



agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Interim*

Bovine Tuberculosis Scheme Manual

6 December 2013

* This manual is to be used as an interim manual while the Tuberculosis Advisory group revises the control of Tuberculosis in South Africa

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INTRODUCTION

The Bovine Tuberculosis Scheme was officially introduced by the Division of Veterinary Services during 1969 with the purpose of eradicating the disease in the Republic of South Africa.

At the initial stage it was already realised that the need for testing for tuberculosis in herds varied and that it was largely determined by the type of farming. In order to incorporate as many stock owners in the scheme as possible the testing procedures and the various testing programmes were adapted throughout the years. It also necessitated a change in the administrative processes which meant that the manual had to be adapted accordingly.

The various testing programmes as set out in the manual should be regarded as different means of eventually achieving the final objective namely the total eradication of tuberculosis. In order to reach this objective, thorough planning and the use of available funds and manpower are necessary. A thorough knowledge of the scheme through studying this manual is a prerequisite for sensible planning and execution.

It begins with effective guidance to the stock owner who wishes to join the scheme so that his herd can be incorporated into the correct testing programme. The previous accreditation programme has been discarded. Stud herds & dairy herds should for example be incorporated into the Maintenance (old Annual Diagnostic) Programme as many of them require a declaration for the sale of stud animals or milk. Where there is no need for a tuberculosis-free declaration or where such a declaration is not of much value for the owner the incorporation should be into the Herd Diagnostic Programme and *not* the Maintenance Programme. A single negative herd test, under the Herd Diagnostic Programme makes it possible to test many more herds to track down bovine tuberculosis and thereby achieving the final objective sooner.

The Bovine Brucellosis Eradication Scheme, launched by the Division of Veterinary Services during 1978 primarily has the same final objective, namely the eradication of brucellosis in cattle in South Africa. The Bovine Brucellosis Eradication Scheme is conducted in much the same way as the Tuberculosis Eradication Scheme and the possible synchronising of tests within the same herd under the two testing schemes should be borne in mind.

1 TUBERCULOSIS: THE DISEASE

Definition: Tuberculosis is a chronic disease caused by infection with a member of the *Mycobacterium tuberculosis* complex which comprises *M. tuberculosis*, *M. bovis* (incl. BCG and *M. caprae*), *M. microti*, *M. africanum*, *M. canettii* and *M. pinnipedii*. All mycobacteria are acid fast and can affect the majority of vertebrates.

Three types of tuberculosis are important, namely bovine tuberculosis caused by *M. bovis*, avian tuberculosis caused by *M. avium*, and human tuberculosis caused by *M. tuberculosis*. Humans may also develop tuberculosis due to *M. bovis* as it is a zoonotic pathogen. Although these diseases are related, the causative organism differs in all three cases and can be identified by laboratory tests. The organisms can also cause the disease in animals other than cattle, birds and humans.

Avian tuberculosis (caused by *M. avium*) is rather referred to as mycobacterioses and not discussed in this document.

1.1 Susceptibility of animals to the three Mycobacterium strains

Animal	<i>M. bovis</i>	<i>M. avium</i>	<i>M. tuberculosis</i>
Cattle	XXX	X	X
Fowl	-	XXX	
Human	XXX	XX	XXX
Pig	XXX	XX'	XX'
Sheep	XX'	XX'	XX
Goat	XXX	XX	XX
Horse	XX'	XX	XX
Cat	XXX	XX	
Dog	XX'	XX	XXX
Wild animals			
Kudu			
Deer and others	XXX	XX	XX
Guinea pig	XXX	-	XX
Rabbit	XXX	XX	X

Meaning of symbols:

XXX	susceptible - visible lesions develop
XX·	susceptible - visible lesions sometimes develop
Xx	susceptible - visible lesions seldom develop
X	visible lesions usually do not develop but animals react to the tuberculin test

1.2 History

Tuberculosis has been known since classical times when it occurred in Europe and Great Britain. In South Africa the disease was recorded for the first time in 1880. It is apparent that tuberculosis was introduced into the local cattle population by the introduction of European breeds of cattle.

In 1902 Natal was reported to be free of tuberculosis but tuberculosis was found in a large number of imported animals. These animals were destroyed. However, the disease still spread through the country, mainly in dairy

herds, but as farming systems became more intensive the disease was commonly found in beef herds as well. Due to the increasing prevalence, tuberculosis was declared a notifiable disease in 1911. Various programmes were introduced to control the disease, the present one being introduced in 1965. The Bovine Tuberculosis Eradication Scheme had as its aim the total eradication of tuberculosis from the country. With large numbers of tests being performed, the prevalence of the disease was reduced to being performed, the prevalence of the disease was reduced to 0.04% by 1991. The number of tests performed since 1991 has declined dramatically due to various factors, including budget constraints, changes in farming systems and a shortage of manpower. The prevalence of tuberculosis in the communal farming areas of the country appears to be very low. In addition it would seem that Zimbabwe, Namibia and Swaziland all have a very low prevalence of tuberculosis.

1.3 Pathogenesis of the disease

With cattle and humans the organism enters the body via the respiratory system in most cases, but it can also enter the body via the digestive system. After the organism has established itself its activities stimulate the formation of lesions called tubercles. Tubercles are typical granulomas containing a central core of caseous necrotic tissue. The centres of tubercles may later calcify. The tubercles are pale orange in colour. When bacteria escape from this original focal point, they can spread to other parts of the body via the lymphatic ducts and lymph nodes or the blood stream to other parts of the body and again form tubercles there. If many organisms find their way into the bloodstream in this way, general diffusion takes place through the body and it forms multiple lesions which can lead to toxemia, debility, weakness and death. Sometimes lesions are limited to such an extent by the dense connective tissue that further diffusion does not take place and the disease is limited to that area. Lesions usually also develop in the lymph nodes which drain the lymph of the affected part of the body or organ, and therefore the lymph nodes are usually examined to determine the presence of tuberculosis.

1.4 Transmission

The most common method of transmission is by direct aerosol contamination of the environment. An animal which has open lesions will shed many millions of organisms.

Direct infection of the respiratory system mainly occurs in cowsheds and other farm buildings. Cattle with open lung lesions cough up infected mucus or the bacteria may be carried by means of small exhaled moisture particles and be inhaled directly by other animals in the immediate vicinity. The bacteria may, however, also land on the ground and thereafter become airborne together with dust particles and then again be inhaled and thereby indirectly infecting other animals.

Infection through the mouth also occurs via infected milk or where the food, water and grazing especially irrigated pastures - as well as mineral and feed licks are contaminated by bacteria from open lung lesions where mucus is coughed up and swallowed again. It can also be transmitted where the saliva or food in the alimentary canal is infected by lesions in the canal itself are excreted in the dung as well as in the milk, urine and vaginal excretions if these organs are infected. Infected lymph nodes that erupt (burst) on the skin may also contaminate the food and water sources mentioned.

The possibility exists that infection can be transferred through infected teat needles, milking machines, speculums and other instruments or during service if the genital organs are infected. Wound infection can also occur. Transmission to the unborn calf when the uterus is infected or when the cow has developed general lesions is also possible.

The period that the bacteria remains infectious outside the body depends on climatic conditions.

Dessication and direct sunlight are detrimental to the organism and will reduce the length of time that the organism remains infectious.

The organism can remain infectious in stagnant water for up to 18 days while it can remain viable in moist soil for up 8 weeks.

The main route of infection between herds is by the introduction of infected cattle.

Humans most commonly become infected with *M. bovis* by drinking unpasteurised infected milk.

1.5 Symptoms

The vast majority of infected cattle that react positively to the tuberculin test show no clinical symptoms. Animals with diffuse lesions in the body may later gradually become emaciated and anorexic with a fluctuating temperature and a dull coat (systemic reactions). Such animals become lethargic but the eyes remain clear. The symptoms become evident during stress and when the body is subjected to physical exertion as in calving. These symptoms will take many months to develop.

With advanced infection of the lungs a single suppressed moist cough may occur especially early in the morning after exercise or when it is cold or in dusty conditions. A lack of breath is apparent in advanced cases where much lung tissue has already been damaged. Enlarged bronchial lymph nodes exert pressure on the lung passages and this can lead to dyspnoea (difficulty in breathing). Continuous bloat can occur as a result of pressure on the esophagus through enlarged mediastinal lymph nodes.

If the lymph glands such as the submaxillary, prescapular, precrural and supramammary are infected they may sometimes be seen or felt.

Metritis (uterine infection) as a result of tuberculosis can hamper conception or it can result in abortion at advanced pregnancy.

Infection of the udder entails danger for the calf or people who drink the infected milk if it is not boiled or pasteurised. It is sometimes difficult to distinguish this type of mastitis from mastitis as a result of other organisms. In cases of tuberculosis-mastitis hardening and/ or enlargement of the top part of especially the udder hind-quarters takes place. In such cases the supramammary lymph nodes become enlarged. If the lymph nodes become enlarged without palpable lesions in the udder itself it often points to tuberculosis. Initially the milk itself looks normal; later flakes may become visible if the milk has been standing for a while and in advanced cases the excretion (milk) becomes a light yellow-brown colour and transparent.

1.6 Diagnosis

1.6.1 Field tests

a.) Intradermal tuberculin test

This is the definitive SCREENING test. It is the most common and most practical test and is also a prescribed test by the OIE (World Organisation of Animal Health) for international trade. Tuberculin consists of a solution of protein material extracted from the cell wall of the mycobacteria organism. When this is injected into the skin of an infected animal, the body's defenses will cause an inflammatory reaction that leads to the typical signs of a positive tuberculin test. Animals that have not been exposed to tuberculosis will not mount an inflammatory reaction. (see Chapter 2 Immunology)

The preferential site for conducting this test is the neck area (see Chapter 3) (A caudal fold test using tuberculin has been used in the past)

The various types of mycobacteria all have lipid cell walls with closely related chemical compounds. It is therefore not surprising that the various types of mycobacteria cause a degree of sensitivity for mammalian tuberculin. Fortunately the reaction to mammalian tuberculin is less than the reaction to a tuberculin of the mycobacterium concerned. This enables us to determine the sensitivity caused by *M. avium* or related mycobacteria by means of the comparative test where mammalian and avian tuberculin are used simultaneously.

The following tests (where tuberculin is used) have been used in the past but are no longer used as they have no compelling advantage over the intradermal tuberculin test Stormont Test

- Double Intradermal test
- Temperature test

1.6.2 Laboratory Diagnosis

a.) Direct Examination

Smears can be made from: bits of mucus that have been coughed up; the sediment after milk has been centrifuged; lymph nodes; or other excretions and organs. These smears are then stained according to the Ziehl-Nielson method and examined under the microscope.

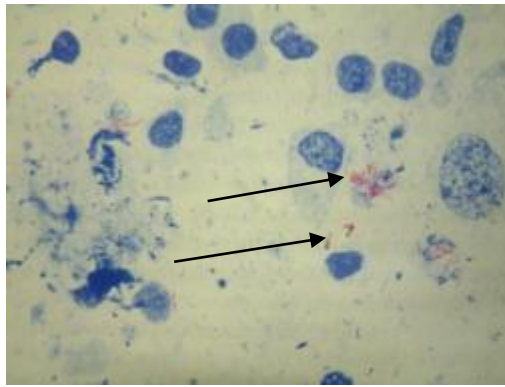


Figure 1 Acid Fast organism

According to the OIE manual, the fluorescent acid fast test and immunoperoxidase could also be used for direct examination.

b.) Biological Test.

Suspensions of the above samples can be injected into guinea pigs and then a post mortem is conducted 6 weeks later to identify typical lesions.

c.) Culture Test

This is the definitive DIAGNOSTIC test and should be used for all suspect and positive herds. The same type of material that is used in the previous two tests is plated onto special media and cultured. This is a very difficult procedure as Mycobacteria are very slow growing and it can take months before a diagnosis can be made.

d.) Gamma Interferon Test

This is an in vitro test for cellular immunity. It is a useful auxiliary test as used in combination with the intradermal tuberculin test, it increases the sensitivity by up to 20% (number of positive animals that test positive). This test cannot be used in isolation to make a diagnosis.

e.) Elisa

A newly developed test. It measures humoral antibodies which develop later in the course of the disease. Shows promise in identifying anergic reactors. According to the OIE Manual, ELISA could also be useful for detecting *M. bovis* in wildlife.

f.) Flourescent Polirization Test

A newly developed test still under investigation.

g.) PCR

An auxiliary test that is very sensitive but expensive and time consuming. It is used more for research purposes at the moment.

1.6.3 **Post Mortem**

In performing a post mortem for tuberculosis attention is mainly focused on the lymph nodes because they are usually infected if the organ or the part of the body from which they drain the lymph is infected. The expected degree of infection of the lymph nodes and organs are given in descending order:

Lymph node / Organ	Expected degree of infection
Bronchial, Mediastinal or Mesenteric Lymph nodes	90%
Lungs	75%
Pleura	55%

Peritonium	50%
Liver	50%
Spleen	50%
Pericardium, Uterus and kidneys	10%
Trachea	5%
Udder and ovaries	0.5 - 1%

When the lymph nodes are infected tubercles are formed that enlarge and later on coalesce eventually becoming caseous and calcified.

In buffalo it has been found that the lesions can be extremely small and the cut surfaces of the lymph node must be carefully examined in good light.

When doing a post mortem all lymph nodes of the head, neck and thoracic cavity must be carefully examined and numerous cuts made into the lymph node tissue. Whenever signs of systemic tuberculosis are seen, all other lymph nodes in the body must be examined as well.



Figure 2 Tuberculosis in lymph node (Buffalo)

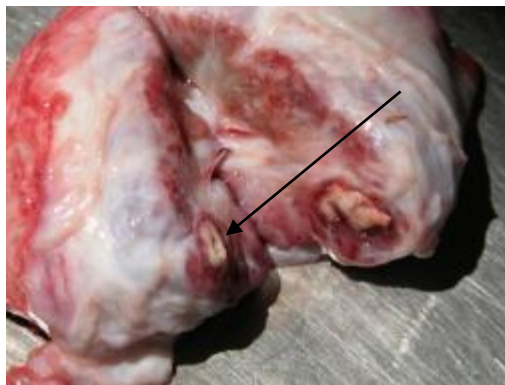


Figure 3 Discrete lesion in lymph node

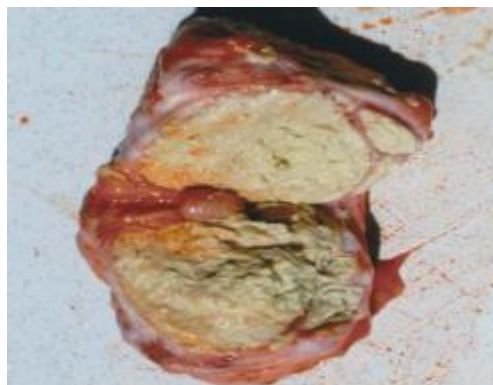


Figure 4 Lymph node showing caseation

In the lungs of infected cattle, large nodules are seen with normal lung tissue between them. These nodules increase in size and often coalesce to form large areas where the normal lung tissue is completely destroyed. When cut these nodules have a caseous centre and often older lesions are 'gritty' when cut into due to the calcification.

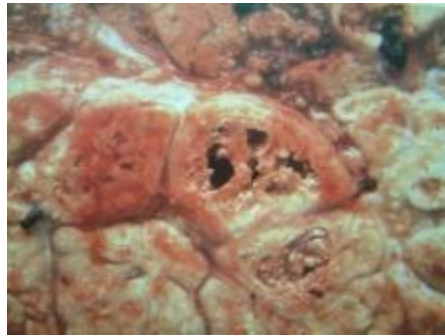


Figure 5 Tuberculosis in lungs

These lesions can also be very small and only felt if the lung tissue is carefully palpated



Figure 6 Tuberculosis in lungs

Chronic lesions in the lung are often surrounded by a thick fibrous capsule

In cattle tuberculosis pleuritis and peritonitis are often found. This condition can occur without the lungs, liver, spleen or kidneys being infected, in other words when infection has taken place through the lymphatic ducts. Granular tuberculosis nodules that form on the pleura consolidate to form clusters almost like a bunch of grapes. Such lesions are also surrounded by connective tissue with caseous and sometimes calcified content. The same lesions are found on the peritoneum. There can also be adhesions to other organs.

Organs such as the liver, spleen and kidneys must also be examined as well as the reproductive system of both sexes. Infection of the bones, especially the ribs, vertebrae and joints must not be overlooked.

In the case of infection of the udder it is sometimes found that only one quarter, especially a hind-quarter, near the attachment of the udder, is involved. In chronic cases the lesion develops slowly with: hardening and enlargement of that quarter without pain being elicited. The normal grey-white elastic udder tissue changes and becomes yellowish brown with a firm and hard consistency.

Samples for laboratory examination

The following samples are taken:

- Unstained smears of the lesion, excretions or / and secretions;
- Lesions on ice or
- lesions in a water solution containing 10-20mg of chloramphenicol/ ml;
- 15-20 ml milk with 10-20 mg/ml chloramphenicol;
- samples of the infected parts of the body, organs and glands in 10% formalin. Samples should not be

larger than 25 mm x 12 mm x 6 mm.

1.7 Reasons why Tuberculosis is combated and eradicated

Tuberculosis is one of the controlled diseases in terms of the Animal Diseases Act, 1984 (Act 35 of 1984) and the Standing Regulations enacted in terms of this Act. Specific regulations in terms of the Bovine Tuberculosis Scheme were also proclaimed in GN R1953 of 30 September 1988.

In Table 2 of the Standing Regulations all animals except fish, reptiles and amphibians are indicated as susceptible animals. Over and above the compulsory notification of the disease, the Regulations also entitle Veterinary Services to make tests compulsory where the disease occurs or is suspected and to apply other Regulations such as quarantine, the slaughtering of animals, disinfection, etc. in order to combat and eradicate the disease.

The total waste and damages from an infected herd result from meat and milk condemnation, loss of offspring, decrease in average age, insufficient utilisation of food, decreased milk production, infertility, poor condition and market value.

The overall productive efficiency of infected cows may be reduced by 10 to 25%. Milk production is reduced by 10 to 12%. Involvement of the genital organs is reported to be present in 5 to 11% of cases, and sterility of tuberculous cows increases by 5 to 10%.

Under extensive farming conditions tuberculosis usually spreads slowly. Today it is, however, the tendency to farm more intensively and more and more animals are kept on smaller areas on cultivated pastures, in feed lots and in dairies around the larger cities. Thus favourable conditions are created for the faster spreading of the disease in cow sheds, at feeding troughs and water and by means of licks put out and mass grazing.

The eradication of the disease is also important to protect the country's export trade in animals, meat and animal products. The developed countries of the world are setting increasingly high tuberculosis free demands to countries from where exports are allowed.

As humans, especially young children, are also susceptible to bovine tuberculosis we have a duty in respect of public health to eradicate the disease. In a nationwide study in the USA it has been shown that patients of Hispanic origin aged 15 years or less that were HIV positive, were more likely to be infected with *M. bovis* than by *M. tuberculosis*. Person-to-person transmission of bovine tuberculosis has been reported. In herds where the disease is prevalent the danger exists that people may inhale contaminated air where they come in close contact with the cattle such as in cow sheds. The drinking of unboiled or unpasteurised contaminated milk is possibly the greatest danger for infecting people. A few cases are, however, also known where people have become infected through wounds for example, knife wounds during a post mortem on an infected animal.

1.8 Other animals as a source of *M. bovis* infection for cattle or of non-specific reactions

1.8.1 Humans

People infected with *M. tuberculosis* can also infect cattle. Although progressive lesions usually do not develop, the cattle are in fact sensitised and this makes the interpretation of the tuberculin test all the more difficult. Where people become infected with *M. bovis* the lesions usually occur in the bones, mesenteric (and sometimes other) lymph nodes. There are however also known cases where pulmonary infection occurred in such people with open lesions becoming a source of infection for cattle and other humans. Re-infection of tuberculosis-free herds by people who are infected with *M. bovis* should therefore not be overlooked.

All staff working on infected farms should be tested by the Department of Health.

1.8.2 Pigs

Pigs are susceptible to infection with *M. bovis*, *M. tuberculosis*, *M. avium* and *M. avium-related* bacilli. The lesions that develop are usually not of a progressive nature. The lesions tend to diminish in size over time and to disappear or to become inactive. Where pigs become infected with *M. bovis*, it is usually through the intake of contaminated dairy products, especially milk. The disease does not have the tendency to spread from one pig to

another and it usually disappears if the source of contamination, such as contaminated milk, is removed.

Pigs are usually slaughtered under six months of age, hence it is normally the older breeding pigs that could be infected with *M. bovis* and can pose a danger for re-infection if there is close contact with cattle.

