



agriculture,  
forestry & fisheries

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Department:  
Agriculture, Forestry and Fisheries  
REPUBLIC OF SOUTH AFRICA

# BOVINE BRUCELLOSIS MANUAL

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A handwritten signature in black ink, appearing to read 'M. Maja', written over a horizontal line.

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# Part 1 – General Information on disease & control

## 1 Introduction

### 1.1 Principle:

Brucellosis is a bacterial disease of cattle caused by *Brucella abortus*, which may cause abortions. The disease is most commonly spread between herds by the movement of infected animals and between animals by contact of susceptible animals with infective discharges at the time of calving or abortion of infected animals, and for up to 1 month thereafter. Other less common means of spread occur.

Infected animals are generally detected by serological tests, in particular the Rose Bengal Test (RBT), and complement fixation test (CFT), which measure the presence of antibodies to *B. abortus*. Other tests such as the milk ring test (MRT), ELISA and culture and typing may be used in certain situations.

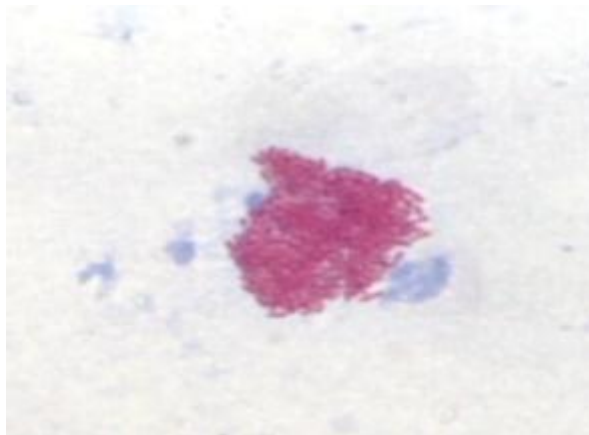
Most infected animals become serologically 'positive' soon after infection but some may not sero-convert until after the first abortion or calving post-infection. Some may revert to being serologically negative after a short interval. Very rarely animals may be infected but not detected by serological tests. Not all infected animals become infective and contaminate the environment. Factors other than exposure to infection (e.g. strain 19 vaccination or infection with antigenically similar organisms) may result in the production of antibodies, which are detected by the serological test.

In cattle the disease is controlled in terms of the Animal Diseases Act, 1984 (Act 35 of 1984). This is as a result of the economic impact and the public health hazard the disease poses. Abortions storms lead to large financial losses to the dairy and beef farmer alike. Additional financial losses occur due to decreased milk production, extended inter-calving period and decreased value of breeding stock. Disease in humans is debilitating and can become chronic if not treated efficiently and timeously. The first phases of schemes for the eventual total eradication of Bovine Tuberculosis and Brucellosis in both cattle and small stock were put into operation during 1969.

National, provincial and area guidelines have to be developed to guide Veterinary Services towards successful detection and eradication of brucellosis. However, these goals will not be achieved without sound, considered and professional input from all staff and continued support from industry.

## 1.2 General:

*Brucella abortus* is a gram-negative bacterium that can be described as a coccus, a coccobacillus or short rod. It is approximately 0,5 microns in diameter and 0,6-1,5 microns long. Although it is not a true acid fast organism, it can resist decolourisation by weak acids. It therefore stains red when the modified Ziehl-Neelsen (Stamp) staining technique is used.



Animals become infected by ingestion of infected material per os. The organisms enter the body's cells and live as intracellular organisms. Large numbers of organisms are excreted when an infected cow or heifer calves or aborts. Infected cows periodically excrete organisms in the milk. The excretion of *Brucella* in the bull string has not been shown to be of importance in the spread of this disease. Should semen from an infected bull be used for AI, the risk of spread of the disease is great, but if used for natural service does not play a role in spread of the disease. The pathogen can survive for some weeks in a cool, moist environment.

*Brucella abortus* causes abortion in cattle, and occasionally in sheep and goats.

