



agriculture, forestry & fisheries

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TUBERCULOSIS TESTING IN SHEEP AND GOATS MANUAL

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Table of Contents

1. Purpose	3
2. Scope	3
3. Introduction	3
3.1 Abbreviations	3
3.2 Background	3
3.2.1 Goats	4
3.2.2 Sheep	5
3.2.3 New World Camelids	5
4. Legislation	5
5. Testing procedures	6
5.1 SIT and SICT	6
5.2 The IFNy assay	8
5.3 Agent identification – culture	9
6. Interpretation	9
6.1 Skin test interpretation in previously negative herds	10
6.2 Skin test interpretation in infected herds	10
6.3 Reactions	11
6.4 Herd approach	11
6.5 Complicating factors	12
7. Certification	12
Acknowledgments:	12
References:	13

1. Purpose

This manual serves to be used as a guideline for Tuberculosis (TB) testing in small stock. This is a working document and future findings will be incorporated into future drafts to improve on the current scientific and practical knowledge available.

2. Scope

This manual serves to compliment the Bovine Tuberculosis Manual by providing testing methods and interpretation for small stock, and includes: goats, sheep and new world camelids (NWCs). TB testing relies on the experience of the tester, as well as the level of care taken when handling the thin skin of small stock and insuring that the tuberculin injection is in fact administered intra-dermally. This manual should be read in conjunction with the comprehensive Bovine Tuberculosis Manual. The manual contains essential information relating to TB as a disease in animals and humans, tuberculin and interpretations of skin test reactions.

3. Introduction

3.1 Abbreviations

AHT	Animal Health Technician
DAFF	Department of Agriculture, Forestry and Fisheries
DAH	Director Animal Health
NTMs	Non-Tuberculous Mycobacteria
NWC	New World Camelid
SICT	Single Intradermal Comparative Tuberculin Test
SIT	Single Intradermal Tuberculin Test
SV	State Veterinarian
TB	Tuberculosis

3.2 Background

Tuberculosis (TB) is a chronic, contagious disease of vertebrates causing the formation of granulomatous lesions, specifically in the lungs and associated lymph nodes, but also in other organ systems. The disease is caused by bacteria belonging to the *Mycobacterium tuberculosis* complex. Mycobacterial species that have been shown to cause tuberculosis in small ruminants and camelids include *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae*, *M. microti*, *M. pinnipedii* and *M. tuberculosis*. It is also possible for nontuberculous mycobacteria (NTMs) to cause clinical signs similar to TB in these species.

In the absence of a test that allows a definitive diagnosis of tuberculosis in live animals, several diagnostic methods will be considered in these guidelines, including:

- **The single intradermal tuberculin test (SIT):** Performed by measuring the cell-mediated immune response to intradermal injection of purified protein derivative (PPD) of *M. bovis*.
- **The single intradermal comparative tuberculin test (SICT):** Performed as the SIT above, but with the addition of intradermal injection of *M. avium* subsp *avium* PPD at a different site in order to differentiate between animals infected with *M. bovis* and those sensitised by exposure to other species of Mycobacteria.
- **The interferon-gamma (IFN γ) assay:** Measures the release of IFN γ from lymphocytes when incubated with bovine, avian or other PPD. Whole blood samples need to be taken and transported to a laboratory equipped to perform this test on the same day as the sampling took place.

The majority of these tests were developed for detection of bovine tuberculosis caused by *Mycobacterium bovis*, and therefore adequate validation of the tests has not been performed in non-bovine species. With this current lack of data upon which to base testing guidelines, veterinarians should be aware that the final diagnosis of a herd or individual animal is based on their sound professional discretion.

3.2.1 Goats

Goats are highly susceptible to infection with *M. bovis*, *M. tuberculosis* and *M. caprae* (to date detected only in central Europe), as well as NTMs. They are usually infected through exposure to other infected animals. For example, in a pastoralist setting where close contact between goats and people occurs, there is a risk of infection with *M. tuberculosis*. The same risk occurs for *M. bovis* infection in situations where there is close contact between goats and cattle at shared grazing or water. After infection, goats have the potential to become a reservoir of TB and can transmit it back to cattle or other susceptible species. Routine TB testing in goat herds that share camps or pastures with cattle, or have other epidemiological relationships with them, is therefore recommended, as is routine testing in dairy goats for public health reasons.