Pigs can be tested for tuberculosis using the intradermal test done in the soft skin at the base of the ear. The test is read after 24 or 48 hours. In pigs with active lesions a skin reaction of 5 mm and more may be observed - sometimes even with necrosis. The test on pigs is, however, not reliable because the animals often overcome active infection. Where pigs are the source of re-infection for cattle, measures will have to be taken to separate the pigs and their products totally from the cattle on the farm. The possible transmission of infection by workers will also have to be prevented. The slaughtering of the pigs, if feasible, will possibly be the best solution with proper meat inspection.

1.8.3 Poultry

Poultry and birds can become infected with *M. avium*. If there is contact between infected poultry or birds and cattle, the cattle can become infected. In cattle *M. avium* usually does not cause visible lesions, but small non-progressive lesions can appear especially in the mesenteric lymph nodes. This is because the main route of infection is oral through the contamination of cattle feed by infected poultry. In exceptional cases *M. avium* causes metritis and mastitis. Lesions in the lungs and general tuberculosis may also occasionally be seen. Infection with *M. avium* sensitizes the cattle and they will react to the Intradermal tuberculin test. When a comparative Intradermal test is done, these animals show a stronger reaction to the avian than to bovine tuberculin. In order to determine the source of infection post mortem examinations can be done on suspect poultry or they can be tested with avian tuberculin in their combs. If poultry are suspected of infecting cattle all poultry should be slaughtered. Further sensitizing of cattle can be prevented by preventing cattle from coming into contact with poultry or their products (such as the feeding of chicken manure to cattle) provided the chickens are the only *M. avium* source of contamination.

1.8.4 Sheep and horses

These animals have a natural resistance and are seldom infected. It therefore only happens by means of exception that the cause of re-infection of cattle will be found among these animals if all the other causes have been eliminated.

1.8.5 Goats

Goats are not infected often. The natural resistance of goats is not high and where they are exposed to a high degree of infection with *M. bovis* large numbers can be infected. Broncho-pneumonia can develop and the gastrointestinal lymph nodes and other lymph nodes can be infected. Where infection in cattle is determined it will be necessary to pay attention to goats especially if there is close contact between them and cattle. Where possible a post mortem examination can be undertaken and the tuberculin test applied if the hair is clipped short. *M. avium* infection has also been found among goats. The infection causes a disease that develops slowly in goats but may be the cause for the disease spreading to other animals.

1.8.6 Pets

The possibility that dogs and cats can be a source of infection to cattle is very slight. Dogs, which are readily susceptible to *M. tuberculosis* infection, are possibly a greater danger to humans than to cattle. Cats, however, are readily susceptible to *M. bovis* infection. On many farms it is customary that cats are kept at the cow sheds to kill off rodents. Usually unboiled milk is given to cats and if the milk is contaminated the cats can become infected and later become a source of reinfection to cattle.

1.8.7 Wildlife

Distribution and history (free-ranging)

- World
- 1919-, Canada: bison and elk
- 1981-89, Australia: fallow deer, water buffalo
- 1984-, USA: bison, elk, deer

- 1993, Sweden: deer
- 1995, Italy: wild boar
- New Zealand: deer, possum, ferret
- England, Ireland: badgers
- 2004, UK: yellow-necked mouse, wood mouse, shrew, polecat, muntjac and stoat
- Africa
 - 1963-, Uganda: buffalo, warthog (1% mortality)
 - 1972-, Zambia: lechwe (20% mortality)
 - 1987, Kenya: baboon
- South Africa
 - 1928, Eastern Cape: kudu, duiker, springbuck?, bushbuck?, bushpig?, hare?, giraffe?
 - 1940, Eastern Cape: kudu
 - 1970, KwaZulu-Natal: black rhinoceros
 - 1977, Kruger National Park: impala (avian?)
 - 1990, KNP: buffalo
 - 1992, Hluhluwe-Umfolozi Park: buffalo, lion
 - 1995, KNP: lion, cheetah, baboon, kudu
 - 1996, Mpumalanga: kudu
 - 1997, Mpumalanga; buffalo, baboon
 - 1998, KNP: leopard
 - 1998, Mpumalanga: lion
 - 1999, Mpumalanga: large-spotted genet
 - 1999, KNP: spotted hyena
 - 2000, Mpumalanga: warthog
 - 2001, Mpumalanga: bushpig
 - 2001, KNP: honey badger
 - 2002, Mpumalanga: eland
 - 2002, KNP: Lichtenstein's hartebeest
 - 2003, Mpumalanga: impala
 - 2004, Mpumalanga: blue wildebeest?
 - 2006: KNP: Bushbuck

Epidemiological factors in transmission amongst wildlife

- Social behaviour
 - Group size
 - Group stability
- Territoriality
 - Size of territorium
 - Overlapping of territoria
 - Migration
- Population density
- Feeding behaviour
 - Predation
 - Scavenging
- Potential for maintenance host
- Stress - nutrition, drought, predation, trauma, other diseases, weaning

Host – either maintenance- (reservoir) OR spillover hosts.

Spillover host = accidental infection, cannot maintain the infection within the population without a constant source of infection from another species in the ecosystem. Faster progressive disease, do not shed for long periods, poor transmitters depending on lesion location. (leopard, cheetah, hyaena (very resistant), african badger, blesbok, meerkat, baboon, spotted genet). Maintenance host = can maintain the infection within the population without constant re-infection from an outside source and without the infectious agent totally eliminating the host population. (cattle, buffalo, kudu, lion, warthog, possibly bushbuck, European badgers). Important in spread of the disease.

Both can be a source of infection both intra-species as well as trans-species (buffalo-buffalo vs. kudu-lion). Same species can be maintenance- / spillover host depending on habits. Baboon at Skukuza sleeping in pump shed

maintained the infection. The prevalence only decreased when the shed was closed to them.

Effect on wildlife

- Clinical signs
 - intestinal – emaciation
 - lymphnodes – abscesses
 - respiratory – coughing
 - lagging behind herd
- Normal reproduction
- Death
- Group structure
- Population size

Indirect

- Contaminate environment
- Infect other wildlife species

Implications

- Movement restrictions
 - Limited marketing of live game
 - Isolated “conservation islands”
- Eco-tourism
- Hunting
- Spread to free areas
- Infect cattle etc.
- Infect humans (zoonosis)

Surveillance

WHAT?

- Post mortem examinations
- Intradermal tests (buffalo, lion)
- Gamma interferon tests (buffalo)
- Bacteriological culture

WHEN?

- Hunted animals
- Culled animals (sick, reduce numbers)
- Movements
- Abattoir
- Dead animals

HOW?

- Training
- Extension > awareness

Control options

- “Laissez faire” (= do nothing)
- Movement control
- Prevalence reduction
- Test and slaughter
- Depopulation
- Vaccination? (not yet!)
- No treatment!

In advanced cases Kudu show a typical swollen parotid lymph node at the base of the ear as below.



Figure 7 TB in parotid lymph nodes of Kudu



Figure 8 Lion with TB.

2 BASIC IMMUNOLOGY

2.1.1 INTRODUCTION

Definition:

Immunology is the study, in our case, of how the body of an animal protects itself from infectious agents such as bacteria, viruses, fungi and other harmful materials (internal and foreign).

The immune system of the body can be compared to the security forces of a country. Around the country there is a border area that is fenced (skin, mucous membranes) and is patrolled regularly by the border soldiers (macrophages, neutrophils, etc.). If they find any intruder (e.g. bacteria or virus) that does not have the right passport, it is either destroyed immediately or it is taken to their headquarters (lymph node, spleen, etc) for further identification. If it is a new type of intruder, soldiers are then specially trained to identify similar intruders more quickly in future and to kill them (T- and B- cells that transform into memory cells after interaction with a specific pathogen).

The police also take action against inhabitants of the country that do not obey the rules of the country (cancer cells). The security forces must therefore have the ability to distinguish between that which is "own" and that which is foreign and potentially dangerous. Just as people carry passport and ID- documents to identify themselves, every cell of the body has identification markers (tissue compatible antigens). They can thus be distinguished from intruders and cancer cells.

Not all people (foreign substances) entering the country are necessarily harmful (food) and must be tolerated by the security forces (tolerance). Sometimes the security forces overreact and act against non-harmful substances that enter the country (e.g. pollen is not harmful but can cause hay-fever and allergies).

All security forces have a variety of weapons at their disposal. Depending on the weapons the enemy is using, the size of the enemy, etc. different types of weapons and methods are used by the security forces to combat the attack. These include chemical weapons (interferon, porforins and complement), hand-to-hand combat (macrophages and neutrophils), inactivation (antibodies) and encircling and smothering actions (macrophages that stick together and form granulomas).

2.2 TYPES OF IMMUNITY

2.2.1 NON-SPECIFIC IMMUNITY

Non-specific immunity includes those defenses directed against pathogens, foreign material etc that are not specific to each pathogen or that are not directed against specific invaders. For example: physical barriers, chemical barriers, some cellular defenses, inflammation, fever, etc.

2.2.2 SPECIFIC IMMUNITY

Specific immunity is that aspect of the body's defenses directed against specific pathogens and foreign material and usually requires that the immune system learns the properties of the specific pathogen over a number of days or weeks before mounting an effective response against it. Typically a specific immune response against one pathogen will be largely ineffective against a different pathogen, even if the second pathogen is closely related to the first one (but can get some response).

Specific immunity includes humoral and cell-mediated immunity. A number of body organs, tissues and cell types are involved in effecting each of these forms of specific immunity (see below).

Specific immunity is further described as being naturally acquired (colostrum) or artificially acquired (vaccination) and actively acquired (disease challenge, vaccination) or passively acquired (colostrum, antiserum).

Innate Immunity (Genetic Immunity/Species Immunity) is present before an animal is exposed to a pathogen. It is due to the pathogen's inability to cause disease in a species because it has not adapted to that species e.g. horses do not contract swine fever.

2.3 THE ORGANS OF THE IMMUNE SYSTEM

2.3.1 BONE MARROW:

All cells of the immune system are initially derived from bone marrow. They form through a process called hematopoiesis. During hematopoiesis, the bone marrow-derived stem cells differentiate to either mature cells of the immune system or into precursors of cells that migrate out of the bone marrow to continue their maturation elsewhere. The bone marrow produces B-cells, natural killer cells, granulocytes (neutrophils, eosinophils, basophils) and immature thymocytes, in addition to red blood cells and platelets.

2.3.2 THYMUS:

The function of the thymus is to produce mature T-cells. Immature thymocytes, also known as prothymocytes, leave the bone marrow and migrate into the thymus where they mature into T- cells. The mature T-cells are then released into the bloodstream.

2.3.3 LYMPH NODES:

The lymph nodes function as an immunologic filter for the body fluid known as lymph. Lymph nodes are found throughout the body. They are mainly composed of T-cells, B-cells, dendritic cells and macrophages.

2.3.4 SPLEEN:

The spleen is the immunologic filter of the blood. It is made up of B-cells, T-cells, macrophages, dendritic cells, natural killer cells and red blood cells. In addition to capturing foreign materials (antigen) from the blood that passes through the spleen, migratory macrophages and dendritic cells bring antigens to the spleen via the bloodstream. An immune response is initiated when macrophages or dendritic cells present the antigen to the appropriate B or T-cells.

2.3.5 MUCOSA-ASSOCIATED LYMPHATIC TISSUE (MALT):

Organ-like aggregated lymphatic nodules are found in the digestive, respiratory and urogenital tract. Those found in the pharynx and caudal oral cavity (the back of the mouth) are called tonsils.

In the gut or intestines they are called gut-associated lymphatic tissue (GALT), which includes solitary and aggregated lymphatic nodules (Payer's patches).

2.4 THE CELLS AND MOLECULES OF THE IMMUNE SYSTEM

The Immune system is comprised of a variety of different cell types and proteins. Each component performs a special task aimed at recognizing foreign material (antigen) and/or reacting against foreign material. For some cells recognition of the material as foreign to the body is their primary and only function. Other components function primarily to react with the foreign material whereas others function to both recognize and react against the foreign material.

2.4.1 **B-Lymphocytes (B-Cells):**

These cells mediate humoral immunity. The major function of B lymphocytes is the production of antibodies in response to foreign protein (antigen) of bacteria, viruses or tumour cells. Antibodies are specialized proteins that specifically recognize and bind to one particular protein.

For every foreign antigen, there are antibody molecules specifically designed for that antigen. Antibody production and binding to the foreign substance or antigen is critical as a means of signaling other cells to engulf, kill or remove that substance from the body. There are five major classes of antibodies or immunoglobulins (Ig): IgG, IgA, IgM, IgE and IgD.

2.4.2 **T-Lymphocytes (T-Cells):**

These cells mediate cell-mediated immunity. T-lymphocytes do not produce antibodies. The specialized roles of T-Cells are to directly attack foreign antigens such as viruses, fungi or transplanted tissues and to act as a regulator of the immune system.

2.4.3 **Macrophages:**

They are often referred to as scavengers because they pick up and ingest foreign materials (antigens) and present these antigens to other cells of the immune system. This is one of the important first steps in the initiation of the immune response.

2.4.4 **Dendritic Cells:**

They also capture and present antigens to cells of the immune system. They are mainly, but not exclusively, found in the structural compartment of the lymphoid organs such as the thymus, lymph nodes and spleen.

2.4.5 **Granulocytes (Polymorphonuclear Leukocytes):**

These are commonly known as Neutrophils, eosinophils and basophils - based on their staining characteristics with certain dyes. They are predominantly important in the removal of bacteria and parasites from the body, which they do by engulfing them and degrading them using powerful enzymes.

2.5 THE IMMUNE RESPONSE

(See also accompanying diagrams)

An immune response to a foreign antigen requires the presence of an Antigen-Presenting Cell (APC) – usually a macrophage or dendritic cell which engulfs the antigen, processes it internally and then displays parts of the antigen on its surface, thereby “presenting” the antigen to either a B-lymphocyte or T-lymphocyte.

a) Cell Mediated Immunity (TB)

Helper and Killer T-cells are activated (sensitized) and multiply. Killer T-cells, with the help of the Helper T-cells and other cells such as macrophages, kill any body cell infected with that specific kind of antigen. During the replication of the T-cells, Memory T-cells are produced. These circulate in the body and result in an improved response rate to any subsequent infection by the same kind of antigen.

b) Humoral Immunity (CA)

B-cells are activated and multiply to form plasma cells and memory cells. The plasma cells produce highly specific antibodies (also called immunoglobulins) which inactivate the antigen using a variety of tactics. The memory cells circulate and give rise to a faster and bigger response should that specific antigen invade the body again.

Tuberculosis anergy

(see chapter 3 for more detail)

The largest and most typical reactions to an intradermal tuberculin test occur in the early stages of infection with *M. bovis*. This sensitivity lessens later on during the chronic (advanced) phase of the disease when the animal’s immune system gradually switches from a cell-mediated reaction (responsible for the skin reaction) to a humoral antibody – based reaction. Thus the intradermal test becomes less effective in detecting infected animals and in some cases the sensitivity disappears completely and the animal, although still infected, shows no reaction to the tuberculin test at all. This is known as anergy.

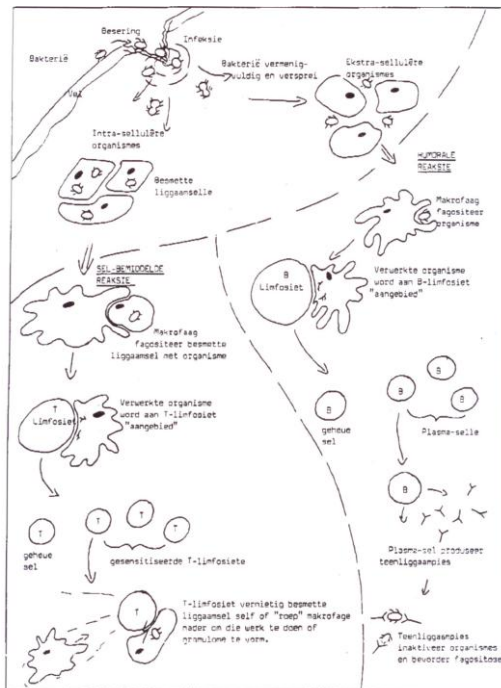


Figure 9 the immune reaction – cell-mediated: humerol

Fig 10:

This diagram illustrates the movement of cells after the intradermal administration of tuberculin.

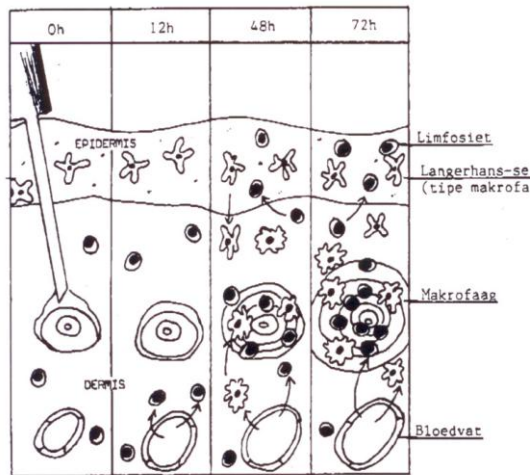


Figure 10 Tuberculin-reaction

- 12 h - Lymphocytes start to migrate from the local blood vessels.
- 48 h - Lymphocytes and macrophages migrate from local blood vessels. Langerhans' cells migrate out of the epidermis. Lymphocytes and macrophages start to clump together at the injection site.
- 72 h - The congregation of cells result in the thickening of the skin. This is a visible and measurable reaction.

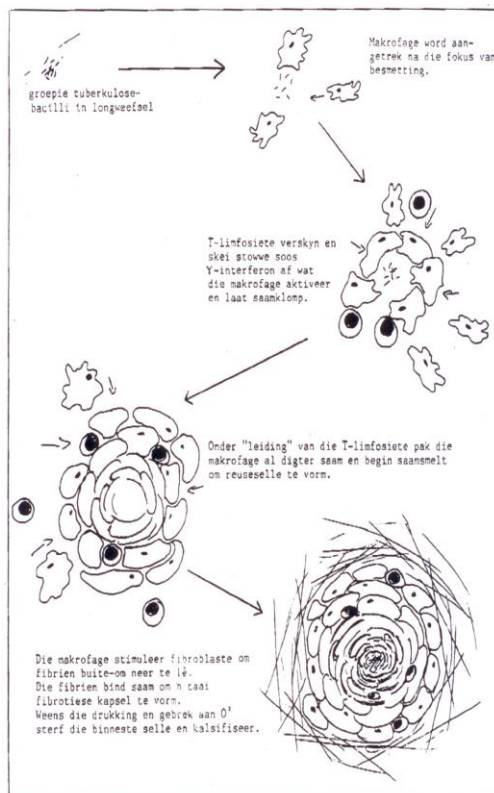


Figure 11 Granuloma formation

3 THE INTRADERMAL TEST FOR BOVINE TUBERCULOSIS

3.1 INTRODUCTION

The tuberculin test is today possibly the biological test used most often by veterinary & para-veterinary personnel throughout the world for diagnostic purposes. The test enables one to determine which animals in a herd are infected with bovine tuberculosis. The countries that have already combated and eradicated the disease successfully and efficiently made use of the tuberculin test.

The test is prescribed by the OIE for international trade and used in tuberculosis control programmes worldwide but there are different approaches in the interpretation of test results. Within a country it is important that all officials and private veterinarians use the same standards of testing and interpretation.

It must also be remembered that the test is a **HERD TEST** and the first and most important diagnosis that must be made is whether the herd is infected or not. Only after this decision has been made can the individual animal's reaction be assessed.