Apart from *B. abortus*, seven other species are known, including *B. melitensis*, *B. suis*, *B. neotomae*, *B. ovis* and *B. canis*. Their importance with respect to infection and their incidence in South Africa is as follows:

*B. melitensis* causes abortion in sheep and goats, and Malta fever in humans. It also occasionally causes abortion in cattle and wildlife. Cattle are affected when they live in close contact with infected sheep and goats. Serological testing cannot differentiate between *Brucella* species and hence a positive serological result can indicate any of the following causative smooth strained *Brucella* species: *B. abortus*, *B. melitensis*, *B. suis*.

*B. canis* causes brucellosis in dogs. People can become infected.

*B. ovis* causes epididymitis in rams and infertility in ewes. Sheep brucellosis has been found in all sheep breeds in South Africa. Goats and other animal species are apparently not affected. This is **not** a controlled disease.

*B. suis* is a pathogen of pigs that can also infect humans, dogs and horses. To date it has not yet been isolated in South Africa.

*B. neotomae* causes brucellosis in desert rats and has also not been isolated in South Africa.

*B. cetaceae* has been found in marine cetaceans. Its significance in South Africa is unknown.

*B. pinnipediae* has been found in marine pinnipeds. Its significance in South Africa is unknown.

Humans can become infected when they come into contact with: infected excretions of cattle, fetuses or abortions, foetal membranes or with infected carcass material in abattoirs. Brucellosis vaccines should be

handled with care. Humans can also be infected by ingesting infected unpasteurized, unboiled milk. The disease in humans caused by *B. abortus* is also called undulating fever.

**Classification of the genus *Brucella***

Species	Biovar(s)	Colony morphology	Host(s)	Pathogenicity humans
<i>B. melitensis</i>	1-3	smooth	goats, sheep, cattle, wildlife	high
<i>B. abortus</i>	1-6, 9	smooth	cattle, sheep, goats, wildlife	average
<i>B. Suis</i>	1 et 3	smooth	pig	high
	2	smooth	hare	not
	4	smooth	reindeer, caribou	average
	5	smooth	rodents	high
<i>B. neotomae</i>	-	smooth	desert rat	not
<i>B. ovis</i>	-	rough	sheep, experimentally in goats	not
<i>B. canis</i>	-	rough	dog	low
<i>B. cetaceae</i>	-	smooth	cetaceans	average
<i>B. pinnipediae</i>	-	smooth	pinnipeds	average

*B. ovis* and *B. canis* are rough strain brucella and are not diagnosed with the same serological tests as *B. melitensis* and *B. abortus* which are smooth strains.

**1.3 Immunology:**

**1.3.1 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 and kill similar intruders more quickly in future (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 country’s rules (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. Similar to persons carrying 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 (Interferons, fibronectin, and complement), hand-to-hand combat (macrophages and neutrophils), inactivation (antibodies) and encircling and smothering actions (macrophages

and lymphocytes that stick together and form granulomas).

## NON-SPECIFIC IMMUNITY

Non-specific immunity includes those defences 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 defences, inflammation, fever, etc.

## SPECIFIC IMMUNITY

Specific immunity is that aspect of the body's defences 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 (General Immunity/Non-specific 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.

The Immune system comprises 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.

### 1.3.2 CELLS AND MOLECULES OF THE IMMUNE SYSTEM

#### *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 antigen, usually a protein, polysaccharide or lipopolysaccharide. B-lymphocytes contact antigens via Antigen Presenting Cells (APC) and split to form memory cells and plasma cells. Plasma cells produce antibodies that bind and inactivate pathogens and memory cells enable the immune system to react quicker when exposed to the same pathogen in future.

For every foreign antigen, there are antibody molecules specifically designed for that particular 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. On the first exposure to an antigen, IgM production levels are much higher than IgG levels. On subsequent reactions, IgG levels will be higher than IgM levels.

#### *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. Antigens need to be presented to T-lymphocytes by Antigen Presenting Cells

(APC) whereafter T-lymphocytes then split into memory cells and sensitized (killer/ helper) cells. Sensitized cells release chemical mediators that attract phagocytic cells to destroy infected cells and memory cells enable the immune system to react quicker when exposed to the same pathogen in future.

*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.

*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.

*Granulocytes (Polymorphonuclear Leukocytes):*

These include 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. They may also be effector cells in antibody-mediated auto-immune or hypersensitivity reactions, causing host tissue damage.