Clinical signs in goats with TB include dry coughing, progressive emaciation and sometimes diarrhoea. Post-mortem examinations usually show circumscribed, sometimes encapsulated, caseous lesions in the lungs, mediastinal lymph nodes or mesenteric lymph nodes.

3.2.2 Sheep

Cases of TB in sheep are considered to be rare, occurring only when animals are exposed to a high level of challenge by close contact with infected cattle. Even in these situations, a low incidence of cases in sheep usually occurs. TB testing in sheep is therefore not recommended unless there is a suspicion of TB infection, or if sheep have been in close contact with other infected livestock or if it is required for export purposes.

Infected sheep show non-specific clinical signs and are usually noticed as “poor-doers”. On post-mortem examination, lesions usually occur in the respiratory tract.

3.2.3 New World Camelids

Domestic species of New World camelids (NWCs) include llamas (*Lama glama*) and alpacas (*Vicugna pacos*). NWCs rarely contract TB in the wild, but are highly susceptible to infection with *M. tuberculosis*, *M. bovis* and *M. microti* when kept in intensive conditions. As they are not considered maintenance hosts, infection occurs as a result of contact with infected animals or people. As for sheep, routine TB testing in NWCs is therefore not recommended.

NWCs infected with TB show non-specific signs including general distress, wasting, anorexia, respiratory signs, enlargement of superficial lymph nodes and sudden death.

4. Legislation

Tuberculosis is a controlled diseases in terms of the Animal Diseases Act, 1984 (Act No. 35 of 1984). All confirmed and suspected cases in any susceptible animal have to be reported to State Veterinary Services. The Animal Diseases Regulations (R.2026 of 1986) state the following regarding Tuberculosis:

Table 2 - Control Measures relating to Controlled Animal Diseases

Animal Disease	Nature, causal organism and symptoms	Susceptible animals	Controlled veterinary act to be performed in respect of-		
			Susceptible animals	Contact animals	Infected animals
1	2	3	4	5	6
Tuberculosis	Chronic contagious animal disease to which man is also susceptible, caused by the bacteria <i>Mycobacterium bovis</i> , <i>M. avium</i> and <i>M. tuberculosis</i> , and in an advanced stage in some animals characterised by emaciation, coughing and enlarged lymph nodes	All animals, except fish, reptiles and amphibians	Susceptible animals may be tested by an officer, authorised person or veterinarian	Contact animals shall be isolated and be tested by an officer, authorised person or veterinarian	Infected animals shall be marked as contemplated in regulation 29 isolated and be slaughtered in the manner determined by the responsible State Veterinarian: Provided that the director may in a particular case approve that specific contaminated animals may be treated with an efficient remedy

For Bovine Tuberculosis control, please refer to the Bovine Tuberculosis Scheme (R. 1953 of 30 Sep 1988) and the most recent Bovine Tuberculosis manual (both available on the DAFF website, www.daff.gov.za).

5. Testing procedures

5.1 SIT and SICT

Of all non-bovine domestic livestock species, SIT and SICT has been most thoroughly evaluated in goats, revealing sensitivities ranging from 45-94% and 43-84% respectively according to literature (see references at end of document). Not enough information is currently available to estimate specificity. The SIT and SICT are regarded by the OIE as the prescribed test for international trade.

In a presumed TB-free herd where the goal of testing is certification of freedom from TB, the more specific SICT should be used instead of the SIT. The test is performed on both sides of the neck in the mid-cervical region.

- Palpate both test areas of the neck very gently to feel for any lumps or adhesions present. The skin test should be done on a healthy patch of skin preferably in the mid-cervical region.