In South Africa tuberculosis is a controlled disease and all testing is done under the control of the Director of Animal Health of the Department of Agriculture, Forestry and Fisheries, and according to the Animal Diseases Act, 1984 (Act No 35 of 1984) and the Regulations pertaining to this Act. (See Chapter 4)

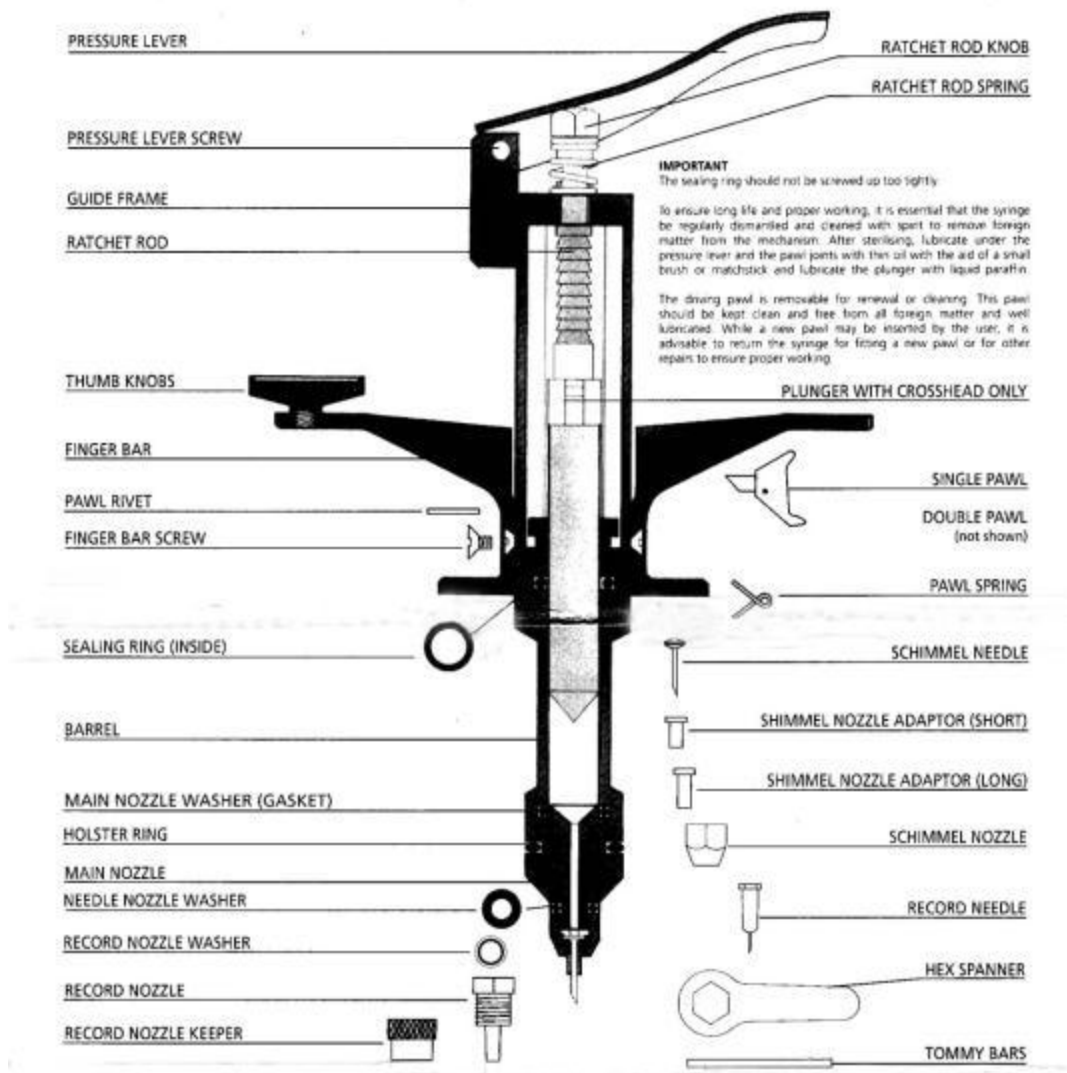


Figure 12 Parts of McClintock syringe

M. Maja
 Approved: Dr M. Maja

3.2 TESTING EQUIPMENT

3.2.1 McCLINTOCK SYRINGE

Although injection of 0.1 ml of tuberculin can be done using a plastic disposable syringe, when testing large numbers of cattle, it is more practical and accurate (the tuberculin is definitely injected intradermally, whereas with a disposable syringe it may be injected subcutaneously) to use the specially designed McClintock syringe. This syringe is specifically built for tuberculosis testing and is relatively robust and automatically injects 0.1ml of tuberculin.



Figure 13 McClintock syringe and accessories

Before use, the McClintock syringe must be checked and the plunger oiled with small amounts of the lubricant supplied with the syringe or liquid paraffin. The plunger should move smoothly and easily. The syringe should then be rinsed out by filling it with distilled water and injecting this water out. By doing this, the working of the syringe is checked and any leaks or loose parts can be identified and remedied before starting the tuberculin test.

Two syringes are needed for the comparative test and the appropriate coloured disks should be placed on the handles to avoid confusion during the test. A third syringe should be available in case one breaks.

The syringe with the red disk is used for avian tuberculin and one with the blue disk for bovine tuberculin.



Figure 14 McClintock syringes showing coloured discs

Once a comparative test has started it is essential to ensure that the syringes are not swapped i.e. the syringe used for avian tuberculin must only be used for avian tuberculin.

It must be remembered that these syringes are precision instruments and are expensive to replace. They must therefore be handled with care and maintained on a regular basis.

Spares for the McClintock syringes can be ordered from the suppliers. The most common breakage is to the handles. (See figure 12 for parts of the syringe)

DISINFECTION OF SYRINGES

Before using a syringe test the functioning as described in section 3.2.1.

Where large numbers of cattle are tested, the syringes should be cleaned and disinfected after every 100 animals, or if it has fallen in dung. In the latter case the outside of the syringe should first be rinsed in clean water. The syringe is then filled and rinsed out using sterile water, then filled with 70% ethyl alcohol and left for at least 5 minutes. The alcohol is then ejected and the syringe is again rinsed using sterile water. The rinsing of the syringe should be repeated a number of times to ensure that all traces of the alcohol are removed. At the same time the

condition of the needle should be checked and replaced if necessary.

If more than one herd is being tested on a day, syringes must be cleaned and disinfected as above between herds.

Once back at the office, the McClintock syringe must be rinsed externally under running water and then filled out with sterile water to remove all traces of tuberculin, dismantled and sterilized by boiling in water for at least 20 minutes. The rubber O rings and plastic handles must be removed from the syringe before boiling, and placed in 70% ethyl alcohol. After the syringes have cooled, they are reassembled and the plungers oiled with liquid paraffin or the special supplied oil. They are then stored in their cases with the plunger in the middle. A new needle can also be inserted at this stage if necessary.

3.2.2 NEEDLES

Only the special needles (Record or Schimmel) designed for the McClintock may be used. The correct needle is 3.9 mm long (5/32") (Record). This length helps to ensure that the injection takes place in the correct level of the skin and avoids subcutaneous injection. Needles should be examined at regular intervals during a test and replaced if bent or damaged in any way. It is of vital importance to check the needle and syringe if it has been dropped or hit against the crush pen by the sudden movement of an animal. It is important to develop a feel for a sharp needle and as soon as it is felt that the needle is blunt then it should be changed.

Never use a needle that has developed a burr on the point or that has been bent or damaged in any way. Do not try to straighten a bent needle, rather replace it.



Figure 15 Showing bent needle

The number of needles required will depend upon the type used. A short firm needle such as the above-mentioned should last long so that it shouldn't be necessary to have more than ten needles during tests and a supply of five dozen needles at the central office should be enough. The use of types of needles other than those manufactured for the McClintock syringe is unacceptable.

3.2.3 CALIPERS

To measure the skin thickness, a pair of calipers is used. There are a number of different types available.



Figure 16 Hauptner calipers being used to measure skin thickness

The Hauptner pistol grip (broad lipped type) with a dial indicator which can measure one tenth of a millimeter is the standard. This instrument has a spring loaded jaw which ensures that a standard pressure is placed on the skin swelling. It does have the disadvantage that this pressure can flatten an oedematous swelling if the reading is not taken quickly enough. They can also be clumsy to use on wilder animals that do not stand still.

The pointer on the dial of these calipers moves completely around the dial for every 10mm of skin thickness

measured. The slide is also marked at 10mm intervals and this needs to be referred to when the skin thickness is more than 10mm. Care must be taken when reading large skin increases as it is easy to make a mistake of 10mm.e.g. a skin reaction with a thickness of 25mm will show 5mm on the dial but the markings on the slide would show a position between 20mm and 30mm. the final reading would then be the 20mm shown on the slide and the 5mm shown on the dial =25mm.

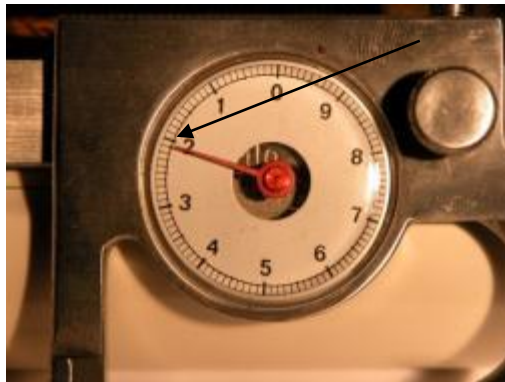


Figure 17 Dial showing a reading of 12.1mm

(Note marking on slide)

Other calipers that have to be opened and closed by hand can be used by testers who have more experience. These are faster and easier to use but it is only with time and experience that testers learn how to apply the same pressure on the swelling every time. With these calipers it is difficult to read in fractions of a millimeter. In order to maintain a standard it is essential that the same person reads both the normal skin and the reaction. Only one instrument is required for tests but it is advisable to have an additional pair of calipers handy in case one is damaged.

3.2.4 HAIR CLIPPERS

Before the injection of tuberculin, an area on the side of the neck must be clipped free of hair. This is to identify the injection site as well as to ensure the injection can be done intradermally and cleanly.

It is not acceptable to identify the injection site with paint.

Before shaving an area it must be inspected and palpated to ensure that there are not any existing lumps, adhesions or other skin damage that could confuse the interpretation of the test.

The area clipped should be large enough to be visible and allow easy estimation of the centre point for the injection.



Figure 18 Animal showing existing lumps on neck & how NOT to shave

A square area the width of the clippers is suitable



Figure 19 Suitable clipped area

The standard way to clip this area is to use portable hair clippers, either rechargeable or those that can run from car batteries. The rechargeable ones are easier to use without the problems of cables and needing a vehicle close to the crush pen.

The clippers should have a fine blade (0 or OO) installed to ensure that all hair is removed from the injection site. Clippers need constant cleaning and oiling to avoid jamming and the blades should be sharpened by a professional company at regular intervals.

When testing large herds ensure that enough clippers are available to avoid the rechargeable batteries running flat.

Clippers with fine blades cannot be used on very dirty animals with long hair. Also the noise that clippers make often scares animals, especially Brahmins!

After use the blades should be removed, thoroughly cleaned and oiled and the battery recharged.

Before a test is done, check the clippers and ensure that the batteries are fully charged.

Hand clippers can be used but are clumsy and very slow. They also do not shave long and dirty hair well.

A pair of curved scissors can be used as a backup and can be useful for very long and dirty hair. They are often used to first remove long hair before using a clipper to shave the site.

Farmers should be advised before the test not to dip animals on the day of the test as wet animals should not be tested.



Figure 20 Rechargeable clippers and razor

An ordinary double sided razor can be used by testers with experience. This method is NOT recommended as it is dangerous to the operator and also can damage the skin at the injection site which complicates the interpretation of the test reaction.



Figure 21 Damage caused by razor

3.3 OTHER EQUIPMENT

3.3.1 BELT AND HOLSTER

A belt with holders for the McClintocks and calipers can be useful to hold the equipment securely and out of harms way whilst not being used. This avoids the need for an assistant. If the McClintock syringes are not going to be used for a longer period, they must be replaced in the cooler box.

3.3.2 CLIPBOARD

It is essential to have a clipboard to hold writing paper and the TB 10 forms. The clipboard should have a pen or pencil attached to it.

3.3.3 COTTON WOOL

Cotton wool is handy to clean the injection site if it is soiled. It should be used dry if possible. If the injection site is so dirty that it needs to be washed, then the cotton wool is used to dry off the site. The injection site must be clean and dry before injecting tuberculin

3.3.4 DISINFECTANT

Usually no disinfectant is used on the injection site. If the site is very soiled, then it can be disinfected using ethyl alcohol (or methylated spirits). If the site is disinfected then it must be given enough time to dry completely before the tuberculin is injected.

Bottles containing 70% ethyl alcohol and sterile water must also be taken with in order to disinfect syringes during the test as described under 3.2.1.1 above.

3.3.5 FOLD UP TABLE

A sturdy fold up table is essential to enable equipment to be stored off the ground and can also be used for writing of notes and completion of TB 10 forms.

3.3.6 TOOL BOX

A sturdy steel or hard plastic box to hold all the equipment needed for tuberculin testing should be used. This not only protects the equipment, but also ensures that all equipment is kept together. A record of the contents of the box should be taped to the inside of the lid. This assists with the checking and packing of equipment after a test is completed.

3.3.7 UMBRELLA

Most tuberculin tests are performed outdoors, often without any shade. An umbrella provides shade for the equipment and cool box, thus ensuring the correct temperature is maintained. It also provides shade for officials whilst waiting for animals to arrive.

3.3.8 HEBCOOLER

A cooler box is an essential part of testing equipment and the tuberculin must be kept between 4 - 8°C in the dark during the test. The cooler box should be large enough for the amount of tuberculin needed for the testing as well as sufficient ice packs to ensure the maintenance of a temperature of 4 – 8°C at all times. NOTE: Tuberculin bottles should be surrounded by, but not in direct contact with ice packs to avoid freezing!

3.3.9 CHECKLIST

A checklist is provided as an annexure to this manual. This can be reproduced and used in the field.

3.3.10 FIRST AID KIT

Performing a TB test can be dangerous and officials should always carry a basic first aid kit so that minor injuries can be correctly treated.

3.3.11 PLIERS & THIN SCREWDRIVER

Always good to have for assisting in fixing McClintock syringes and thin screwdriver also useful for assisting in filling McClintock from the 18 dose vials.

3.4 ANIMAL HANDLING EQUIPMENT

The normal animal handling equipment should be available during tuberculin testing, i.e. nose tongs, ropes, suitable crush pen and neck clamps. It is often difficult to perform a tuberculin test on animals restrained in a neck clamp as it interferes with access to the side of the neck. This should only be used for animals that cannot be tested in the crush. If cattle are correctly and firmly packed into the crush pen then the shaving and injection should be possible with few problems.

Facilities on farms vary greatly and care must be taken to ensure both the safety of the persons testing as well as to ensure that the test can be performed properly.



Figure 22 Farm with excellent facilities

(Photo by Dr. Davey)



Figure 23 Farm with reasonable facilities



Figure 24 Animal in neck clamp

3.5 IMMUNOLOGY OF TUBERCULOSIS

3.5.1 GENERAL INTRODUCTION TO IMMUNOLOGY

(See Section 2)

3.2.2 TYPES OF IMMUNITY

a.) CELLULAR IMMUNITY

It is also called non-specific immunity and is designed to form a quick and first line of defense in response to the intrusion of pathogens into the body. The means of defense are uniform regardless of the type of pathogen.

b.) HUMORAL IMMUNITY

It is also called specific or antibody-based immunity because it is based on the formation of specific antibodies to a particular pathogen.

c.) DETECTION OF IMMUNE REACTIONS IN THE DIAGNOSIS OF TUBERCULOSIS

The type of immune response which is predominantly elicited during the course of a particular disease depends on the initial interaction of the causative pathogen with the animal's immune system. Tuberculosis infection typically induces a cellular response and this limits the initial spread of disease in the animal. The formation of microscopic and small visible lesions represents the stage of equilibrium between replicating mycobacteria and local cellular host defense. If the host fails to contain the mycobacteria in this way and the formation of multiple lesions increasing in size continues, the humoral immune response and production of antibodies is induced. For a certain period of time both cellular and humoral immune responses are detectable before the cellular immunity decreases and eventually disappears (anergic phase, see also 'Anergic reactors').

Among a group of cytokines involved in anti-tuberculous host response, gamma interferon is the principal mediator of cellular immunity. It is released by T-cells of an infected animal upon presentation of mycobacterial antigens such as tuberculin. Both the tuberculin test and the gamma interferon test are based on the detection of gamma interferon and its effects.

In contrast, antibodies to *M. bovis* can only be detected by serological techniques. The application of such tests is particularly relevant:

- In tuberculosis outbreaks which are not eradicated within a period of several months. It is likely that anergic reactors (infected but tuberculin test negative cattle with advanced disease) have remained undiagnosed but may serve as large scale spreaders of the disease.
- If single or very few animals from herds with unknown or positive infection status have to be certified free of tuberculosis. There is, strictly speaking, a risk of underlying anergic reactions which can only be eliminated by the use of a combination of cellular based and serological tests.

3.6 TUBERCULIN

Bovine tuberculin PPD (purified protein derivative) is prepared from protein contained in the culture filtrate of the *M. bovis* tuberculin production strain AN5.

When injected into the skin of a bovine infected with *M. bovis*, this tuberculin causes a delayed hypersensitivity reaction. This results in an inflammatory reaction at the site of injection which causes the typical signs seen in positive animals.

According to the OIE Terrestrial Manual 2009, a dose of tuberculin injected must be no lower than 2000 International Units (IU) of bovine and avian tuberculin. The OIE further advises that in cattle with diminished allergic sensitivity, a higher dose of bovine tuberculin is needed and in national eradication campaigns, doses of up to 5000 IU are recommended.

All tuberculin is issued with a lot number and an expiry date. These must be checked and recorded before the start of a test.

NB: Tuberculin must never be used after its expiry date.



Figure 25 Old type OBP Tuberculin showing batch number and expiry date

Tuberculin must be stored and maintained at 4 - 8⁰ C in the dark at all times. In the office it must be stored in a refrigerator and never frozen. Any tuberculin that has been frozen must be discarded. The temperature in the office fridge should be checked regularly (every week) using a laboratory thermometer.

When transporting tuberculin it must be kept in a coolbox at all times surrounded by sufficient ice packs to ensure that a temperature of +/- 4⁰C is maintained. This coolbox must be kept in the shade in the coolest place near the crush pen.

Tuberculin should never be shaken or exposed to direct sunlight. When filling syringes the bottle should be protected from direct sunlight.

Avoid shaking tuberculin when filling syringes.

Before filling a syringe examine the tuberculin, check the expiry date and ensure that the fluid is clear without any sediment and that the correct tuberculin is filled into the correct syringe. Tuberculin that has a cloudy appearance must never be used.

At the end of a test any tuberculin left in the McClintock syringes must be discarded and the contents of the vial must also be used on the day that the vial is opened and not thereafter.



Figure 26 Old Type Bovine and Avian tuberculin bottles



Figure 27 German Avian tuberculin



Figure 28 German 18 dose bovine tuberculin vials



Figure 29 20 dose bovine tuberculin

3.7 TESTING PROCEDURE

3.7.1 SELECTING THE TYPE OF TEST

Before a test is organized the history of that herd must be determined and recorded.

The comparative intradermal test is generally used for herds:

- that are tested for the first time;
- where the tuberculosis status or the history of the herd is unknown or vague
- where non-specific reactions occur;
- where problems are experienced with the interpretation of tests.

The simultaneous use of both the avian and bovine tuberculin is therefore mainly a diagnostic aid to obtain a more definite diagnosis in respect of the presence or absence of bovine tuberculosis in a herd.

The single intradermal test on the other hand is mainly used where:

- the negative tuberculosis status of the herd is known;

Positive herds can be tested with the single intradermal test, depending on the previous reactor rate and the use of additional tests such as gamma interferon test and serology.

3.7.2 SITE OF INJECTION

It is known that the skin of an animal does not show the same degree of sensitivity to tuberculin everywhere on the body. There is an increase in sensitivity from the back towards the front and from the bottom to the top of the body. A sensitivity of 1 in the skin of the hindquarters increases to a 3 in the neck.

The same dosage of bovine and avian tuberculin is injected during a test. It is therefore important to inject both tuberculins in an area with the same sensitivity. This will prevent the sensitivity of the skin from having an effect on the interpretation of the test.

CERVICAL TEST: The tuberculin is injected on the side of the neck, midway between the head and the shoulder and halfway between the top and bottom of the neck. **In South Africa this is the only approved site for intradermal TB testing in cattle.**

CAUDAL FOLD TEST: Tuberculin is injected into the deeper layers of the skin on the lateral aspect of the caudal fold, midway along the fold and midway between the hairline and the lower aspect of the fold.

NOTE: The caudal fold should not be the first choice for injection because of decreased sensitivity and potential complication in the interpretation of results. **If the caudal fold is used double strength tuberculin must be used.**

a.) SINGLE INTRADERMAL TEST

A place on the side of the neck halfway; between the juncture of the head and neck and the fold in front of the shoulder and halfway between the top and bottom of the neck is chosen. The place is palpated to determine whether the skin texture is normal without lumps in or under the skin and to ensure that there are no adhesions between the skin and the subcutaneous tissue.

It is necessary that all the animals of one herd be tested on the same side be it on the left or the right hand side.

b.) COMPARATIVE TEST

When a comparative test is being performed then two sites on the same side of the neck need to be prepared. The bovine tuberculin is injected closer to the shoulder, whereas the avian tuberculin is injected closer to the head. The two sites should be at least 15cm apart and both sites must be palpated and found free of lumps, scars or adhesions.

It is sometimes recommended that the avian tuberculin is injected on the opposite side of the neck. This method

is difficult to perform under field conditions.

For practical purposes preference is given to the method where both tests are performed on the same side of the neck.

c.) DISINFECTION OF SYRINGES

Before using a syringe test the functioning as described in section 3.2.1.

Syringes must be disinfected after use, between herds and also after every 100 animals See Section 3.2.1.1 for details

d.) FILLING OF SYRINGES

With old type tuberculin bottles as in figure 26 as well as new type bovine tuberculin bottles as in figure 29:

Withdraw the plunger of the syringe to approximately the 0.5ml mark. The needle is then inserted into the bottle and the air expelled into the bottle. This increases the pressure inside the bottle and makes the filling of the syringe easier and faster. The syringe and bottle must be held upright and the plunger is slowly withdrawn, drawing the tuberculin into the syringe. Whilst filling the syringe the tuberculin must not be exposed to sunlight.