### **1.3.3 Cell mediated Immunity**

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 host 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.

### **1.3.4 Humoral Immunity**

Humoral Immunity (BR) (Immunity conferred by antibodies directed against a) bacterial capsules that are antiphagocytic e.g. polysaccharide capsules of *Streptococcus pneumoniae* and *Neisseria meningitidis* groups A and C in humans or to the polypeptide capsule of *Bacillus anthracis*; b) antibodies to bacterial toxins e.g. *Clostridium tetani* and *Corynebacterium diphtheriae*; and c) antibodies to viruses [viral neutralization])

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



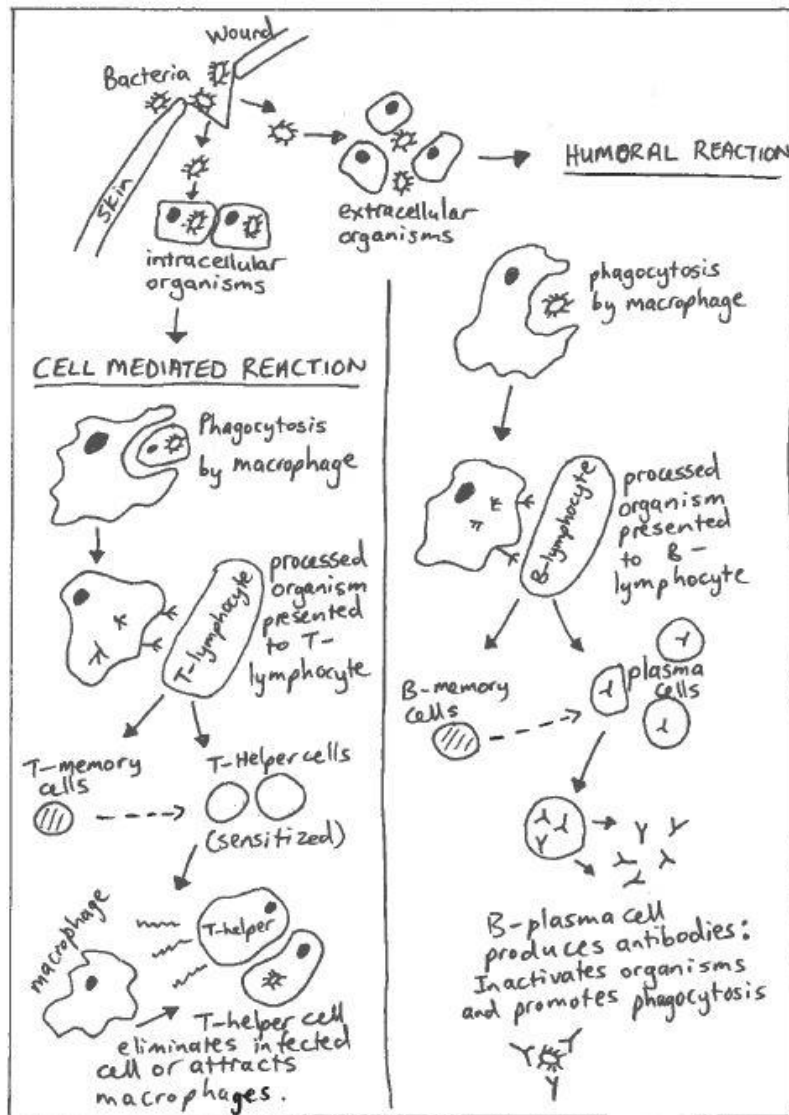


Image showing cellular versus humoral immunity

### 1.3.5 Brucellosis Immunology

*Brucella abortus* antigenic stimulation of the host immune system includes the Lipopolysaccharides (LPS) of its gram negative cell wall. Field strains and the S19 vaccine strains have an O-side chain LPS. The RB51 vaccine strain does not have an O-side chain. 8 different biovars exist and may be identified on phage biochemistry with monospecific antiserum. Cross reactions to other bacteria may occur (e.g. *Yersinia*, *Chlamydomphila*, *Coxiella*). The brucella bacteria typically enters the body through the mucous membranes (nose, mouth, conjunctiva), where the reticulo-endothelial system (macrophages) picks it up and drains it to the local lymphnodes. From here a bacteremia usually ensues which may be recurrent. The organism typically targets the synoviae of joints, the testes and seminal vesicles, the udder and the gravid uterus (erythitol sugar) which includes the endometrium, placenta and foetus. Cell destruction and inflammation occurs which may lead to abortion. *Brucellae* are intracellular bacteria that stimulate both the cellular and humoral immune systems.

