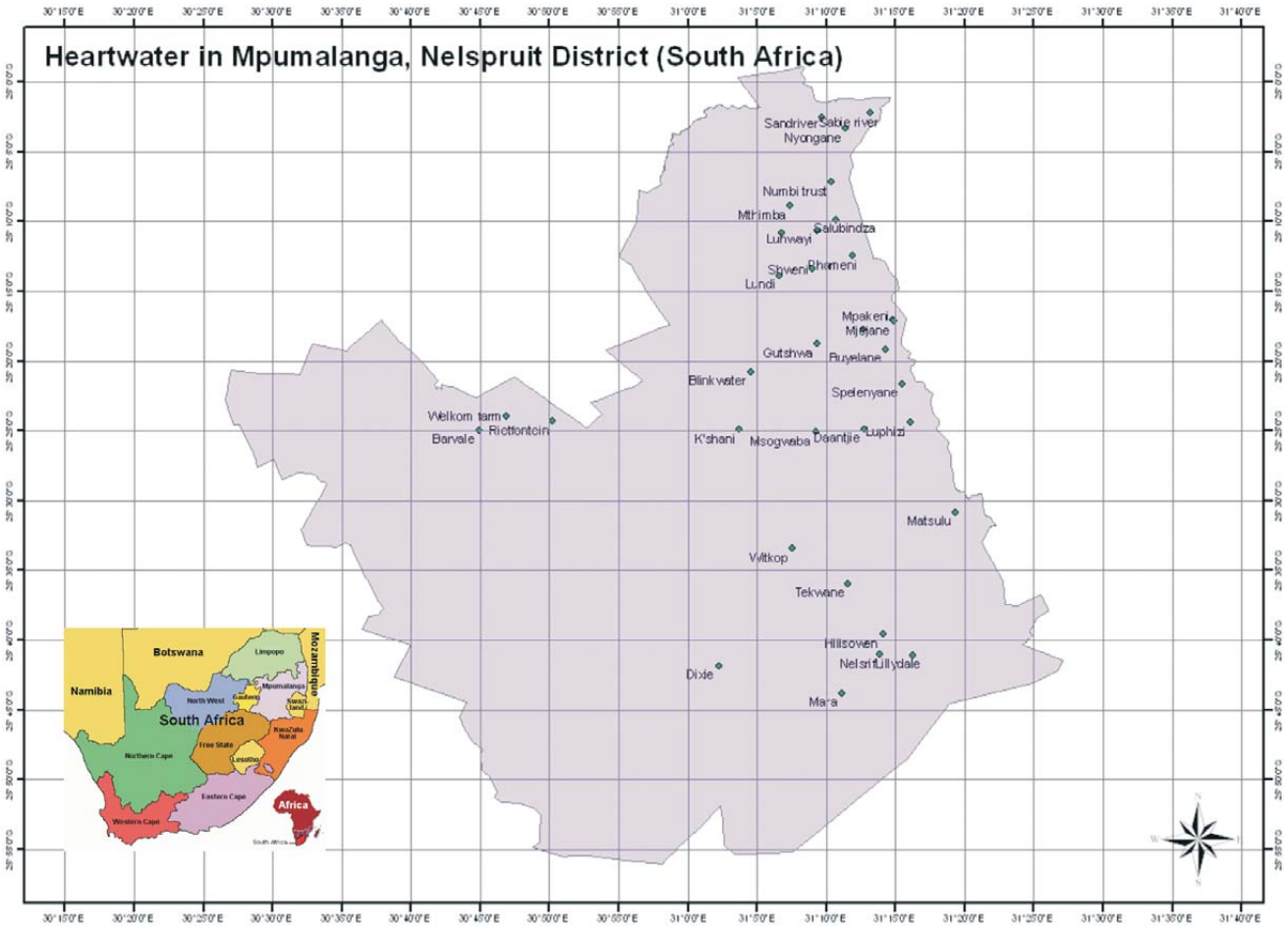

Fig. 1: **The randomly selected commercial farms and dip tanks where blood and tick samples were collected.**

for the identification of *A. hebraeum* in all its stages. However, no extension materials for training AHTs in the correct methods of handling animals, collecting and dispatching samples were found. It has been suggested that a great deal of attention be paid to this[3], as it can be a major constraint to the successful surveillance of animal diseases. The learning outcome was thus the correct collection, labelling, handling and dispatch of blood and tick samples by AHTs for the study of heartwater epidemiology.