Filling syringe from German vials: (fig 27 & 28)

- Empty syringe completely
- The needle is then inserted into the vial and whilst withdrawing push rubber stopper down gently with a thin screwdriver or needle
- The syringe and bottle must be held upright and the plunger is slowly withdrawn, drawing the tuberculin into the syringe.
- Make sure that tuberculin is not exposed to sunlight

When all the tuberculin is in the syringe, then one dose is injected back into the bottle. This should appear as sharp solid jet of fluid. If there is any air in the syringe then the air can be seen as it is injected into the bottle. If air is present then extra doses must be injected into the bottle until no air is present in the syringe at all.

In most cases this will only be one or two injections.

Do not waste tuberculin by injecting into the bottle un-necessarily.

NB: If any air is left in the syringe then a full dose will not be injected into the animal and the test could show false negative results.

This will be seen as a dribble when the syringe is moved away from the animal

e.) INJECTING TUBERCULIN

Tuberculin must be injected *intradermally* at the shaven site. The injection should be made in the centre of the shaven site. This can be estimated by taking diagonals from each corner, where they meet is the centre of the site. The syringe is held in the hand in such a way that the short needle can be pushed into the skin at an angle from the top. This ensures that the tuberculin is at a lower level than the injection wound so that the tuberculin will not flow out spontaneously after the needle has been removed.

If the syringe is held in the hand, and the knuckles are held against the neck of the animal and moved smoothly downwards the angle is correct.



Figure 30 Correct angle to inject

It is only with experience that the correct injection technique can be perfected. The needle is pushed into the skin with a smooth movement, it must not be jabbed or stabbed into the skin.

Potentially complicating factors:

- A rough injection often does not go intradermally and can also cause skin damage that will interfere with the interpretation of the test reactions.
- If the injection is too shallow the tuberculin will be injected into the hard horny tissues of the epidermis. This gives a very high resistance when the injection is made. The reaction to the tuberculin will also be poor in this layer of the skin due to the poor blood supply.
- If the needle is too long or the injection made with too steep an angle, the needle may penetrate the skin and the tuberculin deposited under the skin. In this case there is very little resistance to the injection. The reaction caused by subcutaneous injection of tuberculin is very variable and cannot be used in interpreting the test.
- The correct site of injection is into the lower layers of the epidermis, in this case there is a measure of resistance when injecting and the animal often shows a pain reaction. This site will also cause a pea sized nodule in the skin. It is essential that after every injection, the site is gently palpated to ensure that this nodule is present. If no nodule can be felt the injection has not been made correctly and must be repeated.

f.) TEST PERIOD

After the tuberculin is injected the test is read (interpreted) after a period of 72 hours (3 days) without exception, otherwise the test will have to be repeated after 3 months.

There are cases where the reaction peaks earlier or later than 72 hours but these are the exception and not the rule. If a large oedematous swelling is present it may have lost a lot of fluid before the 72 hour reading and will be smaller. However this reaction will still be interpreted as a positive reaction after the 72 hours.

3.8 READING OF RESULTS

A diagnosis is not made only on the skin measurement. Every reaction must be examined and fully described.

The difference between reading the results and the interpretation must be understood.

Reading the results means looking, feeling and measuring the reactions and then fully describing them.

Interpretation is done after the whole herd has been examined and all the reactions are taken into account. Using this information the decision is made as to the status of the herd.

Only once the status of the herd is established can interpretation of the individual animals be performed.

NB: Animals must not be allowed to run through the crush and only a visual inspection done. Every animal must be held in the crush and each injection site must be examined and palpated. If there is any sign of a reaction in any animal then the skin measurement must be taken.

It is ideal to measure each injection site on the day of injection, but if this cannot be done due to the number of animals to be tested, then the normal skin measurement can be taken from a site directly above the injection site with no lumps, adhesions or other skin damage. The same person must take the pre-injection and post-injection readings.

It is essential when reading a test that every single animal that was injected is again present on the day of the reading. This emphasizes the importance of identification of animals and proper recording of these numbers on the day of injection.

All the senses must be used when examining a reaction.

- Observation
- Feeling (Palpation)
- Hearing
- Measurement

3.8.1 Observation

By looking at the injection site a swelling or lack of swelling can be noticed. If a reaction is seen then the following can be checked:

- The appearance of the swelling may be round or flat.
- The swelling may be clearly demarcated from the surrounding normal skin (circumscribed) or gradually runs into the surrounding tissues with no clear boundary (diffuse)
- Colour changes such as blue and red may be seen in light coloured skin
- Signs of oedematous fluid oozing from the swelling (exudation)
- A central area of dead skin (necrosis) may be seen
- Where the lymphatic system is involved the swollen lymph ducts can be seen.

3.8.2 Palpation

The injection site of every animal that was injected must be handled when reading a test. Many reactions may not be readily visible to the naked eye but can be felt when the skin is palpated.

The following signs of a reaction can be felt:

- Consistency, hard or soft swelling
- Oedema
- Heat
- Pain when site is palpated
- Adhesions between the skin and the subcutaneous tissues

3.8.3 Hearing

Listen to any animals that cough and make a note of their identification. The reactions in these animals should be carefully checked. Make enquiries about the herd's history in respect of tuberculosis.

3.8.4 Local Reactions

The local reactions that can be seen and felt at the tuberculin injection site must be carefully and correctly recorded on the TB 10 in the remarks column when reading a test.

These signs and the skin measurements must be recorded for each animal with a reaction and the TB 10 must be completed as each animal is described.

a.) Redness (Rubor)

This reaction must be described in the test report.

Redness can only be seen in animals with a lighter coloured skin. Such a change usually indicates infection.

b.) Oedema (O)

This is indicated on the TB 10 by the abbreviation O.

Oedema is the abnormal accumulation of fluid in a tissue and can be felt as a soft or hard swelling. If it is pressed then a pit remains when the pressure is released. This is called pitting on pressure.

Oedema can be felt when the lesion is palpated, it may also be circumscribed or diffuse.

Oedema is a very positive sign and almost always indicates infection. In an infected herd oedema should always be considered to indicate a positive reaction, even if the skin thickening indicates a negative reaction.



Figure 31 Large diffuse oedematous swelling

c.) Necrosis

The reaction must be described on the TB 10.

Necrosis, or the dying off of cells can be seen as a round dark area in the centre of the reaction. It is normally surrounded by an area of intense inflammation.

The necrosis is caused by the swelling of the tissue and the interruption of blood supply to the central areas of the reaction.

Necrosis is regarded as a strongly positive sign and is oftenseen in animals that have recently become infected and whichshow a severe reaction to the tuberculin test.

d.) Exudation

The reaction must be described on the TB 10.

Exudation is the leaking of fluid from the swelling.

Such an exudation of fluid may reduce the size of the skin thickening but exudation is always regarded as a strongly positive sign.

The exudates may dry on the skin surface and then it appears as a dry scab or clot on the skin surface.

e.) Lymph ducts/nodes

The reaction must be described on the TB 10.

The lymph nodes of the area next to the reaction may be enlarged and painful in the case of infected animals.

The lymph ducts draining the area are swollen and may easily be seen. The hair over the swollen lymph ducts stands erect and can easily be seen.

The prescapular lymph node should be firmly palpated between the fingers and the thumb. The size of the lymph node on the side of the tuberculin injection should be compared with the lymph node on the opposite side of the body.

Many animals will show a pain reaction when a normal prescapular lymph node is palpated.

The involvement of the lymphatic system is a very positive sign.

f.) Pain (T) (Dolor)

This is indicated on the TB 10 by the abbreviation T (Tender).

During the examination of an animal that shows a reaction the swelling is also handled and palpated. If the animal stands still and shows no signs of uneasiness it can be accepted that the swelling is not tender. However, if the animal moves about and pulls away this may indicate that the swelling is painful. The measurement of pain is very subjective as some animals will show signs of pain when normal skin on the neck is palpated. This can often be tested by first handling an area of normal skin and then the reaction site. The reaction of the animal can be compared and an indication obtained whether the swelling is painful or not. Because of the subjective nature of this sign, it cannot be relied upon too much when making a diagnosis.

g.) Heat (H) (Calor)

This is indicated on the TB 10 by the abbreviation H.

There is no practical mechanical method of determining whether the swelling is warmer than the normal skin. The only subjective way is to compare the reaction site with an area of normal skin away from the injection site.

This is still a very subjective method and as most TB tests are carried out in the open and sunshine, the animal's skin may be normally very warm.

If there is without doubt an increase in heat at the reaction site it is a positive sign, but in most cases heat cannot be used to help make a diagnosis.

3.8.5 Systemic Reactions

In an animal with advanced tuberculosis signs such as coughing or signs of pneumonia will sometimes occur one to two days after the injection of the tuberculin. The following signs may also be seen:

- Increase in temperature (fever)
- Shivering
- Ruffled hair
- Listlessness
- Decreased production in lactating cows

3.8.6 Measurement

The most reliable information is obtained by measuring the increase in skin thickness as this is measured in millimeters with the calipers. This measurement is objective and not subject to human judgement such as signs of pain, heat, redness oedema etc.

The increase in skin thickness is therefore one of the main criteria used in interpretation.

The normal skinfold is measured just above the reaction or on the other side of the neck opposite the thickened skinfold.

If no observable reaction is apparent it may be difficult to find a place where the tuberculin has been injected, especially with animals where a large area has been shaven. With animals where a small square has been shaven and the injection is made at the point where the lines from the corners of the square intersect, no problem is experienced.

The caliper is placed in position over the thickest part of the swelling and the lips of the caliper moved into position until they touch the skin in such a way that the same pressure is exerted as when measuring the normal skinfold without disturbing and flattening the swelling.

The skin reading is only measured in millimetres and tenths of millimetres for the sake of uniformity. This reading as well as the abbreviations describing the reaction are noted. An individual diagnosis is made and noted after all the tested animals have been examined and a decision made on whether the herd is infected, suspect or negative.

In all cases skin reactions are observed and palpated at the place of injection of tuberculin. Irrespective of the testing programme the following should be done:

- the normal skinfold is measured above the place of reaction or on the opposite side of the neck where tuberculin was not administered and noted on TB 10;
- the thickened skinfold is measured at the thickest place(s) of injection reaction and noted on TB 10;
- the type of reaction is observed and palpated at the place of injection of bovine tuberculin and described

by making use of the recognised abbreviations.

No measurement is made and no description of the reaction site is filled in on the TB10 form where there are no palpable reactions at the injection site after 72 hours. The fact that no skin thickening readings need to be given in respect of cattle that show no reaction to the injection of tuberculin does not indemnify the tester against the fact that each injection site regardless of the testing program must be examined and palpated at the time of reading the test.

It is however of vital importance that **ALL SIGNS ARE TAKEN INTO ACCOUNT** when interpreting a test result.

3.8.7 Other criteria

The following criteria are used to describe the swelling along with the increase in skin thickness, as well as those already mentioned above under section 3.8.4.

a.) Hard

Describe in the test report as "Hard"

The swelling feels like a firm round nodule. Other signs are normally absent and these hard circumscribed nodules are often caused by non-specific reactions.

b.) Circumscribed (C)

This is described on the TB 10 as C.

There is a very clear demarcation between the reaction zone and the normal skin. This is seen as a distinct line separating the two.

This type of swelling often occurs with a hard cold non painful reaction and is often caused by non-specific reactions.

It can also occur in infected animals with necrosis and oedema.



Figure 32 Circumscribed lesion with sharply defined edges



Figure 33 Small hard, circumscribed reaction

c.) Diffuse (D)

This is described on the TB 10 as D.

If the reaction zone gradually runs into the surrounding tissue it is diffuse. It is normally associated with oedema and indicates towards a positive reaction.



Figure 34 Diffuse type swelling. No distinct edges

d.) Flat (F)

This is described by the abbreviation F.

When a reaction is flat and one looks at it without handling or measuring it, there is a danger that it could be missed.

If a flat reaction is hard and cold, infection is not expected but it can also be associated with oedema and other signs as shown below, when it will be associated with infection and this is one of the reasons why it is so important to palpate every single animal when reading a test.



Figure 35 Flat, Diffuse, Oedematous swelling

e.) Adhesions (Ad)

This is described by the abbreviation Ad.

In typical positive reactions there are often adhesions formed between the skin and the subcutaneous tissues. The adhesion can be felt when palpating a reaction by trying to lift the skin away from the body.

Because adhesions are a very positive sign it is essential to palpate the site before injection to ensure that there are no adhesions present before the test.



Figure 36 Palpation of swelling – no adhesion



Figure 37 Palpation of swelling - adhesion

3.9 INTERPRETATION OF TEST RESULTS

3.9.1 Herd Approach

The test is conducted on the whole herd. The only exception is when a small number of animals have to be tested for diagnostic or export purposes, in which case these should preferably be carried out by a private practitioner at the owner's expense.

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 cattle 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 cattle incl. external parasite load as well as the overall quality of herd management can provide additional useful information towards a final diagnosis.

NB:

Remember that all signs, history and the possibility of non-specific reactions must be taken into account. Do not rely only on the increase in skin thickness.

3.9.2 Herd History

If the history of the herd is known this will simplify the interpretation. The following information is of great value:

- Results of previous tuberculin tests
- Post mortem/abattoir results on cattle, pigs and poultry
- Closed herd or not
- Recent introductions of cattle
- Tuberculosis bacteria found in milk (culture)
- Avian tuberculosis found in poultry or feeding chicken litter
- Human tuberculosis in farm workers
- Johne's disease diagnosed on the farm
- Skin lesions in the herd
- Clinical suspect or positive cases
- History of TB in contact farms
- Systemic reactions after doing TB test
- Drop in milk production since doing TB test

On the basis of the history, herds can be provisionally divided into three groups

- I. Negative for tuberculosis
- II. Possibly negative but uncertain TB status
- III. Infected with bovine tuberculosis

3.9.3 TB Negative herds

There will be herds where the above-mentioned data are not available. Such herds are regarded as being TB negative until the contrary is proven.

Maintenance herds where animals are tested regularly and where it is ensured that additions are tested negative before they may mix with the herd.

Infected herds where the disease has been eradicated by means of regular tests, the elimination of reactors and disinfection.

3.9.4 Tuberculin test interpretation in a suspected negative herd

It must be stressed again that a final diagnosis on the status of a herd must not only be made on the increase in skin thickness. This is only one of many factors to be taken into account.

The possibility of non-specific reactions must be taken into account as well.

The age of the animals tested must also be taken into account. Animals under 12 – 18 months tend to show more non-specific sensitivity in respect to environmental mycobacteria.

Animals older than 5 years have a higher chance of being infected for a long time and may even be completely insensitive (anergic) to the tuberculin test.

Skin lesions also cause most non-specific sensitivity in animals between 2 – 5 years old.

The same skin thickening in millimeters in animals with a thin normal skin is of greater significance than in animals with a thicker skin.

The density of the skin can also have an influence on the reaction, particularly in respect of fluid accumulation (oedema)

a.) Single Intradermal test with bovine tuberculin only (supposed negative herds)

Negative Herds

In a herd with no history of tuberculosis infection, especially in closed herds, it can be expected that most animals will be negative. Where animals show skin thickenings after 72 hours a reason will be looked for i.e. non-specific reactions, or the possibility of human tuberculosis sufferers etc.

In these herds an increase in skin thickness of up to **6mm** is regarded as negative. Once again the type of reaction must also be considered.

A herd with a negative history showing a number of hard circumscribed reactions, even with increases of up to 6 mm or more can be regarded as negative. If there is any doubt as to the status of the herd it must be retested after three months using the comparative test or gamma interferon test.

Suspect Herds

When a herd is tested for the first time and a number of reactions are found with an increase of more than 6mm but with signs of a positive reaction i.e. oedema, necrosis, exudation should be regarded as a suspect herd and retested in 3 months using the comparative intradermal test. Alternatively, blood samples in heparin can be collected from the suspect animals and submitted to the laboratory for gamma interferon testing.

Positive herds

When large typical reactions are found in a number of animals in a herd tested for the first time and a few animals show increases of more than 20mm the herd can be regarded as positive. The positive reactors should be slaughtered for post mortem examination and affected tissue samples sent to the laboratory to confirm the diagnosis.

Infection of animals with *M. tuberculosis* can in some cases also give this picture and the possibility of sensitization by infected workers should be eliminated first. Such animals, if tested after 3 months, usually show a decrease in skin thickness but in some cases a positive test result may persist.

b.) Comparative intradermal (negative herds)

In interpreting the comparative test two extra criteria are used in addition to all the normal criteria as described in Section 3.8

- The increase in skin thickness at the bovine tuberculin site
- The difference between the bovine and avian increases (the avian increase is subtracted from the bovine increase)

The table below shows the interpretation of the comparative test in negative or suspected negative herds.

A positive reaction will be characterised by the following measurements:

1. A bovine increase of more than 4mm
2. A positive difference in increase of skin thickness between bovine and avian injection sites of more than 4mm

Skin thickening in millimeters		
Bovine	Bovine minus avian increase	Diagnosis
Less than 4	Less than or equal to 0	Negative
More than 4	1 to 2 mm	Negative
More than 4	3 to 4 mm	Suspect
More than 4	More than 5	Positive

Examples:

1

Normal skin 10mm
 Bovine reaction 13mm
 Bovine increase 3mm
 Avian Reaction 18mm
 Avian increase 8mm
 Bovine minus Avian (3 – 8) = -5
 Therefore Negative

2

Normal skin 10mm
 Bovine reaction 18mm
 Bovine increase 8mm
 Avian Reaction 12mm
 Avian increase 2mm
 Bovine minus Avian (8 – 2) = 6
 Therefore Positive **provided** others signs point towards positive reaction.

It is evident from the above that where the avian increase is greater than or equal to the bovine increase then a diagnosis of negative is made.

In cases of (non-specific) avian reactions the avian reaction is much larger than the bovine reaction.



Figure 38 Animal showing reactions at both bovine and avian injection sites

3.9.5 CAUSES OF NON-SPECIFIC (FALSE POSITIVE) REACTIONS

The purpose of the tuberculin test is to trace animals that are infected with *M. bovis* (bovine tuberculosis) and to differentiate such animals, if possible, from other who do in fact have a thickening of the skin after the tuberculin

test but who are not infected with *M. bovis*.

Generally non-specific reactions are caused by temporary sensitisation with (for cattle) non-pathogenic mycobacteria. In most cases retesting of animals after 3 months produces a conclusive result. The purpose of the comparative tuberculin test is also to differentiate animals that show some reaction to the tuberculin test but are not infected with *M. bovis* from those which are infected with *M. bovis*. In most cases non-pathogenic mycobacteria will cause a strong reaction to avian tuberculin PPD and can be readily identified using the interpretation key under 3.9.4.2. Due to the close antigenic relatedness among mycobacteria it is possible, however, that

- non-specific stimulation results in a temporary significantly stronger reaction to bovine tuberculin PPD
- specific stimulation by *M. bovis* infection results in temporary excessive reaction to avian tuberculin PPD
- a mixed infection with both *M. bovis* and non-pathogenic mycobacteria results in temporary excessive reactivity to avian tuberculin PPD.

Care must therefore be taken not to ignore non-specific reactions in cattle herds, especially those with a positive or unknown infection status (not tested for at least 3 years).

Cattle submitted to severe prolonged nutritional stress have been found to react to bovine tuberculin as a consequence of penetration of saprophytic mycobacteria through a weakened intestinal wall.

a.) Mammalian reactions

With its natural resistance to *M. tuberculosis*, the bovine often shows no visible lesions after infection and only small lesions are formed. These lesions tend to get smaller and later, over time, disappear.

The large antigenic similarity between *M. bovis* and *M. tuberculosis* results in the fact that infection of a bovine with human tuberculosis leads to considerable sensitivity to bovine tuberculin. This is often called pseudo-specific reactivity.

Where infection with *M. tuberculosis* in cattle is suspected, enquiries may bring to light that known cases of human infection have occurred on the farm amongst workers who have had contact with the cattle herd.

Arrangements should be made for the workers to be tested for tuberculosis at a Department of Health Clinic.

Eight to ten weeks after a bovine contracts the infection, a maximum skin thickening of up to 10mm at the bovine tuberculin site is observed. Sensitivity lessens gradually and especially if the source of the infection is removed and reactions of 2 - 4 mm could be expected after 4 months. After 9 months these reactions will have disappeared. In exceptional cases it has been reported that animals still reacted slightly after a period of 2 - 4 years.

When *M. tuberculosis* infection is suspected, the reactors should be made suspicious and retested at regular 3 month intervals. With each test the skin thickening should decrease. If the source of infection is not removed other animals will start reacting during this period.