**The humoral immune component** is driven by B-lymphocytes. Antigen Presenting Cells (macrophages) present brucella specific antigens to B-lymphocytes. Memory B-cells are formed as well as active Plasma cells that produce specific antibodies to neutralize *Brucellae*. These plasma cells die after a few days and the antibody titre starts dropping. Memory cells retain the immunity for future recognition of the pathogen. IgM is produced

first (primary response) during the natural immune response, followed by IgG after a short lag period which reaches higher concentrations. IgM declines once IgG starts spiking. During vaccination IgM antibodies persists longer and reaches a greater peak than IgG (compared to normal infection). This phenomenon may be useful in differentiation of infected versus vaccinated cattle if paired serum samples are collected for serology (SAT test). Experience is needed in interpreting results.

**The cellular immune component** consists of macrophages and neutrophils. APC's present *Brucellae* antigens to T-lymphocytes. T-Memory cells are formed, as well as active T-helper/ killer cells that are able to recognize *Brucellae* infected cells. These T-helper/ killer cells then attach to the infected cells and secrete mediators to attract phagocytic cells (macrophages) to destroy the infected body cells. Cellular mediated immunity is a type-IV hypersensitivity reaction (principle that the brucellin intradermal test is based on, that works in similar fashion to the tuberculin intradermal test).

## 2 Pathogenesis and symptoms

The incubation period can be long and variable, depending on the animal's sex, age, sexual maturity and stage of pregnancy. The following periods are averages for what may be found in practice:

- A heifer calf becoming infected in utero or at birth (latent carrier) -18 months or longer
- A cow five months pregnant - 14 days.
- A cow that recently conceived - 225 days

A susceptible herd is normally infected when an infected female that has been brought into the herd, calves or aborts in the new environment. Brucellosis spreads in such a herd by contamination of the environment by aborted fetuses, afterbirths and uterine secretions (lochia). Infections can also occur if cows are inseminated using semen from infected bulls, because the semen is placed directly into the uterus.

The establishment of infection is influenced by the size of the infective dose, virulence of the bacteria, resistance of the host, the age, sex and reproduction status of the animal.

*Brucella abortus* readily penetrates membranes such as those of the nasal, oral or pharyngeal cavities and conjunctiva and survives and multiplies in the cells of the reticuloendothelial system (RES). After penetration, the organisms are phagocytosed by neutrophils and macrophages, which carry them to the regional lymph nodes where the brucella organisms multiply and cause lymphadenitis, which may persist for months. Multiplication of the organism may be followed by a bacteraemia, which may last for several weeks, resolve itself, or may be recurrent for at least two weeks in 5-10% of animals. Recurrence occurs particularly at the time of parturition.

During the bacteraemia, organisms are carried intracellularly in neutrophils and macrophages, or free in the plasma and localize in various organs, particularly the pregnant uterus, udder and supra-mammary lymph nodes. Localisation may also occur in other lymph nodes, the spleen, testes and male accessory sex glands. *Brucella* organisms have a predilection for, amongst others, the pregnant uterus and foetal and testicular tissue. This is apparently linked to the presence of a sugar-alcohol, erythritol, occurring in these tissues.

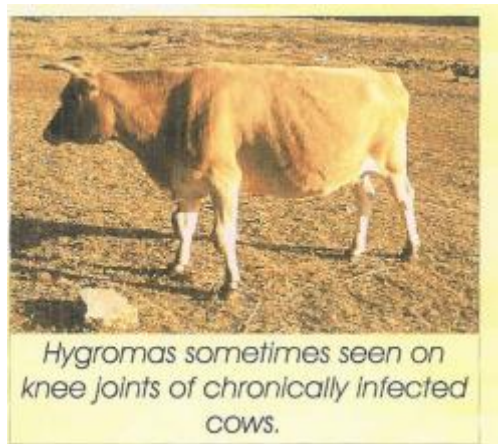
In the uterus infection causes varying degrees of placentitis (inflammation of the placenta). As a result of a disturbed gaseous exchange between the dam and foetus, the foetus dies and is aborted. In herds where brucellosis is already endemic (chronic disease situation), the birth of weak and also healthy, but infected calves, may occur.