Veterinary extension makes use of adult learning and community development techniques to improve food safety and control animal dieases[2,13,14,15,20,21]. A previous study[20] showed that an essential part of successful transfer of skills and knowledge to farmers and rural communities involved the correct in-service training of AHTs.

Effective in-service training aims to improve skills and change actions, beliefs and knowledge to promote better extension and communication by veterinary staff[2,11]. It is also essential for successful research projects[6]. Methods developed for veterinary extension and communication provide a good model for the training of both para-veterinary staff and end users[10,16,21].

Outcomes-based education is based on a framework that breaks down learning objectives and attaches measurable assessment criteria to each objective[5]. Evaluation of the performance of both the trainees and the training programme itself are necessary to identify constraints and to ensure success[10].

This paper describes the development, implementation and evaluation of a programme using veterinary extension techniques and outcomes based education to train AHTs in the correct methods of collection, handling and dispatch of whole blood and *A. hebraeum* ticks required for a study related to the molecular epidemiology of heartwater. Humane handling techniques while restraining and bleeding ruminants were also addressed, in line with the modern focus on livestock welfare[10].

MATERIALS AND METHODS

The study area was selected randomly from veterinary districts within the endemic areas for heartwater in South Africa[12]. The geographical coordinates of Mbombela (formerly Nelspruit) are 25°29′S, 30°59′E. The area is situated in the northeastern part of the Mpumalanga Province and has bushveld vegetation (Fig. 1). The area borders the Kruger National Park on the eastern side and Swaziland on the southern side. The southwestern region of Nelspruit consists of mountains and plantations, such as banana farms. It has summer rainfall. The area has a high incidence of heartwater in cattle, sheep and goats.

Ten AHTs employed by state veterinary services in the Nelspruit area participated in the project. For initial evaluation prior to the development of the programme, they were issued with the prerequisite materials listed in Table 1.

The blood samples had to be collected from the medial coccygeal vein of cattle and from the jugular vein of sheep and goats. Ticks were collected from these animals, as well as other animals in the herd, if none were seen on sampled animals. The AHTs (n = 10) were taken to the local dip tanks where they were asked to collect blood from sheep, cattle and goats. After a verbal description of what was required, AHTs were observed during bleeding and dispatch of samples using a checklist (Table 2).

The correct techniques used and criteria for assessment, as listed in Table 2, are described in more detail below. Prerequisites had to be unpacked and set out neatly and collection tubes correctly and clearly labelled in pencil or with a laundry

marker. All tubes were correctly numbered and correlated on an information sheet labelled with the farm name, GPS reading, owner name and contact details, number and description of individual animals and the date. Correct and humane restraint and handling of animals was expected to be in line with international animal welfare norms and South African legislation (Animal Protection Act of 1962).

Sheep and goats were restrained manually and 10 mℓ of blood was collected from the left jugular vein using 18-gauge Vacutainer® needles and 10 mℓ capacity EDTA BD Vacutainer® K2E tubes. Cattle were restrained in a crush-pen and blood was drawn from the medial coccygeal vein using 18-gauge Vacutainer® needles and 10 mℓ capacity EDTA BD Vacutainer® K2E tubes. The medial coccygeal artery and vein lie on the ventral side of the coccygeal vertebrae. Samples were taken from the vein or artery at the level of the 3rd or 4th coccygeal vertebra. The needle was inserted ventral to the vertebral body (not ventral to intervertebral joint) so that no nerve damage would occur. All tubes were correctly numbered and placed immediately (not left lying in the sun until all the animals were bled) in a zip-lock plastic bag in a polystyrene cooler box, next to a frozen ice brick, and the lid of the box closed.

A maximum of 5 adult *A. hebraeum* ticks were collected from each animal that was bled and these were stored in a correctly labelled, screw-topped plastic container, with holes in the lid, inside a larger zip-lock plastic bag (a maximum of 15 containers) and kept in the same cooler box with an ice brick for transport to the regional veterinary offices or laboratory.

Once they reached the regional centre, the blood sample tubes were wrapped separately in order to prevent contact between them. A triple layer packaging system was used (absorbent, leak-proof bag, cool bag or freezer) to meet the relevant packing instructions. The documentation accompanying the parcel included the delivery address, sender's details, emergency contact details (name and telephone number) and parcels had to be clearly marked as diagnostic specimens transported for research and diagnosis. Tick specimen bottles were placed in a special portable acaridarium containing a saturated saline solution. To ensure humidity and prevent spilling of the saline solution a thick layer of cotton wool was moistened with 40 % saline at the bottom of the acaridarium.