Skin lesions (dermatitis nodosa – acid fast lymphangitis) Lesions are caused by saprophytic mycobacteria and appear as hard or soft nodules in the subcutaneous tissue and seldom as open sores. Nodules are usually found on the bony areas of the body. Sometimes the lymph glands of the shoulder and neck are affected. The nodules are small and sometimes not noticeable but can be up to 10mm in diameter.

The nodules form typical granulomas with necrotic and caseous centres. Large numbers of acid fast bacteria can be found on smears made from these lesions.

In the Western Cape, Eastern Cape and Kwazulu Natal, skin lesions can complicate the interpretation of the tuberculin test.

Cattle recently infected show the greatest reaction and it appears in particular in the 2 – 3 year old group. As the lesions encapsulate the sensitivity gradually decreases and disappears between 6 months and 3 years after initial infection. Young animals without noticeable skin lesions can show reactions of up to 20mm on bovine tuberculin whilst older animals with clearly noticeable lesions show little or no reactions depending on how inactive the lesions have become.

It appears that the organisms causing skin lesions are allergenically more closely related to *M. tuberculosis* and one cannot depend upon the comparative test to distinguish the condition from *M. bovis* infection.

All animals in a herd should be examined for these skin lesions if non - typical reactions are found i.e. sudden positive reactions in a herd that has tested negative for years with no history of introductions.

b.) Avian type reactions

***M. avium* infection** in cattle usually does not elicit visible lesions, if lesions do occur they are small without a tendency to become progressively larger and are found mainly in the mesenteric lymph node. In exceptional cases the uterus, udder and lungs may be infected.

Fowl tuberculosis is sporadic in South Africa and direct infection of cattle from poultry is probably rare. However, there are a number of other causes of sensitivity to avian tuberculin in cattle.

If justified an investigation to determine the possible presence of avian tuberculosis and even post mortem investigations on poultry may supply valuable information. One should also take note of the contact between chickens/poultry and their products and cattle such as feeding chicken manure to cattle.

In cattle infection with *M. avium* does cause sensitivity to bovine tuberculin, but these reactions are not typical with hard cold circumscribed lesions. If the comparative test is used a much greater reaction will be seen at the avian site.

If the source of infection is removed the sensitivity diminishes after 6 – 12 months and each test should show a decrease in skin thickening

***M. avium* complex bacteria** - In South Africa it was found that up to 75 % of lesions in the lymph glands of pigs are caused by organisms closely related to *M. avium* i.e. *M. intracellulare*. These organisms have been isolated from humans, birds, cattle, soil, plants, wood shavings etc. These organisms can also cause sensitivity in cattle.

As in the case of *M. avium* a comparative test will distinguish these from true *M. bovis* infection in most cases.

Other Mycobacteria- Other species of non-pathogenic mycobacteria can cause temporary sensitisation in cattle. These organisms generally cause a greater reaction to avian tuberculin and the comparative test can be used to distinguish these infections from specific tuberculin reactions.

Johnes Disease- Infection with *M. avium* subsp. *paratuberculosis* is rare in cattle in South Africa but the disease is widespread in sheep flocks in several provinces in the country.

Infected animals that are tested with bovine tuberculin usually do not show typical reactions but more of a suspect type of reaction.

Sensitivity in respect of the tuberculin test is usually the greatest immediately after infection has taken place, thereafter it decreases and animals with noticeable symptoms will probably react poorly or not at all to the bovine tuberculin.

M. paratuberculosis is antigenically related to *M. avium* and accordingly greater reactions are to the avian tuberculin than to the bovine tuberculin.

Saprophyte- Saprophytic mycobacterium which are usually found in food, water, and therefore also in the gastrointestinal tract of herbivores play an unimportant role but may sometimes cause tuberculous mastitis.

These organism can build up in numbers when animals are held for long periods of time in enclosed bomas. Animals sensitized by these organisms will generally show a greater reaction to the avian tuberculin but in exceptional cases they may cause a false positive bovine reaction.

c.) Non-Mycobacterium organisms

These organisms are rare and their involvement in non-specific reactions should only be considered after all the other causes have been eliminated.

Nocardiosis – The acid fast *Nocardia spp.* can cause problems regarding the differential diagnosis of tuberculosis and cultures of nocardia can be confused with mycobacteria. Histopathologically it is similar to tuberculosis and a degree of cross sensitivity can be experienced against mammalian and avian tuberculin which in experimental cases disappears after 72 days. A retest after 3 months will eliminate *Nocardia spp.* as a possible source of sensitivity.

The following organisms have been suspected of causing non-specific reactions but there is insufficient proof to regard these as of practical importance in the field

- Actinomycosis
- Actinobacillosis
- Brucellosis
- Corynebacteriosis
- Trichophyton

d.) Pathological conditions

Liver fluke infection, peritonitis, abscesses, cysticercosis, pneumonia, mastitis, nephritis, and lumpy skin disease are sometimes blamed as causes of non-specific sensitization. However these are not proven and the reactions caused will show a decrease on repeated testing.

Herds with a liver fluke infestation should be dosed before the next test, to eliminate liver fluke as a potential problem.

e.) Physiological conditions

In the advanced stage of pregnancy or during the peak of the oestrus cycle cows sometimes show an increased sensitivity to the tuberculin test.

Non-infected oxen between the ages of 2 - 4 years show up to 3 time more suspect reactions than non-infected cows of the same age. This increase in sensitivity is attributed to the influence of sex hormones.

f.) External conditions

Factors such as damage to the skin during the shaving of the hair, use of dirty needles and syringes, rough injection technique, may cause a local reaction at the site of injection which could be confused with a reaction to the tuberculin.

The cause of these local reactions are often obvious on closer examination of the area.

3.9.6 Infected herds

a.) Herds are regarded as infected if any of the following examinations indicate infection.

i) Meat Inspection

When lesions typical for infection with bovine tuberculosis are found during routine meat inspection at an abattoir, it must be immediately reported telephonically to the State Veterinarian in whose area the cattle originated as well as on the meat inspection form in terms of the Meat Safety Act. The State Veterinarian is responsible for the testing of the infected herd and the tracing of all movements. After a positive meat examination the testing of the herd is compulsory.

ii) Infection in milk

Some municipalities and distributors conduct random sample testing of milk. If acid fast organisms are detected in a milk sample then testing of the herd of origin by the State Veterinary Services is compulsory.

iii) Post Mortem examination

Infection can also be detected when *post mortem* examinations are conducted on the farm, either by the State Veterinarian or a private veterinarian. If infection is suspected by a private veterinarian then it must legally be reported to the local State Veterinarian. Testing of such a herd is compulsory.

iv) Clinical cases

Clinical cases may be detected by Veterinarians or Animal Health Technicians during routine inspections. Old animals with a cough and emaciation should be suspected of being infected.

v) Tuberculin test

The tuberculin test is the commonest way in which tuberculosis is detected. This test could be performed as part of the Maintenance programme, the Herd Diagnostic programme or Diagnostic tests. These tests may be performed by State officials or by private veterinarians. If a positive reaction is found by a private veterinarian, then he/she is legally obliged to report this immediately to the local state veterinarian. The state will then take control of that herd and will remain responsible for all testing and other control measures until the herd is declared negative again.

vi) Introduction of animals from an infected farm

When infection is confirmed on a farm, all movements of animals off that farm must be investigated. Any farm that has received animals from the infected farm must be regarded as infected and the state veterinarian of the area of destination must be informed. The testing of such farms is compulsory and such herds are regarded as positive until proven negative.

b.) Interpretation of single intradermal test(positive herds)

A number of animals in an infected herd, will only have become infected fairly recently. This means that they will show large reactions to the tuberculin test. Such animals will show typical reactions with skin thickening of more than 20mm with some or all of the following positive signs:

- Oedema
- Pain
- Redness
- Necrosis
- Adhesions
- Enlargement of prescapular lymph node

In an infected herd the average of all skin increases will normally be 10 - 12 mm.

Once the herd has been declared positive, then all animals with a skin thickening of 4mm or more on the bovine tuberculin are regarded as positive.

The following exceptions to the above must also be taken into consideration:

- Old animals with a skin increase of less than 4mm but with other positive signs i.e. oedema, pain, heat etc.
- Old animals with skin increase of less than 4mm with hard and circumscribed lesions but with clinically suspect signs i.e. coughing, emaciation, swollen lymph glands etc.
- Animals younger than 5 years with a skin thickening of 4mm and more where the reaction is circumscribed and without heat. With such animals a diagnosis of suspect can be made and the animals retested after three months. These animals should, ideally, be kept separate from the rest of the herd until their status is confirmed.

c.) Interpretation of comparative tuberculin test (positive herds)

Skin thickening at injection site(s) in mm		Diagnosis
Bovine injection site	Bovine reaction minus avian reaction	
Any	Less than or equal to 0	Negative
4mm or more	Plus 1 or plus 2	Suspicious
4mm	Plus 3 or greater	Positive

The exceptions mentioned in 3.9.6.2 must also be considered in the interpretation.

Old animals with a suspicious diagnosis can be regarded as positive if the bovine reaction shows any other positive signs i.e. oedema, heat etc.

Animals up to 5 years old with a positive interpretation according to the table above may be considered suspicious if the bovine reactions are hard, circumscribed with no pain or heat, and there are indications that other causes of sensitivity are present on the farm. This would include skin lesions, liver fluke etc.

The success and speed with which tuberculosis will be eradicated in an infected herd is closely related to:

- the ability of those doing the tests to identify all infected animals and to remove these from the herd as soon as possible and
- adherence to strict testing schedules of the entire herd.

The stricter the interpretation in the first positive test, the quicker the disease will be brought under control. At the beginning of a control operation, caution must be exercised in making a diagnosis of suspect animals, it is often better to remove such animals from the herd. If they are kept for retesting in three months, they should be kept in a separate herd until their status is confirmed.

Note: Successful eradication of an outbreak requires the synergistic application of available diagnostic and epidemiological tools to identify infected cattle but also to identify and analyse other factors which can potentially complicate the outbreak.

In a positive herd, record keeping is essential and all animals have to be identified with ear tags or brands. This allows the state veterinarian to follow the individual animals through the testing procedure and improves the accuracy of the interpretation.

In controlling the disease in any farm, buy in and cooperation from the farmer is essential and the better the cooperation, the faster the disease can be eradicated. Ideally cooperation should be achieved by positive encouragement rather than by threatening actions.

3.9.7 CAUSES OF FALSE NEGATIVE TUBERCULIN REACTIONS

These are cases where the animal has bovine tuberculosis infection but does not react to the tuberculin test.

a.) Tuberculosis anergy

Infection with *M. bovis* causes the largest and most typical reactions in the early stages after infection. This sensitivity lessens later on during the advanced stages of the disease. As the immune system of infected cattle gradually switches from a cell-mediated type (which is primarily detected by skin test and gamma interferon test) to a humoral antibody-based reaction, the skin test becomes less sensitive in detecting infected animals. If the eradication campaign fails to remove such animals in time or in the absence of regular routine testing, they will eventually become totally non-responsive to the skin test (no reaction at all). This is known as anergy. These anergic reactors often show generalized spread of tuberculosis throughout the body and are therefore a great danger and source of infection to the rest of the herd.

In order to eradicate the disease in a herd, it is important that these anergic animals are identified and removed.

Indications that there may be anergic reactors in a herd are continual new infections being found even after a number of test-and-slaughter actions were performed.

Anergy is more often found in the older animals and special attention must be given to this group. The following tests can be applied to try and identify these animals:

- Clinical examination, any older animal showing signs of emaciation, coughing should be regarded as positive. In tuberculosis the cough is short and dry at the beginning of the disease but can become moist with a lot of exudates being produced. Smears of this exudate can also be collected and submitted to a laboratory for Ziehl Neelson staining
- Udders of animals showing chronic mastitis must also be examined and particular attention paid to those with enlarged and hardened quarters which could be painful or painless. The organ's shape can be deranged with a reduction in milk secretion. The milk can even be watery; with small clots and flakes. Abscesses may be present in the udder or growths as large as a child's head. Where in the case of ordinary mastitis the udder is usually affected in the region of the milk cisterns and are therefore closer to the teat, lesions as a result of tuberculosis mastitis often occur closer to the adhesion of the udder. A suspicion such as this can be verified by examining milk samples (especially milk sebum) microscopically or in the laboratory by making cultures or by doing biological tests on guinea pigs.
- Uterus: Up to 20% of animals with generalized tuberculosis show lesions in the uterus with a mucoid discharge which also contains numerous organisms.
- Infected animals almost always show some degree of lymph node enlargement and all lymph nodes should be examined. Periodic bloat in some cattle may be an indication that lymph glands exerting pressure on the oesophagus are infected.
- The following tests have been used in the past to try and identify anergic reactors but are not used in the field
 - The double intradermal test
 - Stormont test
 - Temperature test
- A new ELISA test which measures humoral antibodies is at present being validated, which may prove to be of great value in identifying anergic animals

b.) Inactive Tuberculosis

In some cattle infection with *M. bovis* can be self-limiting resulting in the encapsulation of the microgranulomatous lesions. These animals react poorly and the reaction often fluctuates in respect of the tuberculin test. These animals are not a great danger to the rest of the herd as long as the tuberculosis remains inactive. Under stress conditions the tuberculosis can become active again.

c.) Pre-allergic phase

After an animal has become infected it takes from 8 – 65 days (average 21 days) before an allergic condition develops and the animals react to the tuberculin test. These animals will however be detected at the following tuberculosis test in three months' time.

d.) Drugs

The application of illegal practices such as the injection of cortisone and other drugs causing vaso-constriction could affect the interpretation of the tuberculin test.

e.) Calving

Shortly before and after calving infected cows may show a false negative skin test result. Such animals will return to a positive test status four to six weeks later. Such doubtful reactors should be retested after three months.

3.9.8 Test interpretation - summary and table

Considering all that has been said and taking into consideration skin thickening, reaction signs, the history of the herd and other information the following table may help to give a better idea of how a combination of skin thickening and other information can be used to come to a decision whether the animal concerned is positive, suspect or negative. On the right hand side of the list (median) of the table are the signs that tend to be negative

and on the left hand side are the signs that indicate a positive diagnosis.

For a specific type of herd (negative or positive), the number of millimeters skin thickening which is the recommended differential between positive or negative are placed on the median according to the type of test (single or comparative) to distinguish between negative or suspect on the one hand and negative/suspect or positive on the other. As far as a specific animal is concerned the reaction signs and other data are then taken into consideration and a decision is made whether the evidence, as far as that animal is concerned, should be put on the right hand side of the median that is to say on the negative side or left of the median that is to say on the positive side. This helps to decide on a positive, suspect or negative diagnosis.

Test Interpretation Summary Table

Type of herd, skin thickening, median and proposed diagnosis

	Greater	Median	Smaller
For a negative herd			
Bovine tuberculin alone	Suspect	> 6mm <	Negative
Bovine & avian tuberculin	Suspect (Provided the reaction to bovine tuberculin is 4mm or more)	> + 3mm <	Negative
For a positive herd			
Bovine tuberculin alone	Positive	> 4mm <	Negative or Suspect
Bovine & avian tuberculin	Positive (Provided the reaction to bovine tuberculin is 4mm or more)	> + 3mm <	Negative or Suspect

Median on which number of mm of skin thickening is placed.

Positive signs	Median	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 cows 8.6 Coughing spells		8. Young animal
9. Adhesion of skin to subcutaneous tissue = Ad		
10. Old animal		
11. Swelling diffuse = D		

4 The Gamma Interferon Assay (IFNg)

4.1 Principle

The IFNg test is, like the tuberculin test, a method to detect an animal's cellular immune response to *M. bovis*. It is therefore in essence an *in vitro* analogue of the tuberculin test.

4.2 Procedure

Samples (10ml) of whole blood in heparin (green top tube) are taken aseptically from individually identified bovines. The blood samples are transported to a laboratory equipped to perform the preparatory stimulation (first phase) of the test. During transport the blood samples are kept between 15°C and 25°C (NO chilling). As the stimulation relies on viable T-cells this step must be done on the day of sample collection. In the laboratory the blood samples are aliquotted and stimulated with bovine and avian tuberculin as well as Fortuitum PPD (available from the OVI TB laboratory). Following incubation at 37°C for 24 hours the clear plasma supernatant is harvested in the laboratory and can be assayed for the presence of gamma interferon (second phase). The entire duration of the test is approximately 2 days.

4.3 Use of the IFNg test

The IFNg test is not intended to replace the tuberculin test because of higher costs in most cases but it is useful as an ancillary test to the tuberculin test in all situations where a sensitive and quick diagnosis is required. A final diagnosis of suspect reactions detected during routine tuberculin testing can be obtained as early as 2 weeks after the skin test.

The major advantages of the IFNg test can be summarised as follows:

- Animals are handled only once, therefore only one farm visit necessary
- No waiting period between IFNg tests
- Test performance is not related to experience of field staff (as for the tuberculin test)
- No unlawful intervention possible (use of drugs, swopping of ear tags etc.)
- IFNg test has the capability of detecting early infections. Parallel use with tuberculin test therefore result in increased overall diagnostic sensitivity

5 ZOOBOTIC ASPECTS

Bovine tuberculosis is a zoonosis and although it is mostly contracted via infected, unpasteurised milk, it can also be transmitted by aerosol during close contact between humans and cattle (e.g. handling, slaughter). The susceptibility of humans must be considered higher in immuno-suppressed patients infected with HIV.

TB in humans - very important, worldwide disease.

Described in literature in 2000 BC. Found in Egyptian mummies.

Common in poor, malnutrition, overpopulated and poor hygiene situations.



Figure 39

In Europe almost eradicated in humans (pasteurisation and immunization)

M. bovis and *M. tuberculosis* cause similar lesions in humans. Can only differentiate by means of culture and isolation.

The World Health Organisation (WHO) estimates that each year more than 8 million new cases of TB occur in the world. More than 3 million deaths annually due to TB. 90% of the cases occur in the developing world.

95.1% increase in reported TB cases in Africa over the past 10 years.

What is the situation in South Africa?

- 20 people die daily in South Africa because of TB. No differentiation is currently being made regarding the cause of the TB, whether it is due to *M. bovis* or *M. tuberculosis*
- SA is currently rated as the fastest growing country in terms of newly diagnosed cases of TB in humans
- Department of Health spends annually >R100 million
- Annual cost of lost man hours to state: R250 million
- South Africa ranks 5th amongst the 22 high burden countries
 - India, China, Indonesia, Nigeria, Bangladesh, Ethiopia, Philippines, Pakistan, South Africa, Russian Federation, DRC, Kenya, Viet Nam, Tanzania, Brazil, Thailand, Uganda, Myanmar, Mozambique, Cambodia, Zimbabwe, Afghanistan

What is the situation of South Africa per province in terms of case finding and treatment outcomes?

TB cases registered per province in S.A

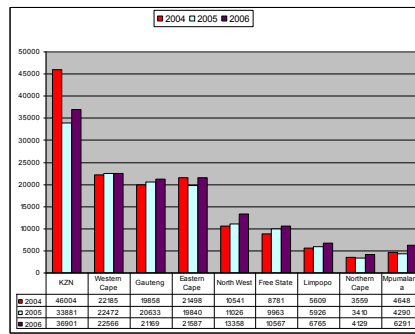


Figure 40: Human TB in South Africa

Why is the TB situation in South Africa so bad?

- TB/HIV/AIDS
- Demographic forces
- Strains of bacteria that are resistant to drugs used to treat TB
- Delay in diagnosis
- Improvement in case-notification

The Burden of TB and HIV

- S.A. is facing one of the worst dual epidemics in the world.
- it is estimated that 4.7 million South Africans HIV-infected.
- Approximately 60% of TB patients are infected with HIV.
- TB is the most common opportunistic infection and leading cause of death in people living with HIV/AIDS.