Acute outbreaks of brucellosis, during which 30 to 40% of pregnant animals may abort, occur in a susceptible herd after exposure to the infection. Abortions usually occur from five months pregnancy to full term. Once brucellosis is established in a herd and has become chronic, the incidence of abortion usually decreases until it is only seen in replacement stock. **If heifers are correctly vaccinated with a *Brucella* vaccine the incidence of**

**abortions also decreases if the infection pressure is controlled.** In herds where brucellosis is already endemic, abortions may not occur as frequently. Instead, alternative symptoms such as retained placentas may occur in cows calving at full term. Usually an infected cow will only abort once, but there are exceptions to the rule. She may, however, continue to spread *Brucellae* in her lochia for the rest of her life after normal calving. Despite the potential absence of abortion storms in infected herds, large numbers of organisms may still be excreted for a month after calving in infected cows' genital excretions and in milk. This ensures maintenance of the infection within a herd.

The testes, seminal vesicles and epididymis of infected bulls are normally affected. Large numbers of organisms are excreted in the semen of acutely infected bulls. This does however not normally play an important role in the spread of the disease within a herd during natural mating. The disease can spread if infected bulls are used for semen collection and artificial insemination. Infected bulls should be tested and one needs to keep in mind that they will become infertile over time.

Hygromas (fluid accumulation in the knee joint) may occur in some cases. This plays no role in the spread of disease in animals, though abattoir workers and individuals who conduct informal slaughter may become infected.



### 3 Brucellosis in humans

#### 3.1 Transmission of the disease

Man may become infected through mucous membranes (eye and mouth tissue) or by handling infected tissue when assisting during calving. Unpasteurised milk and milk products, such as soft cheeses are further sources of infection through ingestion. Contaminated hands may spread the infection to the eyes, eating utensils, etc. Uncontrolled slaughtering where no preventative measures are taken, also add to the risk.

*Synonyms:*

Undulating Fever (*B. abortus*)

Malta Fever (*B. melitensis*)

Mediterranean fever

Bang's disease

*People at risk:*

- a). Veterinarians, animal health technicians, cattle inseminators as well as dairy and abattoir workers
- b). Farmers or staff vaccinating cattle with S19, RB51 or ram lambs with Rev1 vaccine (self-inoculation or contamination of the mucous membranes of the mouth or eyes).

- c) Tourists returning from foreign countries where *B. melitensis* infection is prevalent.
- d) People eating soft cheeses or other milk products as well as urban visitors to the farm coming into contact with infected cows and their products. Souring of milk does not destroy *B. melitensis* organisms. Experimental studies showed that *B. abortus* is able to survive in fermented milk after 10 days of storage with a pH below 4.

### 3.2 Clinical signs

Early signs are chills, tiredness, headaches and night sweats. These may, however, not always be present. A mild syndrome may occur with a fever of 38-39°C in the evenings (undulant fever). Intense pain in the back, arms, legs accompanied by extreme fatigue later in the day may occur. The signs persist in untreated cases. Severe cases may be confused with malaria, glandular fever, influenza and tick-bite fever. The patient has no appetite, becomes exhausted and loses weight. If there has been contact with infected material or animals, the doctor should be informed. Remember that infection with RB51 will not be detected by normal serological tests (only by the Western Blot technique) and this should be brought under the attention of the physician.

#### *Treatment:*

**Treatment must be given under strict specialised medical supervision.** It consists of high dosages of intracellular active antibiotics for extended periods. **Milk products may not be used during the treatment period** (calcium binds and precipitates tetracyclines).