Samples were sent or transported according to the Dangerous Goods Packaging regulations as per UN class 6.2, when transported by road (SANS 10229-1: 2005 edition 1) (Laboratory Specialist Services (Pty) Ltd, 2005). This final packaging and dispatch from the regional laboratory or veterinary centre was done by laboratory technicians or the researcher and thus did not form part of the field assessment of the AHTs.

Table 1: **Prerequisites for successful whole blood and tick sample collection.**

- Vacutainer® EDTA blood collecting tubes(10 mℓ), plastic shoulders, 18-gauge Vacutainer® needles.
- Zip lock plastic bags, labels, 2B pencils (or waterproof laundry marker).
- Polystyrene cooler boxes, frozen ice bricks.
- Sterile plastic containers for tick collection.
- Biodegradable liquid soap, 20-litre plastic container containing clean water, plastic bowl for hand washing.
- Standard information form.
- Geographical Positioning System instruments (GPS).
- Protective clothing and a 'Sharps container' for disposal of used needles.

Table 2: **Checklist for evaluating skills level of animal health technicians prior to training.**

The AHTs were assessed on the following:

- Preparation of pre-requisites prior to bleeding.
- Correct labelling of blood collecting tubes.
- Humane handling and restraint of livestock.
- Correct and efficient bleeding from the jugular vein of sheep and goats or the median coccygeal vein of cattle.
- Correct handling of blood samples.
- Correct labelling of tick containers.
- Correct tick collection and identification.
- Correct handling of tick samples.
- Dispatch of samples in correct container at correct temperature without undue exposure to sunlight.

The level of proficiency in field collection of samples (whole blood and ticks) was assessed and scored (Table 3). The deficiencies seen during this initial assessment of the AHTs were broken down into achievable outcomes and a template developed in line with that described by McCrindle[16] for dairy farming extension. The learning outcomes and assessment criteria for the learning programme are shown in Table 4. Photographs were taken on station to show exact steps in the process (Fig. 2) and these were then used to develop extension materials. Microsoft Power Point® was used as a tool to train learners and each photograph was explained with simple text. In order to test the training materials, a training day was held with the same AHTs and they were then asked to once again sample cattle sheep and goats. A final assessment of the skills of the 10 AHTs was carried out during whole blood sample and tick collection from a total of 465 animals (15 animals per dip tank) and 247 tick samples (either from animals bled or from the herd) collected at dip tanks and commercial farms in the study area (Fig. 1).

RESULTS

During the initial assessment livestock were not always correctly restrained, handling was not always humane, blood samples were left in the sun, incorrect tick species were collected, containers were

Table 3: **Competency score of the animal health technicians before and after training.**

Criteria	Competency score*	
	Before training	After training
1. Preparation of necessities	0/10	10/10
2. Labelling of blood collection tubes	1/10	9/10
3. Humane handling of cattle	5/10	10/10
4. Bleeding cattle from the tail vein	5/10	10/10
5. Handling blood samples	0/10	10/10
6. Labelling of tick containers	0/10	9/10
7. Tick identification and collection	5/10	10/10
8. Handling of tick samples	0/10	10/10
Average competency score	15/80	78/80

*Number of AHTs out of 10 that mastered skill.

Table 4: **Skills training programme compiled after the competency score obtained after the initial animal health technician assessment.**