- A small square (3cm x 3cm) is trimmed with an electric clipper. Avoid shaving as this damages the thin skin. If the skin is very dirty, wash the site gently with clean water and allow to dry before injecting.
- At 0 hours, measure the skin thickness at the intended injection site using a functional calliper. Avoid overstimulation and excessive handling of the skin. Record the 0 hour reading on both the left and right sides of the neck.
- 0.1ml each of bovine (left) and avian (right) PPD should be injected intra-dermally at a concentration of 2mg/ml.
- For each animal, a new disposable insulin or 25G needle should be used. A McClintock syringe may be used as well if care is taken to inject intra-dermally into the thin skin and ensuring that the needle is sharp at all times.
- Care should be taken when performing the test that the tuberculin is not injected subcutaneously, due to the relatively thin skin that goats possess. After the injection, palpate the skin surface gently to feel the pea-sized bump after injecting (if it was done correctly). If there is no bump it means the injection was not intra-dermal. If there is no bump, make a note of this on the TB record /form, as after 72 hours there may be an attached diffuse bump that may not be associated with TB. **Do not inject again.**
- 72 hours after injection of tuberculin, on both sides of the neck the skin thickness should be measured and the injection site should be thoroughly inspected and palpated (and any reactions recorded). For a more comprehensive explanation on reactions, please consult the latest edition of the Bovine Tuberculosis Manual.
- Accurate information on all readings and reactions should be filled in on an "intra-dermal tuberculin test record (TB10)".

Guidelines for interpretation of the SICT test are shown in table 1 and table 2 below. Animals are classified as positive, negative or suspect based on the herd status.



The mid cervical region was used preferentially as the skin was relatively mobile in this location

Image courtesy of KZN Veterinary Services.

Sheep - The SIT test has been evaluated in two small studies on sheep, using the medial thigh as an intradermal injection site and interpreting the results using the same criteria as for cattle (see table 1). These two studies reported a sensitivity of 67.7% and 81.6% respectively and a specificity of 99.6% according to the literature.

NWCs - The SIT and SICT have a low sensitivity (14-58.3%) in naturally infected NWCs according to the literature. The IFN γ assay is capable of detecting camelid IFN γ , but cannot achieve a sensitivity of more than 70%. If used, the skin test should be performed in the axilla, or rather the pre-scapular site for highest sensitivity, avoiding the cervical site due to the thick skin present in this area. 0.1ml of bovine PPD should be injected intra-dermally on the left side and 0.1ml avian PPD should be injected intra-dermally on the right side, using a concentration of 2mg/ml. Reactions should be measured and recorded 72 hours after injection of tuberculin. Interpretation guidelines in table 1 may be used for a previously negative herd.

5.2 The IFN γ assay

The IFN γ assay has been relatively well evaluated in goats, though studies evaluating its use show variable sensitivities (58-92.9%) and specificities (96-100%) according to literature. Its most important application would be its use in infected flocks in combination with the SIT or SICT. When animals are regarded as positive if they test positive to either the skin test or the IFN γ test, this results in a sensitivity and specificity of testing both over 95%.

The IFN γ assay can theoretically be used in sheep. There has, however, been only one small study done on its use in this specie; resulting in a sensitivity of 100% (three out of four sheep that tested positive were confirmed to have TB lesions).

5.3 Agent identification – culture

This is the definitive diagnostic (gold standard) test and should be used for all suspect and positive herds. Culturing is a time-consuming test as Mycobacteria are slow growing and it may take six to eight weeks before a diagnosis can be made. Tissue samples from suspected infected cases are inoculated onto special media and cultured. Tissue samples may include: relevant lymph nodes, lesions from affected organs, the sediment after milk has been centrifuged, and bits of mucus that have been coughed up. These samples should be collected when a positive reactor is slaughtered. In performing a post mortem examination for TB, attention is mainly focused on the lymph nodes as they are usually infected if the organ (or the part of the body) from which they drain lymph is infected.

The following samples should be collected:

- a) Unstained smears of the lesions, excretions or / and secretions;
- b) Lesions on ice for culture;
- c) Samples of infected parts of the body, organs and lymph nodes in 10% formalin. Samples should not be larger than 25 mm x 12 mm x 6 mm.

6. Interpretation

6.1 Skin test interpretation in previously negative herds

Table 1:

Test result	Interpretation
SIT	
Any positive skin changes such as exudation, necrosis, oedema, pain or adhesions	Positive
≥ 4mm increase	Positive
Increase between 2 – 4 mm	Suspect
No reaction ≥ 2mm, nor clinical signs	Negative
SICT	
Only bovine site reacts and there is ≥ 4mm increase	Positive
Only bovine site reacts with an increase of 2 – 4 mm	Suspect
Both sites react; bovine site reaction ≥ 4mm greater than avian site reaction	Positive
Both sites react ≥ 4mm; avian site has an equal or greater reaction than the bovine site	Suspect
Only avian site reacts	Negative
No reactions ≥ 4mm at either site	Negative

6.2 Skin test interpretation in infected herds

In a confirmed infected herd, where the goal of testing is to eradicate TB from the herd, a more severe interpretation should be used, according to table 2 below.