- Treatment of brucellosis is often complicated by treatment failures and relapses.
- The WHO has not updated its recommended treatment regimens for brucellosis since 1986; these regimens have been found to have treatment failure and relapse rates ranging from 4.6 to 25%.
- A meta-analysis has shown that dual or triple regimens, including an aminoglycoside, significantly reduces treatment failure and relapse rates, and are currently recommended as first-line treatment regimens.
- Duration of treatment is 6 weeks for doxycycline and rifampicin, and 2 weeks for aminoglycoside therapy (daily intramuscular injections).
- Patients require prolonged follow-up to monitor for further complications or relapse.
- Relapse rates are lower when aminoglycosides are used in combination with other antibiotics.
- The best choice is probably doxycycline plus an aminoglycoside (streptomycin/gentamycin)
- Doxycycline plus rifampicin is less effective, especially for spondylitis
- Gentamycin can be used instead of streptomycin
- Doxycycline is preferred to tetracycline
- In pregnancy, clotrimazole plus rifampicin may be used
- **Note that RB51(vaccination) infection is not responsive to rifampicin.**

### 3.3 Prevention

- a) In cases where eradication has not been achieved milk must be boiled or pasteurised before use.
- b) All products of abortion and afterbirths must be regarded as highly infectious (including cows calving normally in an infected herd - also the handling of such newly born calves)
- c) Aborting female animals must be isolated from the rest of the herd until they are proved negative by means of serological tests (only test serologically at least three weeks after abortion or calving. Immediate post calving tests will entail sampling for culture purposes).
- d) People at risk should wear full protective clothing (overall or coat, rubber or plastic apron, masks, rubber gloves, boots, eye protection). Aborted foetuses, foetal membranes and carcasses that are not needed for culture, as well as contaminated litter should be collected in leak-proof containers and disposed of by incineration or deep burial in freshly slaked lime at sites away from water sources. All farm implements used for

handling of contaminated material should be disinfected after use with a suitable disinfectant (iodophor, phenolic or hypochlorite). Any area in which abortion has occurred, should be washed down with an approved disinfectant at the appropriate concentration (iodophor, phenolic, or hypochlorite). The protective clothing to be reserved for this purpose and retained on the premises should be disinfected after use and prior to washing by soaking in a disinfectant solution at the appropriate concentration (iodophor, phenolic soap, chloramine, hypochlorite). Cover the ground on which the infectious material has rested with a thick layer of straw and burn it.

e) Abattoir workers handling infected carcasses should wear shoulder-length plastic disposable gloves. Waterproof aprons and boots, as well as approved surgical masks and goggles. Highly infectious material, including lymph nodes, should not be cut, but handled cautiously to prevent the formation of aerosols.

f) Laboratory workers should wear gloves, long-sleeved autoclavable gowns, surgical masks and goggles. They should work in a biohazard cabinet for protection. Cattle inseminators should also wear protective clothing.

g) Extreme care must be exercised when using S19, RB51 or Rev 1 vaccines.

## 4 Brucellosis in wildlife

*Brucella spp* have been documented worldwide in a great variety of terrestrial wildlife species and marine mammals. In South Africa, apart from African Buffalo, several other species of wildlife namely, hippopotamus, zebra, sable, eland, waterbuck and impala, have tested positive for brucellosis. In the past these species probably were of minor importance in the epidemiology of bovine brucellosis in southern Africa because of the infrequent contact between cattle and wildlife. However, contact has increased dramatically in the past few years, with the possibility that transmission can occur more frequently. Wildlife are also being kept more intensively and translocated more frequently, which may also promote brucellosis occurrence in wildlife.

It is always important to ensure that the organism is cultured and typed when a serological diagnosis is made, especially in species other than cattle. Due to the high costs associated with culturing and typing it is advised to send good quality, appropriate samples to the laboratory. Note that culture is difficult and may often be unsuccessful.

## 5 Diagnosis

Brucellosis has no particular clinical features which allow for an accurate diagnosis. It is for this reason that heavy reliance is placed on laboratory diagnosis. Brucellosis can be diagnosed by both direct and indirect methods. Direct methods include techniques whereby the causative organism can be identified by microscopic examination or by culture. Field strains of *B. abortus* can only be differentiated from vaccine strains using culture and biotyping techniques. Serological techniques for the diagnosis of brucellosis are all indirect methods of diagnosis. True infection reactions cannot be distinguished from serological reactions caused by the *Brucella* vaccine strain, strain S19. (See par. 6.1. below).