Impact of HIV and AIDS on TB

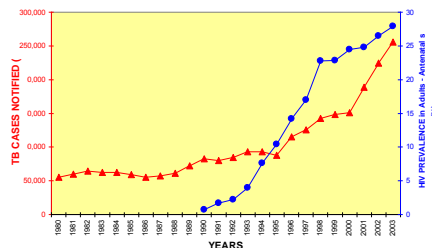


Figure 41: Impact of HIV and AIDS on TB

Epidemiology

- Infected cattle - main source of bovine TB
- mode of transmission
 - ingestion of unpasteurised milk and milk products
 - inhalation (aerosol)
- other reservoirs:
 - dogs
 - cats
 - laboratory animals
 - meat of infected animals

Factors affecting distribution and transmission

Transmission factors:

- Overcrowding

- Social deprivation
- Occupation



Figure 42

Demographic factors:

- Population growth
- Population movements by famine, war, natural disasters, migration



Figure 43

Transmission of TB

- Airborne spread
- Occurs usually indoors
- Droplet nuclei stay suspended in the air for hours
- Ventilation removes droplet & sunlight kills them

Remember: source is a *Smear Positive* TB person or infected animal

Pathogenesis

Two main forms

- pulmonary (lung)
- extra-pulmonary
 - digestive system
 - cutaneous (skin) - mainly from meat

Pulmonary TB in humans

- Enters by inhalation
- Occupational hazard for stable workers
- Establish in lung parenchyma
- Tubercle/granuloma also develops in humans

- Dissemination via regional lymph nodes
- Spreads to any part of the body
- Early treatment can cure lung lesions, but not in other parts of the body

Organs infected by spread from pulmonary TB

- kidneys
- liver
- spleen (milt)
- skeleton -children
- meninges (breinvliese)
- reproductive organs
 - testes

Extra-pulmonary TB in humans

- lesions develop in digestive tract
 - milk/milk products
 - children particularly at risk
- skin lesions
 - meat
 - abattoir workers, SV, AHT
- may also disseminate to other organs

Clinical manifestation of PTB in adults

- Cough is the commonest symptom, early in the course of the illness it may be nonproductive, but as soon as necrosis starts, sputum is usually produced
- Chest pain due to irritation of pleural lining
- Haemoptysis may result from or rupture of dilated veins
- Dyspnoea, common in extensive disease
- The clinical manifestations of TB are variable and depend on a number of factors
- What are the key host factors?
 - Age
 - Immune status
 - Specific immunodeficiency states
 - Malnutrition
 - Coexisting diseases
 - Immunization with Bacillus Calmette-Guerin (BCG)

Organs infected by spread from extra-pulmonary bovine TB

- skeleton
- joints (gewrigte)
- mesenteric Inn.
- meninges (breinvliese)
- lungs
- skin

Prevention in humans

- Extension regarding:
 - the disease
 - effective treatment for humans
 - basic hygiene measures to prevent
- Diagnosis of human cases
 - X-rays
 - BCG-test in children
- Isolation, treatment, rehabilitation
- BCG vaccine - 2 days old

Progression of infection

- Whether the infection progresses to clinical disease depend on two factors:

- The ability of the immune system to control bacterial growth
- Whether the amount of tissue damage in the lesion is too large to heal

Clinical manifestation of TB in children

- In majority of cases - history of contact with an adult positive case.
- All of these also have their own problems:
 - For children living in high burden countries, the absence of a household contact does not exclude the likelihood of TB
 - The symptoms and signs are very vague and common to symptoms and signs in children with other chronic diseases such a cough, weight loss, fever
 - Tuberculin skin tests identify children infected with TB but not necessary those diseased from it. False negative tests can occur in children with severe malnutrition, after measles, HIV
 - Chest X-rays are difficult to interpret
 - Culture of MTB is expensive and has a low yield
 - Scoring systems
 - It is therefore clear that the clinician must have a high index suspicion that a child has TB and then use all the tests to make the best diagnosis

Commonest forms of TB in children are:

- TB lymphadenopathy.



Figure 44

- TB meningitis



Figure 45

TB effusions (pleural, pericardial & peritoneal) and spinal TB

- Extra-pulmonary TB
 - More difficult to diagnose than PTB
 - Extrapulmonary TB covers all forms of TB in which the disease process occurs outside the lungs. Most forms originate from lymphatic or haematogenic spread of mycobacteria
 - Commonest forms are: TB lymphadenitis, serous effusions, pleural effusions, Meningitis, Miliary, Ascites, bones, pericardial effusions



Figure 46



Figure 47

Treatment of Human TB

The management of TB may be summarized as follows:

- Curative chemotherapy. Achieved by:
 - ensuring that the patient receives: at least two drugs that the patient is sensitive to in appropriate dosage for long enough

- Prevention of spread of infection. Achieved by:
 - Rapidly sterilizing anti-TB drugs
 - Identification of close contacts
 - Chemoprophylaxis in certain groups (Children < 5 years)

- Treatment Regimens
 - Treatment regimens have an initial (intensive) phase and a continuation phase
 - Patients can either be new or retreatment cases
 - Treatment should be given not less than five days per week in the intensive phase.
 - It is recommended that treatment should also be given five times per week in the continuation phase, but if that is impossible, then treatment can be given three times per week in the continuation phase only

6 TEST PROGRAMMES

The execution of the tuberculin tests has as its purpose the detection, control, the combating and the eventual eradication of tuberculosis.

The test programs (with their computer allocated codes in brackets) which were used in the past are as follows:

- Accreditation (01)
- Maintenance (old Annual diagnostic) (02)
- Diagnostic herd test (03)
- Ordinary diagnostic (04)
- Import (05)
- Export (06)
- Infected (09)

Joining a program is voluntary (except the infected and import/export programs). If infection is established at the first or later tests, the herd is accommodated under the infected herd program, and although the owner has joined voluntarily, he cannot withdraw from the programme, thereby avoiding further tests and the slaughtering of positive reactors. Tests and further action for the eradication of the infection is compulsory and can be enforced in terms of the Animal Diseases Act, 1984 (Act No 35 of 1984).

It is proposed that only the following programmes be used in future:

- Maintenance
- Diagnostic (would include Herd diagnostic, Ordinary diagnostic, Import & Export of previous system)
- Infected

6.1 Accreditation (01)

One of the main objectives of the eradication programme which was instituted on 14 May 1969 was the expansion of the existing accredited herd programme to form a nucleus of tuberculosis-free cattle from which other herds, be it infected or clean, could be supplemented and to increase this nucleus until it would later function countrywide and all the animals would be tuberculosis-free.

This programme was initiated for owners with a special desire to participate. These herds must maintain exceptional management standards because the State certifies them as tuberculosis free. **Very few herds comply with these strict requirements and thus this program has been discontinued**

6.1.1 Requirements for admittance to the programme

When an owner applies to enter the programme the State veterinarian undertakes an inspection to determine whether the farm meets the necessary requirements:

- The farm must be effectively fenced and all neighbouring herds tested at least once in five years
- Public roads running through the farm must be fenced on both sides.
- A suitable quarantine camp with separate water and adequate grazing must be provided in order to isolate positive, or suspect cattle.
- A suitable crush pen and/or other facilities and sufficient aid where animals are tested must be provided.
- Animals on the farm must not use the same grazing, drinking troughs, cow sheds, kraals, crush pen or dip tank as animals from non-accredited herds.
- The herd must be closed.
- Attention should be paid to hygiene, i.e. the condition of the cows sheds floors, feeding and water troughs, etc. should be of such a nature that they can be thoroughly cleaned and disinfected if necessary.
- Management on the farm is important, because reliable records, indicating all increase and decreases, mutual cooperation and the success of the entire programme depend on it.
- Every animal must be marked in such a manner that individual identification is possible. Identification of cattle is for the owner's personal account.
- Any movement of livestock to the herd is dependant on the approval of the local State veterinarian and the issuing of a transport permit, subject to these conditions.
- Any stillbirths/abortions must be presented for investigation.

This programme was initiated for owners with a special desire to participate. These herds must maintain exceptional management standards because the State certifies them as tuberculosis free. **Very few herds comply with these strict requirements and thus this program has been discontinued**

6.1.2 Application form (TB/CA 1)

If the conditions on the farm comply with the above-mentioned requirements and the herd is accepted, the owner signs a TB/CA1 form after he has made sure of its contents and is prepared to comply with the conditions. The TB/CA1 makes provision and is binding for all tests conducted thereafter on the herd belonging to the same owner. If the herd should change ownership, it will be necessary to complete a new TB/CA1 provided the new owner is agreeable to having the herd subjected to further tests and is prepared to accept and comply with the conditions of the agreement.

TB 1 is submitted in duplicate together with TB 10 and tuberculin usage form to the State Veterinarian and then sent to the office of the Deputy Director: Animal Health or TB & Brucellosis Coordinator after the first test has been conducted on the herd .

6.1.3 Files

When a herd has been accepted under this programme, a file is opened for the herd in the State veterinary office. The file number is given in accordance with the system generally in use, i.e. Province number/ local municipality number/ sequential number of herd for that local municipality.

For example: Mpumamanga/Govan Mbeki/1st herd – would read as follows:
01/152/01

6.1.4 Tuberculosis:free certificates (TB9)

If all the cattle of all sexes of all owners over three months of age in a herd react negatively during the first tuberculin test, the second test is conducted three months later. If this test is also negative the issuing of a certificate is recommended. The certificate is valid for one year calculated from the day on which the test was read and not on the day on which the tuberculin was administered. If suspect cases are found during the first test and such animals react negatively during the second test three months later, the first test will also be regarded as negative and a certificate can be recommended. If there are suspect reactors during the second test or with the annual test the herd will be retested comparatively three months later and if the results are negative the issuing of the certificate is recommended. This certificate is valid for 12 months from the date on which the last herd test was conducted. The whole herd is retested every year and with each negative test a new certificate is issued.

6.1.5 Additions to an accredited herd

A veterinary movement permit, issued by the State Veterinarian or an Animal Health Technician, is required for all movements from and to an accredited herd.

If the cattle are from another accredited herd which is in possession of a valid tuberculosis-free certificate the cattle can be introduced into the herd without restrictions or tests and may mix immediately with the other cattle in the herd. In cases where the herd of origin is not accredited, it is preferred that the purchased cattle are tested at the place of origin at the owner's cost. If the whole herd is, however, tested it is done at the State's cost. If the test is negative, then the animals can in most cases be transported to their destination and mix immediately with the herd.

Where it is impossible to test the cattle at the place of origin, for example where purchases take place at an auction, the cattle are placed in quarantine camp at their destination and will undergo the test there at the cost of the owner before they will be allowed to mix with the rest of the herd.

If the tests are conducted before or after the annual or biennial test for the renewal of the tuberculosis-free certificate, this test must be reported separately on TB 10.

Cattle that were tested at the place of origin before being added to the herd or originated from an accredited herd, must be indicated in the relevant space on form TB/CA8 during the annual or biennial test.

The State Veterinarian or the Deputy Director: Animal Health may in some cases insist on a second test 6 weeks to 3 months after the first test. This can for example be required in cases where the cattle bought in this way, show suspect reactors during the first test or if the cattle are from a risk herd or area. The cattle that must be subjected to a second test in such a case, must be kept in quarantine and may not mix with the rest of the herd until the second test has been conducted with negative results. The second test will take place at the cost of the State.

If positive reactors are found amongst the purchased cattle, slaughtering will take place and the owner will keep his certificate.

In both cases it is required that the purchased cattle should be thoroughly isolated from the herd in accordance with the conditions of the agreement (TB 1).

6.2 Maintenance (Annual diagnostic) herd tests (02)

The purpose of this testing programme is to accommodate herds that were initially included into the accredited programme but which do not meet the laid down requirements as well as those herds where a declaration is required for some or other reason, but the herd does not meet the requirements to be included into the accredited programme. Herds that were infected and did not comply with the requirements for accreditation after infection in the herds was eradicated will also be tested in terms of this programme.

If a herd enters the programme for the first time the agreement (TB 1) is completed in advance and this accompanies the TB 10 together with the tuberculin usage form to the State Veterinarian as well as Deputy Director: Animal Health (or TB & Brucellosis Coordinator).

It is not insisted upon that the animals should be identified individually. However, the owner may identify the animals at his own cost. Cattle that show suspect reactions to the tuberculin test are furnished with official ear tags in order to identify them permanently for further tests.

Should there be cattle that react positively the herd is included into the "Infected Programme" (See 6.7) and all the animals identified by ear tags.

It is not a requirement that the skin thickness readings for every animal (normally and after 72 hours) should be entered on TB 10. However, wherever there are cattle with an increase in skin thickness, whether they were diagnosed as negative, suspect or positive, both readings (normal and after 72 hours) shall be recorded with an indication of the nature of the reaction and the diagnosis.

New herds that enter the programme must undergo two negative tests within an interval of not less than three months before a declaration (TB/CA3) is issued. These initial tests can be done by state officials free of charge.

The relevant declaration only states that the animals were tested negative for tuberculosis on the date of the second negative test and must not be confused with the official bovine tuberculosis-free certificate (TB 9) which is issued in respect of accredited herds.

After a TB/CA3 has been issued, the herd, which includes all animals of all sexes of all owners above the age of 12 months, will thereafter be retested every two years. A TB/CA3 shall be issued after each such negative biennial test.

As in the case of the accredited programme the onus rests with the stock owner to keep his herd free from tuberculosis during the interim period by not infecting his herd, for example through purchases or through contact with infected animals.

It is not compulsory to keep records in respect of reductions and increases.

For each herd a separate file is opened and a reference number allocated as described in paragraph 6.1.3.

The TB 10 is completed in respect of each test as described in 7.2.6

6.3 Diagnostic Herd Tests (03)

Some stock owners are not prepared to subject their herds to the conditions of the accredited or the annual diagnostic herd programme, but are nevertheless desirous to determine the tuberculosis status of their herds. Such owners can be accommodated under the Diagnostic Herd Programme in order to establish reasonably

quickly what the prevalence of tuberculosis is in a herd or even in a certain area or municipality.

Tests in accordance with this programme should preferably be undertaken on an organised basis by for example testing a whole municipal area systematically from a certain point until the tuberculosis status of the whole municipality is eventually determined.

These tests are executed at the State's expense by state officials.

The agreement (TB 1) should be completed in advance and submitted after the test together with the TB 10 to the State Veterinarian for forwarding to the Deputy Director: Animal Health or TB & Brucellosis Coordinator together with the tuberculin usage form.

Separate identification of animals is not required for such tests. However, the owner may identify the animals at his own cost. Official ear tags are only attached to suspect animals.

Should positive reactors be found with the tuberculin test, the herd is incorporated into the "Infected Programme" and, all the animals identified by ear tags.

Skin thickness readings are dealt, with as described in paragraph 6.2.

All cattle of all sexes belonging to all owners on the farm older than 18 months are subjected to the comparative tuberculin test. A herd is only tested once under this programme except:

- when suspect reactors are found in which case the whole herd is again tested after three months;
- when the owner wishes to incorporate his herd either under the accreditation or under the Maintenance programme. (In such cases the conditions and testing procedures as described under paragraphs 6.1 and 6.2 are applicable).

This programme offers an ideal opportunity for stock owners to acquaint themselves in a practical way with the purpose of the tuberculosis tests as well as to identify herds that will later be incorporated into the accredited or maintenance programmes.

Stock owners who are, however, satisfied with a single herd diagnostic test should nevertheless be advised and motivated to keep their herds free from tuberculosis by means of good management practices, by purchasing cattle from accredited or maintenance herds or to have the cattle tested negatively prior to purchasing.

A separate file is not kept for each individual herd tested under this programme except where the test has positive reactors. In such cases the herd is treated further as described under "Infected Programme" (see 6.7).

Test results of negatively tested herds are placed on one local municipality file in numerical order and the following reference number allocated: Province/Local Municipality/HD. The "HD" distinguishes the file from the accredited, maintenance and infected herd files.

TB 10 and tuberculin usage form are completed and submitted as indicated in paragraph 7.2.6

6.4 Diagnostic (04)

All tuberculin tests, no matter under which programme they are conducted, are in fact diagnostic by nature. All the tests that cannot be incorporated in one of the other programmes mentioned, fall under the diagnostic programme.

Such tests are conducted where an owner wishes to test one or more of the cattle in a herd, that is to say, not the whole herd, for example, for purposes of sale, the use of unboiled or unpasteurised milk or merely for human health reasons.

Tests in accordance with this programme are preferably, and where at all possible, executed by Private Veterinarians at the expense of the stock owner. Where no alternative is available, the test can be conducted by State Veterinarians, but the owner must pay for the test. As the tests conducted in terms of this programme are regarded as a clinical diagnostic service it will only be done by Animal Health Technician's in very exceptional cases.

The agreement (TB 1) should be completed in advance by the stock owner and the Private Veterinarian, and the test must be forwarded by the State Veterinarian to the Deputy Director: Animal Health or TB & Brucellosis Coordinator together with TB 10 and tuberculin usage forms.

Permanent individual identification of cattle is preferred, but it is not a requirement. However, if positive and suspect reactors are found during the test, such animals must be supplied with the official or other acceptable ear tags for identification if it has not already been done.

Positive reactors found during the test obviously result in the herd being treated in accordance with the requirements of the "Infected Programme" (see 6.7).

It is not a requirement that the skin readings of every animal (normal and after 72 hours) should be recorded on TB 10. However, in all cases where there is an increase in the skin thickness, be they diagnosed as negative, suspect or positive, both skin thicknesses, normal and after 72 hours must be recorded with an indication of the nature of the reaction and diagnosis.

An individual file is not opened for each herd (except in the case of positive reactors) but all negative diagnostic tests are placed on the same file per municipal area.

6.5 Imports (05)

Cattle that are imported and are kept at one of the quarantine stations undergo a compulsory tuberculin test. The test, a comparative test with bovine and avian tuberculin, is undertaken by the officer in charge of the quarantine station with a report thereafter on TB 10 and tuberculin usage form a copy of which must be submitted to the Deputy Director: Animal Health or TB & Brucellosis Coordinator. Because the test is compulsory for all imported cattle, no TB1 agreement is completed.

These test reports are not placed on a separate file for each owner but in a joint import file as follows per quarantine station

6.6 Exports (06)

Most importing countries demand that cattle should be subjected to a tuberculin test. To meet this requirement cattle that are exported are usually subjected to the comparative tuberculin test at the cattle owner's expense - regardless of whether the test is conducted by a Private- or a State Veterinarian.

Before the test the agreement (TB1) must be completed and the test results forwarded to the Deputy Director: Animal Health or TB & Brucellosis Coordinator together with TB TB10 and tuberculin usage record.

6.7 Infected herd program

A herd is regarded as infected when infection has been determined as indicated in paragraphs 3.9.6.1. to 3.9.6.5 during meat inspection, milk examination, post mortem examination and clinical cases, but especially when positive tuberculin tests have been conducted.

Such a herd is then placed under official supervision and the necessary steps are taken to eradicate infection in the herd and to keep the herd free from infection thereafter. In the execution of these duties the official is backed by the Animal Diseases Act, 1984 (Act 35 of 1984) and the Regulations enacted under the Act as well as the Bovine Tuberculosis Scheme regulations.

This means that in all the mentioned cases where infection has been exposed, the herd concerned will come under official supervision and steps will be taken to eradicate infection in the herd. _

Meat inspection

When infection is found at an abattoir it is often not possible to determine the origin of the herd without difficulty especially not if the animals have been bought at stock auctions or have been marketed by speculators. All efforts must however be made to determine this source of infection.

The State Veterinarian from whose area the cattle originate must be informed directly by means of TB 7, and copies of TB 7 must be sent to the relevant Deputy Director: Animal Health or TB & Brucellosis Coordinator.

Infection found during other investigations

If infection is found in milk, during a post mortem examination, a clinical examination or a tuberculin test it is usually not difficult to find the herd of origin.

6.7.1 PROCEDURES FOR HANDLING POSITIVE HERDS

a.) Introduction

Many State Veterinarians and Animal Health Technicians have never had to deal with a TB infected herd. These procedures must be regarded as a guideline only as every herd will differ and must be treated on the merits of that particular case.

These guidelines will also aid the farmer's private veterinarian to understand the course of control and to advise the farmer correctly.

b.) Duties of State Veterinarian

- Once TB is confirmed in a herd the State Veterinarian of the area must personally assume complete control over all diagnoses and control measures.
- The technician may still perform the tests, but the State Veterinarian must be present on the day of reading and must be responsible for the diagnosis of the readings.
- The SV will open a file for the infected farm and all test results and other correspondence must be kept in this file. The file numbering will be as discussed under 6.1.3
- All contact with the farmer about control measures must be done personally by the State Veterinarian.
- The SV must be present when positive animals are slaughtered.
- The SV must confirm all diagnoses and post mortem results in writing to the farmer as soon as they are available.
- The farmer and his private veterinarian must be kept fully informed of all control measures at all times.

c.) Control over TB positive herd

- As soon as the diagnosis has been made the herd must be put under quarantine with a written quarantine notice.
- A stock register must be opened and full records kept of all increases and decreases. This register must be balanced at every test.
- The farmer must be able to account for every single animal on the farm at all times.
- All conditions in the quarantine notice must be explained fully to the farmer.
- All cattle movements off the farm must take place under cover of a Red Cross permit and in sealed trucks directly to an abattoir or in exceptional circumstances to another farm provided the other farm would also then be placed under quarantine.

d.) Testing procedures

- Depending on the circumstances in the herd either the single or the comparative intradermal test may be used.
- It is important to apply a very strict interpretation in infected herds
- All cattle on the farm must be tested (ie. Beef herd on a dairy farm, labourers' cattle etc.)
- All cattle must have acceptable means of identification (ear tags, brands etc.).
- Reactors must be branded on the day of diagnosis
- These reactors must be removed from the herd without delay (the same day if possible)
- If reactors are not removed on the day of testing, then they must be kept separate from all other cattle on the farm.
- In the case of dairy cattle, these animals must be milked last and all facilities cleaned and disinfected after each milking.
- The State Veterinarian will organize the slaughter of the infected animals and the farmer will be responsible for the transport of the animals to the abattoir.