Learning outcome	Associated assessment criteria
1. Necessities for collecting of blood and ticks	1.1 The following prerequisites prepared and neatly assembled: • EDTA blood Vacutainer® sample tubes • Pencil to label tubes • Ice box with ice brick • Vacutainer® needle shoulder • Vacutainer® needles – 1 per animal • Plastic tick containers (with holes in the lid) • Biohazard plastic waste container for needles to discard used needles 1.2. Information sheet correctly completed: numbers listed on the sheet and a tube labelled with a corresponding number, a few tubes should be left unlabelled for replacements
2. Handle cattle humanely in crush	2.1 Cattle are encouraged to walk slowly into the crush 2.2 Cattle heads must all face to 1 side with their backs closest to AHT 2.3 Cattle must be moved as close as possible to each other
3. Bleeding of cattle	3.1 The animal number is written on the EDTA blood Vacutainer® collection tube including the sample number already on the tube 3.2 The shorter cap of the Vacutainer® needle is removed, but not the rubber needle cover 3.3 The needle is inserted into the Vacutainer® shoulder 3.4 The tube is held in one hand and the cow is approached 3.5 The tail is lifted into a horizontal or vertical position by AHT, or an assistant 3.6 Any excess faecal material on the surface of the tail is removed by wiping it with a papertowel or cotton waste if needed 3.7 The cap of the needle is removed and the needle is inserted between the 2nd and 3rd vertebra into the medial coccygeal vein 3.9 The tube is inserted into the shoulder and held until the tube is full 3.10 The tube is removed 3.11 The needle is removed and discarded into the plastic biohazard waste container
4. Handle sheep and goats humanely	4.1 An assistant is requested to catch and hold the animal while it is bled 4.2 The animal is caught by the hind leg and the other hand is put under the jaw (do not catch by holding the wool) 4.3 The animal is held with the head slightly lifted 4.4 The assistant will either straddle it or hold it firmly with the sheep's rear side against a wall or barrier 4.5 The vein is exposed by using either manual pressure or a bleeding rope
5. Bleeding of sheep and goats	5.1 The number of the animal is written on the EDTA blood collection tube together with the sample number 5.2 The needle is put into the shoulder 5.3 The tube loosely inserted into the shoulder 5.4 The needle is inserted upwards into the vein and the tube is pushed against the needle to begin bleeding 5.5 The tube is removed when full 5.6 The needle is removed and discarded into the plastic biohazard waste container
6. Handle the blood sample	6.1 The blood is mixed with the EDTA slowly by tilting the tube back and forth 6.2 The animal number and description are written onto the information sheet 6.3 The blood sample is returned to the ice box and the ice box is closed
7. Tick sampling	7.1 The plastic container is labelled with the number of the animal 7.2 The container is opened 7.3 Adult *A. hebraeum* ticks are collected form the underside of the tail and inguinal areas of the host where they prefer to attach[18] 7.4 A pair of forceps or gloved hand is used to remove 5 adult ticks which are placed into the plastic container 7.5 The container is closed and placed inside a plastic bag and put into the cooler box 7.6 The cooler box is closed

incorrectly labelled and data sheets incorrectly completed. These observed deficiencies were used to design the training materials and were remedied after training (Table 3). After training, the AHTs correctly restrained the animals and were successful in filling tubes. Tubes were not exposed to sunlight and labelling was done correctly. Only *A. hebraeum* ticks were subsequently collected.

Examples of some of the pictures used as training materials are included (Fig. 2). These will shortly be available from the Onderstepoort Veterinary Institute on CD's with an attached training module, as they can be used for field training in the correct collection and dispatch of whole blood samples required for surveillance or research on other diseases.