Table 2:

Test result	Interpretation
SIT	
Any positive skin changes such as exudation, necrosis, oedema, pain or adhesions	Positive
≥ 2mm increase	Positive
No reaction ≥ 2mm or clinical signs	Negative
SICT	
Only bovine site reacts and there is ≥ 2mm increase	Positive
Both sites react; bovine site reaction ≥ 2mm greater than avian site reaction	Positive
Both sites react ≥ 2mm; avian site has an equal or greater reaction than the bovine site	Suspect
Only avian site reacts ≥ 2mm	Negative
No reactions ≥ 2mm at either site	Negative

6.3 Reactions

Table 3:

Positive signs	Negative signs
1. Skin thickening - large	1. Skin thickening – small or negligible
2. Herd history – positive for TB	2. Herd history – negative or unknown
3. Pain = T	3. Hard
4. Heat = H	4. Circumscribed = C
5. Skin colouring – red, blue, etc	5. Flat = F
6. Oedema = O	6. Skin lesions – see or feel
7. Functional skin changes such as: 7.1 Necrosis = Nec 7.2 Exudation = Ex 7.3 Lymph nodes & lymph ducts swollen = Lnn or Ld	History of: 7.1 Avian tuberculosis on farm – <i>M. avium</i> 7.2 Feeding of chicken litter 7.3 Human tuberculosis – <i>M. tuberculosis</i> 7.4 Johne's disease – <i>M. paratuberculosis</i>
8. Systemic reactions such as: 8.1 Fever 8.2 Shivering 8.3 Dull hair coat 8.4 Listlessness 8.5 Decreased production in dairy goats 8.6 Coughing spells	8. Young animal
9. Adhesion of skin to subcutaneous tissue = Ad	
10. Old animal	
11. Swelling diffuse = D	

6.4 Herd approach

The interpretation takes place on a herd basis and all animals showing reactions must be held back until the interpretation is complete. All the reactions in the herd are now taken into consideration. It will now be possible to determine the herd status. Only after the herd status is determined can the interpretation of individual animal reactions be performed.

When reading a herd test it is of value to walk through the animals before they are packed into the crush pen. By doing this large severe reactions can be seen and should be placed in the crush pen first as this helps to determine the status of the herd. The general body condition of the animals including external parasite load, as well as the overall quality of herd management can provide additional useful information towards a final diagnosis. Note if the animals are kept intensively and if they are housed, note the housing conditions. Intensive confinement could potentially increase the likelihood of NTM infection.

6.5 Complicating factors

Concurrent infection with or vaccination against *M. avium* subsp *paratuberculosis* (Johne's disease) can decrease the sensitivity of both the SICT and the IFN γ assay. In these herds, it is better to use the SIT with the severe interpretation (see table 2) for the best sensitivity in combination with the IFN γ assay using 0.05 as cut-off point for a positive test result. Concurrent infection with *Corynebacterium pseudotuberculosis* can also cause false positives, but its influence on test sensitivity and specificity has not been evaluated.

It is likely that, as in goats, concurrent infection with ovine Johne's disease or *Corynebacterium pseudotuberculosis* will decrease sensitivity of the tests in sheep. If the SIT/SICT test or the IFN γ assay is used in sheep, they should be performed and interpreted using the same guidelines as those for goats.

For NWCs, in a confirmed infected herd, a combination of tests should be used to maximise sensitivity in order to eradicate TB. Recommended combinations include (a) IFN γ + serological testing, (b) SIT (interpreted using table 2) + serological testing or (c) serological testing + observation of clinical signs. An infected animal is defined as one that is positive to either of the two tests used.

7. Certification

The same certification process as for cattle is followed. Please see the most recent Bovine Tuberculosis Manual for more detail:

- Maintenance (annual diagnostic) herd programme
- Diagnostic herd test programme
- Infected herd programme

A TB test declaration (TB3) may be issued to a herd that successfully adheres to the Maintenance (annual diagnostic) herd programme.

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