- The technician will be present for the loading of the animals and will ensure that all the reactors are loaded and the truck sealed.
- The technician will then notify the abattoir of the expected time of arrival of the animals.
- A copy of the Red Cross Permit must be forwarded to the SV office as well as Deputy Director: Animal Health or TB & Brucellosis Coordinator.
- Once the reactors have been removed any feed troughs, feeding parlours etc. should be thoroughly cleaned out and disinfected. This is particularly important in the case of dairy animals. Disinfectant to be supplied by farmer but disinfection to be done under official supervision.
- Testing must be carried out on a strict three monthly interval.
- Only after 2 completely negative herd tests can the quarantine be lifted. Depending on the circumstances in the herd and the level of management on the farm the SV may permit the movement of animals directly to an approved abattoir for immediate slaughter. These movements will be under cover of a red cross permit and in sealed trucks.

6.7.2 Procedure that must be followed after infection has been determined

a.) Reporting

Infection or suspected infection must be reported to the local State Veterinarian in terms of the Animal Diseases Act, 1984 (Act No 35 of 1984). If a Private Veterinarian finds positive reactors during a test and there is no Animal Health Technician present to brand the cattle the State Veterinarian is informed telephonically so that arrangements can be made for the cattle to be branded.

b.) Branding of reactors

Positive reactors are, if at all possible branded the same day as the test is read. This is done by or under the supervision of a State Veterinarian, or an Animal Health Technician. Positive reactors are branded with a hot T-branding iron supplied by the State on the left hand side of the neck approximately 15 cm below the junction of the head and neck. Animals that are to be slaughtered within a week or two can be branded lightly but those where postponement for slaughtering has been allowed must be branded thoroughly.

c.) Quarantine

According to the Standing Regulations in terms of the Animal Diseases Act, 1984 (Act 35 of 1984) the onus rests with the stock owner to keep such animals in quarantine until a State Veterinarian authorises their release in cases where a controlled disease mentioned in Table 2 has been determined or is suspected. The issuing of a written quarantine notice is therefore not required. However, to obviate any doubt also as far as later court cases are concerned it is usual to issue a written notice in the prescribed manner where bovine tuberculosis has been determined.

d.) Permits

A permit is necessary for moving cattle from and to the farm. For the infected cattle a red cross permit is issued to an abattoir for the slaughtering of tuberculosis infected cattle or if positive or suspect animals are slaughtered at the owner's risk at another abattoir provided the meat inspection is done by or under the supervision of a veterinarian.

For the negative cattle a permit can also only be issued to an abattoir. After the positive reactors have been removed from the herd, be it through slaughtering or total separation, to the satisfaction of the State Veterinarian and all the cattle of the remaining part of the herd have undergone two negative tests after an interval of three months the animals may be issued with a permit to any destination, that is to say also other farms. The same applies if a group of animals from an infected herd is placed in quarantine and undergoes two negative tests while infection is still present in the rest of the herd. This condition sometimes arises when an owner has a group of heifers, bulls or cows which he does not want to or cannot slaughter profitably and still has to get them off the farm before the whole herd has undergone two negative tests.

e.) Arrangement regarding reactors

In terms of existing legislation and the present scheme we are compelled to deal with reactors in such a way that

the danger of further spreading of the disease is eliminated as far as possible and that infection will eventually be eradicated in the herd.

One of the following can be applied

- immediate slaughtering
- postponed slaughtering

i) Immediate slaughtering

Animals can be slaughtered according to law to prevent the spreading of the disease and to eradicate it. The owner may apply for compensation which, at present, would be the slaughter value that he would have been paid at the abattoir should the animal be condemned. The State Veterinarian must be present at slaughter, or if slaughtered in another State Veterinarian area, arrange that a State Veterinarian is present at slaughter so as to ensure proper meat inspection. If the carcass is condemned then a statement from the abattoir as to what the owner would have been paid should the carcass have been passed, must be obtained, so that the amount of compensation can be determined.

ii) Trial slaughtering

During the first test on a herd the veterinarian might be doubtful whether infection is in fact present in the herd. In this case is it desirable to select two or three cattle for a trial slaughtering.

Selecting animals for trial slaughtering - Young animals with large and typical reactions are **not** suitable for this purpose. Such animals may be in an early stage of infection and thus not have macroscopically observable lesions. It is advisable to select old animals with reasonably typical reactions or animals older than 5 years with a fair degree of skin thickening. If the older animals in addition also show clinically suspect signs of disease, they are the ideal candidates to select for a trial slaughtering.

iii) Postponement of slaughtering

Under certain conditions the immediate slaughtering of a large number of reactors may seriously affect the owner financially, disrupt his farming business or even hamper the provision of milk to a community.

Postponement of slaughtering is considered when:

- animals of a good quality such as high milk producers are involved;
 - the percentage of animals infected is high, for example 20% to 30%;
 - a large number of positive animals are found for example 30 or more;
 - there is a specific reason for postponement in respect of specific animals such as cows at the top of their lactation or cows with small calves at foot.
- Separation - Postponement can only be granted when the positive animals are separated in such a way that they pose no danger for susceptible uninfected animals.
 - Duration of period - After the beneficial lactation period has passed or a calf has been weaned, the cow must be slaughtered. The period will therefore not exceed twelve months. The purpose of this postponed slaughtering is to enable the owner to make alternate arrangements and not to keep on farming indefinitely with an infected herd.
 - Milk for human consumption - Milk coming from positive reactors must be boiled, pasteurised or sterilised before it can be used or made available for human or animal consumption or before it can be sold. Cows that produce infected milk should be slaughtered without delay.
 - Clinical cases - Advanced clinical cases or reactors that are old and so emaciated that there is little doubt that the animals will be generally rejected as a result of tuberculosis may, if the State Veterinarian so decides, be slaughtered on the farm, thoroughly examined and destroyed. In this way a source of infection is immediately removed and cost of transport to the abattoir saved.

f.) Disinfection

The disinfectant that is normally used in cowsheds is 3% formalin whilst 3% wescodyne or F10 is used for milking equipment.

Formalin – 3%. When 1 part 40% formaldehyde is mixed with 12.5 parts water, it will give a 3% disinfectant.

Surfaces that are disinfected should be kept wet for at least 20 minutes with the disinfectant.

- Phenol & Cresol – 3% for 20 minutes.
- Sodium hypochlorite (NaOCl) – 2% for 20 minutes
- Calcium hyperchloride (Ca(OCl)₂) – active chloride should be at least 30% for 20 minutes
- Chloroxylenol – 2% for 20 minutes.

- Lime (CaO) – 20% can be used to whitewash walls

Caustic soda (NaOH) does not kill tuberculosis bacteria and is therefore not recommended.

The disinfectant must be supplied by the stock owner where infection has been found whilst the State Veterinarian prescribes the type of disinfectant and the method of disinfection.

The process must take place under official supervision and takes place just after the positive cattle have been removed from the herd. i.e. have been sent to the abattoir or have been placed in the quarantine camp. Everything in the cowshed as well as feeding and water troughs outside such buildings which could possibly have been infected by the cattle, must be disinfected. All manure, hay and other refuse are first removed from the building to a place out of reach of susceptible animals. Thereafter it is washed with water where possible. After cleaning, the disinfectant is applied according to prescription.

The disinfecting process is repeated 14 days later.

If new positive cases are found on retesting, the disinfecting procedure is repeated

g.) Tests

In a herd with a considerable number of positive reactors it is strongly recommended that the second test be done six weeks later for the sake of resensitising possible anergic cases. The second test should in any case be conducted not later than three months after the first. There-after further tests are conducted at three monthly intervals as long as new positive cases are found.

- TB-free certificate - Infected herds that comply with the requirements for accreditation receive a certificate after three negative tests at intervals of three and six months after the first negative test.
- Herds that do not comply with the requirement for accreditation - After two negative tests on the herd, movements to other herds may take place after the quarantine has been lifted. However, the herds/remain under official supervision and are incorporated into the annual diagnostic scheme to prevent re-infection of the herd or to trace infection in time before it again becomes prevalent. In this way we want to remain in control of conquered areas.

6.7.3 Files

Irrespective of the purpose for which the first test was done. a separate file must be opened for each herd where infection has been found. A file number is allocated according to the customary method as described under 6.1.3

7 ADMINISTRATION OF THE SCHEME

7.1 The ordering & issuing of tuberculin

At present each province purchases their own tuberculin, and in most provinces private veterinarians purchase their own tuberculin, from various suppliers.

In Mpumalanga & Western Cape the province purchases the tuberculin on contract (at present from Intervet Pty Ltd, who are the sole importers) and supply to State Veterinarian offices & private veterinarians in their provinces.

As a result, in most provinces there is no control of what herds are tested, as if private veterinarians purchase their own tuberculin there is no control of TB tests reaching the state for record purposes. To properly control the use and reporting of TB testing in the country ideally the National Directorate of Animal Health should purchase & control all tuberculin imported into the country. Tuberculin should then be supplied to the provinces via one official appointed to that task (ie TB & Brucellosis Coordinator as in Mpumalanga) who will only be able to order more tuberculin or supply it to State Veterinarian Offices & private veterinarians when they have submitted a tuberculin usage form (see example later) explaining what has been done with the previously issued tuberculin & copies of TB10 forms have been submitted for each test recorded.

In terms of Section 27 (4) (b) of the Bovine Tuberculosis Scheme regulations tuberculin “shall be supplied free of charge by the director if a notice of the result of such tuberculin test is furnished as contemplated in Section 9 of this Scheme.”

7.2 Forms in use (available from local State Veterinarian)

See attached examples

7.2.1 Application for TB/CA testing (TB/CA 1)

This form is used as an application by the stock owner to comply with the requirements for admittance to any of the test programmes.

The use of the form is an undertaking by the stock owner. The agreement is a legal document and should be completed fully (also date and place) and be signed by the stock owner or his authorised representative before a test is conducted on a herd for the first time. The application, together with the first test reports, is completed in Triplicate. The original is sent to the State veterinarian and a copy to the owner and Brucellosis Coordinator (or Deputy Director: Animal Health).

7.2.2 TB/CA test declaration (TB/CA 3)

(Refer also to the Bovine Tuberculosis Scheme Regulations.)

A declaration by means of a TB/CA 3 issued to any negative maintenance herd that has been tested as below and is issued by a State veterinarian.

Maintenance programme: after the second negative test and thereafter biennially following the TB test on the herd.

Infected programme: issued after the second negative test, thereafter annually for 5 years & thereafter biennially following the negative TB test on the herd.

Copy of declaration to the following

- Person who did TB test of herd
- Tuberculosis Coordinator (or Deputy Director) for control purposes

In the case of dairy herds copy of declaration to the following as well:

- Milk buyer
- Dairy Standards Agency (Fax: 012-6653895)
- Environmental Health Officer of the local Municipality

In the case of stud herds copy of declaration to the following as well:

- Stud book association concerned

7.2.3 Monthly Statement of tuberculin tests (TB 7)

a.) Office of the State Veterinarian

Here provision is made for a summary of each test conducted during the month for which the State Veterinarian has received a report which is forwarded to the Deputy Director: Animal Health of the District (and the TB and Brucellosis Coordinator where applicable).

b.) Office of the District Deputy Director: Animal Health

Here a composite summary for the whole district is drawn up & submitted to the Director: Animal Health of the province.

c.) Office of the Provincial Director: Animal Health

Here a composite summary for the whole province is drawn up & submitted to the National Director: Animal Health.

7.2.4 Abattoir findings

The notification of bovine tuberculosis found at an abattoir takes place telephonically and also per the meat inspection report form in terms of the Meat Safety Act as indicated in 3.9.6.1.1 under 'meat inspection'. The State Veterinarian or Meat Inspector where the cattle are slaughtered, completes the form because the infection comes to his attention or should come to his attention and because it is also easier for him to get the information regarding the farm of origin of the animal/s. Meat inspection at abattoirs is an important aid for the tracing of infected herds provided that everything possible is done to get the information without delay from the abattoir and thereafter determining the herd's origin if it is at all possible. The form is then sent to the State Veterinarian at the place of origin of the animal/s with copies to the relevant Deputy Director: Animal Health (and the TB and Brucellosis Coordinator where applicable). A quarantine notice is issued against the owner and the whole herd is tested as soon as is feasible. After the Deputy Director: Animal Health (TB & Brucellosis Coordinator) has received a copy of the TB report it should be kept in abeyance for one month. If after the expiry of a month no report of the examination and test results has been received, enquiries can be made at the office of the State Veterinarian thereby ensuring that the herd has not been forgotten.

7.2.5 Official tuberculosis-free certificate for accredited herds (TB9)

Refer to the Bovine Tuberculosis Scheme Regulations – no longer applies

7.2.6 Intradermal tuberculin test record (TB10)

This form is completed by the testing official or veterinarian in respect of all cattle tested & must be submitted as soon as possible, but no later than 30 days after having performed the test (together with a copy of the tuberculin usage form for both avian & bovine tuberculin as applicable), to the State Veterinarian of the area, (in terms of Section 8 (2) of the bovine Tuberculosis scheme regulations) who must check it, sign it & forward a copy of the signed document to the Deputy Director: Animal Health or TB & Brucellosis Coordinator.

The top portion, in respect of the details of the owner & farm are always to be completed in full. In respect of tests at communal dip tanks the owner could be reported as "various".

- The test from date = date of injection of tuberculin
- The test date to = date of reading the tuberculin test (which **must** always be 3(three) days later)

The second portion of the form must always be completed as fully as possible.

- 1st line – **Printed** initials & surname of tester
- 2nd line – previous test date & previous number of animals tested – to be completed if known. This may

have to be completed in the State Veterinarian office if not completed by the tester (as tester may not have such records but State Veterinarian office should have records if herd previously tested)

- 3rd line
 - No. of animals on farm – ask farmer & always complete
 - No. injected – number of animals actually injected with tuberculin – **keep accurate record and complete in all cases**
 - No. read – actual number of animals presented for reading of TB test on day 3. Should be same as number tested but in some cases (ie communal areas, animal died etc) it will not be & thus **keep accurate record of those read and complete in all cases**
- 4th line – Result
 - No. Neg – total of animals diagnosed as negative i.e. those measured because of skin reactions but diagnosed as negative **as well as** those which have been felt/read but which showed absolutely no reaction at tuberculin injection site (in most cases these will be most of the animals in a herd)
 - No. Pos – total of animals diagnosed as positive. Obviously this **must** agree with the animals diagnosed and recorded as positive in 3rd portion of form
 - No. Susp – total of animals diagnosed as suspicious. Obviously this **must** agree with the animals diagnosed and recorded as suspicious in 3rd portion of form
 - Total Read = No. Neg + No. Pos + No. Susp. This must obviously agree with the No. Read in line 3 of this section
- 5th line
 - Cattle breed – always complete. Ask farmer if not sure. Cattle will always have some breed characteristic i.e. not just mixed beef – rather Brahman X etc. Please ensure if doing TB and CA test on herd that the cattle breed mentioned on CA8 and TB10 agree for same farm and test dates!
 - Delivers milk to: This obviously only to be completed in case of dairy herds i.e. whom is milk buyer if sells to a distributor or of sells locally then mention where
 - Herd test type: Mark what is applicable. Remember that a herd will always be regarded as an infected herd as soon as there are positive reactors, no matter what the first reason for testing the herd was.
- 6th line:
 - Signed: Signature of tester
 - Date: Date signed by tester. Should be the same day or at most the day after reading the test.
 - Contact no of tester: Tester's cell phone number

The third portion of the form is however completed differently for the various programmes.

The fourth portion of the form is completed & signed by the State Veterinarian of the area after checking the form for correctness and whether he/she agrees with the diagnosis of each animal. A copy must then be sent to the TB and Brucellosis Coordinator or Deputy Director: Animal Health.

a.) Accreditation, Infected herds & Cattle tested for Export

The first two portions of the form are completed in full as described above under 7.2.6.

The third portion of the form is completed as follows in respect of these tests. Get into the habit of reading out the information in the order of the form from left to right so that the writer does not become confused where to right what

- No allotted to animal:
 - 1....etc. The allotted number in case of Accredited and Infected herds is always the same for the same animal. Thus normally would write all animal numbers down and then when get back to office organize the ear tags in description column in alphabetical, numerical order and the allocate allotted number, otherwise record keeping at next test becomes very difficult.
 - The number allotted to an animal during the 1st test is kept for all subsequent tests and the number is NOT given to another animal
- Description of each animal tested
 - Ear tag, tattoo or individual animal brand number
 - Breed of animal – if crossbreeds the describe the colour of the animal
 - Sex: cow/heifer, ox, bull

- Age: approx age of animal – very NB for positive reactors
- When doing the test it is not necessary to measure each animal at time of testing but at least mark that is present. Again when reading the test three days later every animal's injection site must be palpated, and the animal marked off as present, but only those with palpable reactions will be measured & recorded – in the cases of animals showing palpable/visible reactions the measurements are noted as follows:
 - Bovine tuberculin injection site:
 - Normal Bovine skin fold: Measure skin above the bovine injection site (if comparative test was performed this will be the rear injection site)
 - 72hrs Bovine skin fold: Measure the skin across the thickest part of the swelling – in cases where there is oedema the swelling may sag down.
 - Bovine difference: Swelling – normal
 - Avian tuberculin injection site:
 - Normal Avian skin fold: :Measure skin above the avian injection site (if comparative test was performed this will be the front injection site)
 - 72hrs Avian skin fold: Measure the skin across the thickest part of the swelling – in cases where there is oedema the swelling may sag down.
 - Avian difference: Swelling – normal
 - Bovine diff – Avian diff: If the bovine swelling is larger this will be written as +, if the avian swelling is larger this will be written as –
 - Remarks: Describe only the bovine reaction site, as well as the enlarged prescapular lymph nodes (if applicable). Use the shorthand as described earlier under 3.8
 - Diagnosis: The diagnosis per animal must be written here, i.e. Pos (Positive), Susp (Suspicious) or Neg (Negative)

b.) Diagnostic tests

The first two portions of the form are completed in full as described above under 7.2.7.

The third portion of the form is completed as described under 7.2.7.1 but **only in respect of all animals showing visible/palpable reactions**. In this case the allotted number is ignored

7.2.7 Instruction (order form (TB/CA11)

This form is only used in exceptional cases, for example if the herds in an area or district are being tested systematically under the diagnostic herd programme and some owners who refuse to fall in line, can be compelled to test. This should be issued by the State Veterinarian personally after discussion with the Tuberculosis Coordinator (or Deputy Director: Animal Health).

7.2.8 Notice: Bovine Tuberculosis infection - TB 12 Quarantine notice

According to the Standing Regulations in terms of the Animal Diseases Act, 1094 (Act 35 of 1984) the onus rests with the stock owner to keep such animals in quarantine until a State Veterinarian authorises their release in cases where a controlled disease mentioned in Table 2 has been determined or is suspected. The issuing of a written quarantine notice is therefore not required. However, to obviate any doubt also as far as later court cases are concerned it is usual to issue a written notice in the prescribed manner where bovine tuberculosis has been determined. It is preferable for the State Veterinarian to deliver it himself. It will enable him to give the necessary guidance and to inform the owner of his duties and the control measures, etc., so as to minimise any misunderstanding.

This quarantine is lifted in writing after two consecutive negative herd tests have been conducted with an interval of three months. The regular tests are however not stopped thereafter.

7.2.9 Notice to private veterinarians and Animal Health Technician's to test herds due for retest (TB/CA 17)

The form enables the State veterinarian to remind a private veterinarian of the tests of his clients herds that are due. This form can also be used to remind the AHT to perform testing on a specific herd. The TB/CA 17 is completed in triplicate and the State veterinarian sends the original copy to the private veterinarian or Animal

Health Technician, one copy is placed on the relevant herd file and one copy is sent to the Tuberculosis Coordinator (or Deputy Director: Animal Health.)

7.2.10 Tuberculin test result card (TB24 (LA27/19))

This card is designed to be fixed to the counterfoil of the file for each herd for which there should be a file, i.e.:

- accredited herds
- Maintenance herds
- infected herds.

The card makes provision for entering the following:

- test date;
- number tested and results;
- meat inspection findings in respect of positive reactors;
- by whom tested;
- claim by Private Veterinarians;
- bovine and avian tuberculin group number.

By keeping this card up to date a continuous test history is maintained and data can be obtained at a glance without having to page through the whole file.

7.2.11 Bovine TB Scheme: Compensation claim form

- Must be accompanied:
 - by proof of what was condemned as well as price of offal/carcasses on day of sale
 - as well as TB10 form showing that animal tested positive
- Only paid out for condemnations

7.2.12 Red Cross permit

Cattle from a positive herd may only be removed from the farm directly to an abattoir under cover of a red cross permit until such time as the quarantine has been lifted.

8 TUBERCULOSIS – LEGISLATION

The Animal Diseases Act, 1984 (Act 35 of 1984), Animal Diseases Regulations (R2026 of 26 September 1986) and the Bovine Tuberculosis Scheme Regulations (R1953 of 30 September 1988), all as amended, give the necessary authorisation for the control, combating and eradication of bovine tuberculosis.