After the initial assessment a training programme was developed using Micro-

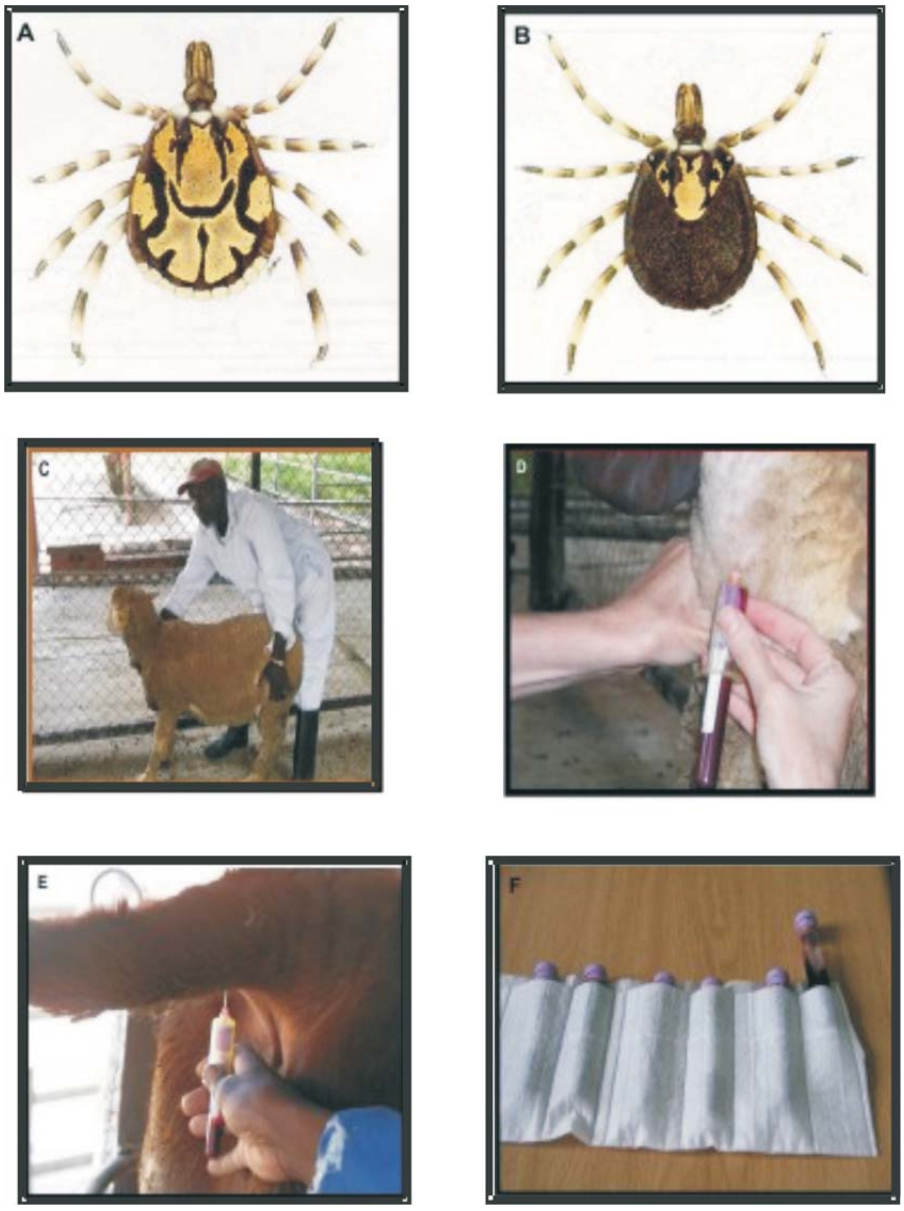

Fig. 2: **Illustration of** (A) **male and** (B) **female *Amblyomma hebraeum* ticks** (illustrations by A. Olwage[25]). C, **Humane handling of sheep;** D, **drawing blood from the jugular vein of a sheep;** E, **drawing blood from medial coccygeal vein of a bovine;** F, **storing blood samples separately in absorbent packaging for transport** (Photographs: J.F. Putteril, ARC-OVI).

soft PowerPoint®. The training materials were saved as a slide show on a computer. These can be printed and put in flip files for field training of the target audience, in this case the AHTs[16].

The outcome of the training showed an increase from 20 % accuracy before skills training to 90–100 % after skills training (Table 3).

DISCUSSION AND CONCLUSIONS

The AHTs were successfully trained in all aspects of correct sample taking and dispatch. These included humane handling of livestock, correct collection of blood and ticks for heartwater epidemiology studies and preventing exposure to heat and sunlight.

Correctly collected samples were subsequently used in another study, for the accurate monitoring of heartwater in problem and endemic areas as well as the identification and characterisation of new genotypes for future vaccine development. AHTs who had undergone skills training also played a vital role in the success of a current study on heartwater epidemiology. This technique also has the potential to improve future sampling for routine diagnosis of heartwater during outbreaks, as using the newly developed technology means that the genotype can be identified and characterised.

The training materials can also be used to train AHTs to collect blood samples for other diseases such as brucellosis and foot-and-mouth disease surveillance programmes. This investigation accentuates the important role of veterinary extension and communication in the correct training of AHTs working in the field, so that poor sampling does not decrease the accuracy of disease diagnosis and research. It is important for the state veterinary services to understand new research technologies and the importance of collecting samples accurately and correctly, not only for research but also for routine surveillance in the field.

In conclusion, the method used for training of the AHTs was successful as the samples taken after training were suitable for epidemiological investigations of heartwater using molecular techniques.

ACKNOWLEDGEMENTS

This research was supported by the National Research Foundation, European Union (EU) and the Sustainable Rural Livelihoods Division of the Agricultural Research Council-Onderstepoort Veterinary Institute. We thank Dr M van Kleef (New Generation Vaccines) and Mr A Spickett (Parasites, Vectors and Vector-born Diseases) for helpful comments on the manuscript.

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