The measures for combating tuberculosis can be summarised as follows, mention being made of the relevant Sections of the Act and Regulations applicable: (with credit to Dr Ben du Plessis for the tables below)

ACT 35/1984 SECTION	ANIMAL DISEASES REGULATIONS	SUBJECT	ASPECTS w.r.t. TUBERCULOSIS
9 (2) (a) 11 (1) (b) (i) 31	11, table 2	Control measures Duties of owners/managers	Testing Isolation Marking Slaughtering Treatment
9 (2) (h) 11 (1) (b) (ii) 31	12	Reporting Duties of owners/managers	Reporting of incidence or suspected incidence of controlled disease by responsible person/veterinarian to State Veterinarian/Animal Health Technician
9 (2) © 11 (1) (a) 31	13	Isolation Duties of owners/managers	Isolation of contact or infected animals
9 (2) (d) 11 (1) (a) 31	14	Prohibition of access Duties of owners/managers	Prohibition of access to places with isolated animals
9 (2) (a) 11 (1) (a) 31	15	Disinfection Duties of owners/managers	Timing, effectivity, concentration, extent, removal and disposal with regard to disinfection of places, conveyances and appliances; inaccessibility of places to animals; washing of person, clothes and equipment.
9 (2) (h) 11 (1) (b) 31	16	Sampling Duties of owners/managers	Taking, preservation, treatment, packing, dispatching and delivery of samples.
9 (2) (b) 31	18	Proof of performance of controlled veterinary acts	Certificate, document, sworn declaration, containers and invoices pertaining to controlled veterinary acts performed in terms of regulation 11.
9 (2) © 31	20 (1) (a) (xiii)	Movement restrictions	Prohibition of movement to accredited herds
9 (2) © 31	22	Slaughter restrictions	Prohibition of slaughter of isolated animals
9 (2) (a) 31	24 (1) (a)	Disposal restrictions	Prohibition of use and disposal of unboiled, unpasteurised or unsterilised milk from infected or suspected infected animals
10		Animal health schemes	See below
15 31	28	Orders	Serving, binding, authority, amending, proof of orders
16		Powers of entry and inspection	Entry upon land and conveyances, assistance, searching, investigation, inspection, marking, testing, interrogation
16 (2) (a) (xiii) 31	29, table 3	Marking	Indication of infection by "T" branding on the left side of neck
19 31	30	Compensation	Extent of compensation for infected and killed animals and infectious or contaminated things
25		Secrecy	Prohibition of disclosing of and access to information, exceptions

8.1.1 BOVINE TUBERCULOSIS SCHEME

(R1953 of 30 September 1988)

established under section 10 of Act 35 of 1984

SECTION	SUBJECT	BRIEF DESCRIPTION
1	Definitions	Meaning of word and expressions
2	Name of scheme	Bovine Tuberculosis Scheme
3	Object of scheme	(1) Eradication of bovine tuberculosis (2) by testing, identification, slaughtering, isolation, prevention of contact and information.
4	Application and scope of scheme	(1) <i>Mycobacterium bovis</i> , cattle (2) Objects of six programs; accredited herd, annual diagnostic (maintenance), diagnostic herd, infected herd, isonicotinic acid hydrazide treatment and diagnostic testing.
5	Manner of infection	(1) Secretion and excretion of <i>Mycobacterium bovis</i> (2) Infection by inhalation, licking, grazing or consuming fodder, water or milk.
6	Characteristics of infection	(1) Mostly no symptoms (2) Advanced stage symptoms (3) Post mortal exhibition of tubercles
7	Tests for bovine tuberculosis	(1) Tuberculin test (2) Interpretation (3) Other tests
8	Requirements relating to a tuberculin test	(1) Only by an officer, authorized person or veterinarian (2) Notification to State Veterinarian of results (3) Prohibition of removal of cattle during test (4) Making cattle available for testing
9	Notification of infection	Written notification of State Veterinarian of infection or suspected infection
10	Measures applying to infected herds	(1) State Veterinarian to order isolation of infected cattle herd or suspect cattle (2) Prohibition of movement of cattle on land with isolated cattle or cattle herds (3) Authorisation to move cattle on land with isolated cattle or cattle herds (4) Identification of isolated cattle (5) Record keeping of isolated cattle (as in regulation 17) (6) "T" brand on left side of neck, separation, prohibition on retesting and authorization of retesting of infected cattle (7) Retesting of suspect cattle (8) Retesting of infected cattle herd
11	Revocation of isolation	(1) Conditions for revoking isolation order of infected cattle herds (2) Conditions for revoking isolation order of suspect cattle
12	Disinfection of certain places and things	Manner, frequency and remedy for disinfection of structures on land with infected cattle herd (as in Regulation 15)
13	Disposal of infected bovines	(1) Slaughtering of infected cattle at an abattoir or on land (2) For account of responsible person (3) Meat inspection by veterinarian (4) Compensation (as in section 19 of Act 35 of 1984) (5) Time of slaughtering
14	Application of INH treatment	(1) Prohibition of INH treatment (2) Permission of INH treatment (3) Agreement, undertaking to comply
15	Requirements for joining scheme	(1) – (6) Requirements for joining each program (see table below)

16	Admission to scheme	(1) Application by responsible person to participate in accredited herd, annual diagnostic (maintenance) herd, diagnostic herd or diagnostic testing programs (2) Admittance of responsible person to infected herd or isonicotinic acid hydrazide treatment programs
17	Refusal of applications	Refusal of application due to non-compliance with requirements or inability to render services
18	Register of responsible persons and herds	State Veterinarian to keep a register with particulars of admitted responsible persons
19	Lapsing and cancellation of participation	(1) Conditions for lapsing (2) Conditions for cancellation (3) Additional conditions for lapsing or cancellation of participation in infected herd and INH treatment programs
20	Switching from one program to another	Conditions for switching from one program to another
21	Measures relating to participating herd	Measures pertaining to contact, sharing, introduction of cattle to herd, making cattle available for testing, applying control measures, recordkeeping and marking
22	Issue of certificates and declaration	(1) Issuing and duration of validity of certificate i.r.o. cattle herds in accredited program (2) Issuing of declaration i.r.o. cattle herds in annual diagnostic (maintenance) or diagnostic herd programs (3) Issuing of declaration i.r.o. cattle herds in infected herd or INH programs (4) Issuing of declaration i.r.o. cattle in diagnostic testing program (5) contents of declaration
23	Renewal of certificates	(1) Application for renewal of certificate (2) Tuberculin testing (3) Conditions for renewal of certificate (4) Duration of validity of certificate
24	Lapsing of certificates	(1) Conditions for lapsing (2) Conditions for issuing of new certificates in case of lapsing
25	Return of certificates	Return of lapsed certificates
26	Restrictions on the use of certificates and declarations	(1) Prohibition of misuse of declarations (2) Conditions for use of certificate
27	Tariffs for services rendered	(1) Fees for services rendered as in regulation 27 (2) Free services (3) Disinfectants and remedies at expense of responsible person (4) INH supplied free of charge, tuberculin supplied free of charge for infected or suspected cattle herd
28	Commencement of scheme	1988-10-01

8.1.2 REQUIREMENTS FOR JOINING BOVINE TUBERCULOSIS SCHEME

(SECTION 15 OF BOVINE TUBERCULOSIS SCHEME)

REQUIREMENT PROGRAM	ACCREDITED HERD	ANNUAL DIAGNOSTIC (MAINTENANCE)	DIAGNOSTIC HERD	INFECTED HERD	INH TREATMENT	DIAGNOSTIC TESTING
Closed herd management	X	X		X	X	
Fencing	X			X	X	
Isolation facilities	X			X	X	
Handling facilities	X	X	X	X	X	X
Written undertaking to co-operate	X	X	X	X	X	

9 Animal Disease reporting codes:

The Disease reporting codes (Province Code, State Vet Code and Local Municipality Code) contained in the Disease Reporting Manual should be used for the TB Scheme.

A copy of the Disease Reporting Manual can be downloaded from www.daff.gov.za
Branches>>Agricultural Production, Health and Food Safety > Animal Health > Epidemiology
OR

Send an email to epidemiology@daff.gov.za requesting a copy of the manual

APPLICATION FOR ADMISSION TO THE BOVINE TUBERCULOSIS / BOVINE BRUCELLOSIS ERADICATION SCHEME
 AANSOEK OM TOELAATING TOT DIE BEESTUBERKULOSE / BEESBRUCELLOSE UITROEINGSKEMA

I
 Ek _____

With postal address:

Met Posadres: _____

Postal Code:
 Poskode: _____

And tel no:

En tel nr: _____

Dialling code:
 Skakelkode: _____

Hereby apply to have _____ cattle on the farm _____ in the Magisterial District of:
 Doen aansoek om _____ beeste op die grond (plaas) _____ in die landdrosdistrik :

_____ tested for tuberculosis and / or Brucellosis in terms of the following programme:
 _____ te laat toets vir tuberkulose en / of Brucellose ingevolge die volgende program:

- Accredited herd programme / Geakkrediteerde kuddeprogram
- Maintenance herd Programme / Kuddeonderhoudprogram
- Diagnostic herd programme / Diagnostiese kuddeprogram
- Diagnostic test programme / Diagnostiese toetsprogram

Purpose of test:
 Doel van toets:

- Stud herd (dairy) / Stoetkudde (melk)
- Stud herd (beef) / Stoetkudde (vleis)
- Commercial herd (dairy) / Kommersiële kudde (melk)
- Commercial herd (beef) / Kommersiële kudde (vleis)
- Mixed herd / Gemengde kudde

I, the undersigned undertake to comply with the provisions of the Animal Diseases Act, 1984 (Act 35 of 1984) regarding bovine tuberculosis / brucellosis eradication.

Ek, die ondergetekende onderneem om die bepalinge van die Wet op Diersiektes, 1984 (Wet 35 van 1984) betreffende beestuberkulose / beesbrucellose uitroeijing na te kom.

 Date / Datum

 Responsible person / Verantwoordelike persoon

TB/CA3 VERKLARING/DECLARATION

Verwys Nr/Ref No: **01/161/126**
State Veterinarian **Albert Luthuli**

Adres: **P O Box 89**
Address: **Badplaas**
1190
Datum: **9 December,**
2013

HEIL DIE LESER
TO WHOM IT MAY CONCERN

Hiermee word verklaar dat die **Fries melk kudde**
This is to declare that the **Friesland dairy herd**

Behorende aan:
Belonging to: **FA van der Walt**
Op die plaas/ perseel:
On the farm/ premises: **Mooifontein**
In die plaaslike munisipaliteit
van:
In the local municipality of: **Albert Luthuli**

Onderwerp is aan die voorgeskrewe toets vir
Was subjected to the prescribed tests for

Beestuberkulose*
Bovine tuberculosis*

Op: Met getal getoets: With
On: **15 January 1990** number tested: **137 (Dr JCI Uys)**
En opvolg toets: Met getal getoets:
And follow-up test: **18 April 2006** With number tested: **106 (Dr JCI Uys)**
(write comments in here – ie first negative test on positive herd or whatever.)

Beesbrucellose*
Bovine brucellosis*

Op: Met getal getoets:
On: **7 February 2002** With number tested: **15 (Serology – MPVL**
0202048)
En opvolg toets: Met getal getoets:
And follow-up test: **7 April 2006** With number tested: **106 (Serology – MPVL**
0604062)

(Brucellosis declaration to be renewed in April 2007)

Met negatiewe resultate
With negative results

Veearts / Veterinarian

MONTHLY REPORT: TB/CA SCHEME FOR COMMUNAL FARMERS

TB/CA 7

PROVINCE/SV AREA: _____

MONTH: _____

	HERDS		ANIMALS			
	TESTED	POSITIVE	TESTED	POSITIVE	SUSPECTED	NEGATIVE
Tests for month						
Previous total						
Total for year						
(1/4/ - 31/3/)						

MONTHLY REPORT: TB/CA SCHEME FOR COMMERCIAL FARMERS

PROVINCE/SV AREA: _____

MONTH: _____

	HERDS		ANIMALS			
	TESTED	POSITIVE	TESTED	POSITIVE	SUSPECTED	NEGATIVE
Tests for month						
Previous total						
Total for year						
(1/4/ - 31/3/)						

Signed:

Date:

INTRADERMAL TUBERCULIN TEST RECORD

TB10

INITIALS _____
& SURNAME OF OWNER _____
Postal Address _____

FARM Name . _____
& Registered Farm No _____
GPS Coordinates: E: _____ : _____ : _____ E: _____ : _____ : _____

District Municipality _____
Local Municipality _____

Contact No: _____ DATE OF TEST FROM _____ TO _____

I, _____ hereby declare that I performed a tuberculin test on the above farm on the dates as shown above:
(Print initials & Surname of tester)

Previous test date: _____ Prev number cattle tested: _____

No. of animals on farm: _____ No. injected: _____ No Read _____

Result: No. Neg: _____ No. Pos: _____ No. Susp: _____ = Total Read: _____ (Reactor identity & descriptions shown below)

Cattle Breed: _____ Delivers milk to: _____ Herd Test Type: Infected/Diagnostic/Maintenance

Signed: _____ Date: _____ Contact No of tester: _____

Signature of Testing Official/Veterinarian

No. allotted to Animal	Discription /Identification (Tag No., Breed, Sex, Age)	Bovine TUB			Avian TUB			Bov Diff - Avian Diff	Remarks i.r.o swelling etc (see abreviations below)	Diagnosis
		Normal Skinfold	72 Hours	Bov Diff (72hrs - normal)	Normal Skinfold	72 Hours	Avian Diff (72hrs - normal)			
1										
2										
3										
4										
5										
6										
7										
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Abbreviations: T= Tender; H = Heat; O = Oedematous; D = Diffuse; C = Circumscribed; F = Flat; S= Slight; A = Adhesions

Guidelines for Neg or Unknown Herds

Single test: > 6mm increase= Susp
Comparative Test Bovine - Avian > 4 mm = Pos
 Bovine - Avian 3 - 4 mm = Susp
 Bovine - Avian < 2 mm = Neg

Guidelines for Infected Herds

Single test: > 4mm increase= Susp
Comparative Test Bovine - Avian > 3 mm = Pos
 Bovine - Avian 1 - 2 mm = Susp
 Bovine - Avian < 0 mm = Neg

FOR STATE VET USE ONLY

State Vet Herd Ref No. _____

Next test Date _____

Comments:

Signature of State Veterinarian

Republiek van Suid-Afrika

Departement van Landbou-

Republic of South Africa

Department of Agricultural

Dr.

.....

.....

.....

Staatsveearts
State Veterinarian

.....

.....

.....

Tel.

Navrae
Enquiries.....

Kuddeverw.
Herd Ref.

Kontraknr.
Contract No.....

Datum
Date

**MAGTIGING OM DIENSTE TE LEWER
AUTHORISATION TO RENDER SERVICES**

U word hiermee versoek en kragtens artikel 4 (1) van die Wet op Dieriesiektes, 1984 (Wet 35 van 1984) om magtig om die dienste met 'n
You are hereby requested and authorised under section 4 (1) of the Animal Diseases Act, 1984 (Act 35 of 1984) to perform the services marked
aangedul namens die Staat uit te voer voor die datum aangedul, ten opsigte van die kudde van:
with an on behalf of the State before the date indicated, with regard to the herd of:

Eienaar
Owner

Posadres
Postal address

.....

Dienste/Services

Datum/Date

- Inspeksie van kudde/Inspection of herd19.....
- TB toets op kudde/TB test on herd19.....
- Bloei kudde vir BM/Bleed herd for CA19.....
- Neem melkmonster vir BM/Collect milk sample for CA19.....
- Toets verdagte beeste vir TB/Test suspected cattle for TB19.....
- Bloei verdagte beeste vir BM/Bleed suspected cattle for CA19.....
- Sit oorplaatjies in/Affix eartags19.....
- Brand positiewe beeste T/C/Brand positive cattle T/C19.....
- Waardeer positiewe beeste/Valuate positive cattle19.....
- Hou toesig oor ontsmetting/Supervise disinfection19.....

Stel my asseblief vooraf in kennis wanneer bogenoemde dienste gelewer gaan word.
Please inform me beforehand when above-mentioned services will be rendered.

.....
Staatsveearts/State Veterinarian

BRUCellosis/TUBERCULOSIS ERADICATION SCHEME

PRODUCER NUMBER

--	--	--	--	--	--	--	--	--	--	--	--

SCHEME (T or B)

HERD REFERENCE NUMBER

HERD TYPE

NAME OF OWNER

ADDRESS 1

ADDRESS 2

ADDRESS 3

ADDRESS 4

POSTAL CODE

NAME OF FARM

CODE: STATE VETERINARIAN

YEAR OF ADMITTANCE

DATE: RECENT INFECTION

PREVIOUS TEST DATE

NUMBER OF CATTLE TESTED

PRESENT TEST DATE

NEXT TEST DATE

FREQUENCY CODE AND TYPE OF TEST

NUMBER OF CATTLE TESTED

NUMBER OF CATTLE NEGATIVE

NUMBER OF CATTLE POSITIVE

NUMBER OF CATTLE INCONCLUSIVE

CODE: OFFICIAL

EXPENSES: OFFICIAL

(i) TRANSPORT

(ii) SUBSISTENCE ALLOWANCE

CODE: PRIVATE VETERINARIAN:

EXPENSES

(i) TRANSPORT

(ii) SERVICES RENDERED

EXPENSES: POSITIVE REACTORS

(i) NUMBER SLAUGHTERED

(ii) VALUATION (@ 80%)

(iii) NETT INCOME/LOSS

(iv) COMPENSATION PAID

(v) DATE SLAUGHTERED

(vi) MEAT INSPECTION

RECOMMENDATION—CERTIFICATE

(i) YES/NO

(ii) TYPE OF CERTIFICATE

(iii) EXPIRY DATE

NATURE OF REPORT:

0 Retest

1 Delete

2 Alter

3 New

CODE

HERD REFERENCE NUMBER

29/Region/District/Numerical number

HERD TYPE

01 = Accreditation

02 = Annual diagnostic

03 = Herd diagnostic

04 = Diagnostic

05 = Import

06 = Export

07 = INH

09 = Infected

FREQUENCY CODE AND TYPE OF TEST

(Purpose of present test and test performed)

1 = First test

2 = Second test

3 = Annual test

4 = Biennial test

5 = Inconclusive reactors

6 = Additions

7 = Infected/INH—herd tests

T = Tuberculin test

S = Serological test

M = Milking test

CODE: OFFICIAL

Region/SV/No

MEAT INSPECTION: NUMBER

L = Local

A = General

P = Passed—No lesions

CERTIFICATE: RECOMMENDATION

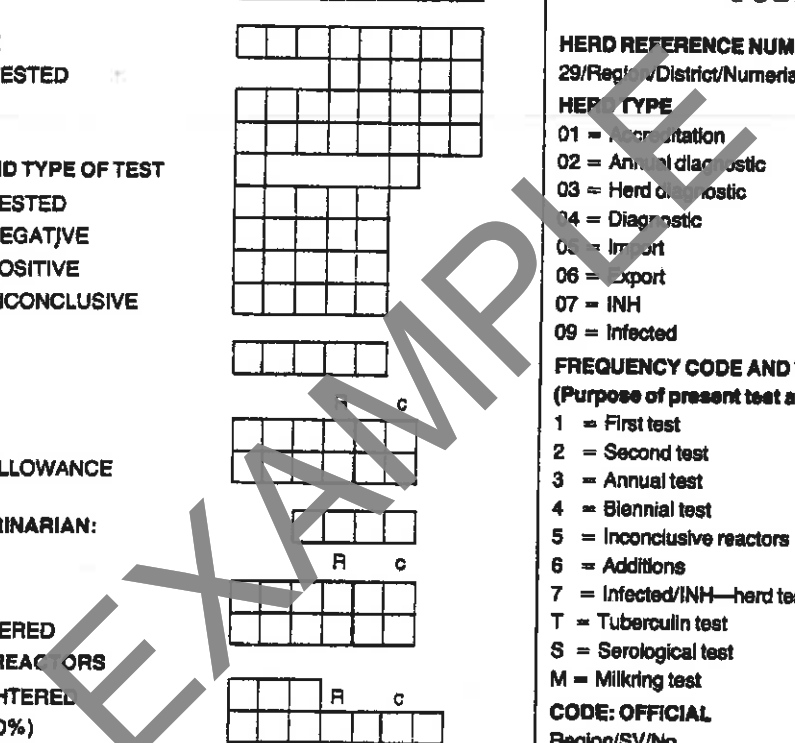
0 = No 1 = Yes

TYPE OF CERTIFICATE

00 = No certificate

01 = Accreditation

02 = Annual diagnostic



CERTIFIED CORRECT		APPROVED	
..... State Veterinarian Date Director: Veterinary Services	
..... Regional Director	 Date	

State Veterinary area.....

Name of testing official (please print).....