### 5.1 Laboratory tests for brucellosis

The laboratory tests used in South Africa may be broadly grouped as follows:

- Tests to demonstrate the pathogen itself (*Brucella abortus*) - direct methods
- Tests to demonstrate the presence of specific antibodies in the blood, milk or semen - indirect methods

## DIRECT DIAGNOSIS

Smears	Culture
Foetal organs	Foetus
Cotyledons	Placenta
Uterine	Uterine
Discharges	Discharge
	Colostrum
	Milk
	Semen
	Lymphnodes
	Udder tissue
	Spleen

## INDIRECT DIAGNOSIS

Antibody detection
RBT – serum
SAT – serum
CFT – serum
ELISA – serum
Fluorescence polarization assay (FPA) - Serum
MRT – Milk
Brucellin skin test – unvaccinated cattle
Calves (latent – cellular)

The brucellin skin test has not been approved by the Director Animal Health as a diagnostic test for brucellosis within South Africa as vaccination is required by law and interferes with test results.

### 5.1.1 Laboratory specimens

Great care must be taken when collecting specimens which may contain viable *B. abortus* organisms!

*Brucella abortus* causes brucellosis (undulant fever) in humans, which can have serious consequences, and it is therefore essential to take all the necessary precautions to protect the sampler and any personnel who may handle the specimens at a later stage. Gloves, a facial mask and eye protection must be worn when sampling.

Specimens must be packaged according to the UN Triple Layer Guidelines as follows:

- A primary container
- If tubes - must be separated
- These containers must be placed in a leak-proof bag
- There must be sufficient absorbent material in the bag so that any leakage is contained within the bag
- The patient documents must be attached to the outside of the bag and inside the rigid outer container
- The bag must be placed in a rigid outer container which complies with all the labelling required as follows:
  - Consignee and consignor details
  - The contact details of the person who packaged the sample
  - An infectious label must be placed on the box indicating "Infectious sample affecting humans and animals UN2814"
  - Graphics of how the samples are packed must be printed on the box

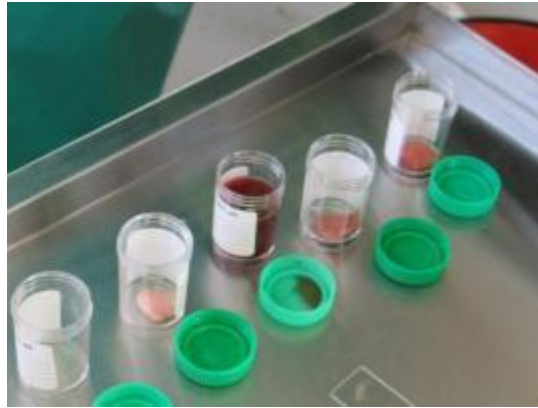
Samples must be transported according to the Regulations of the National Road Traffic Act, 1996 (Act No. 93 of 1996).

#### a.) Foetal specimens

Specimens of choice for culture are aborted fetuses (foetal stomach fluid, lung, spleen, liver) and foetal membranes. Smears should also be made of the foetal stomach fluid, lungs, of the aborting cow's lochia as well as of the cotyledons of the placenta. These samples must be submitted to the laboratory on ice.

#### b.) Bacteriological specimens

These specimens must be submitted to the laboratory on ice (cool pack). They should reach their destination within 48 hours and each organ must be packed separately in sterile specimen jars.



c.) Blood samples

"Vacutest" (7ml or 10ml) tubes, containing no preservatives or anticoagulants, must be used and the tubes should be at least half filled with the blood sample. The quality of the serum sample has an impact on the accuracy of test results. Serum must be clear, not haemolysed. The blood clot should be removed if possible before sending the samples to the laboratory. Each tube must be clearly labelled with a sequential number before going to bleed the animals. A CA 5 form (obtainable from the local State Veterinarian) must be completed in full. All particulars pertaining to vaccination must be given on the CA 5 form. The tubes must be packed in sequence from left to right, filling the polystyrene box from the back to the front. The name of the owner should be indicated on the outside of the box containing the tubes (not on the lid!). Never place blood samples from more than one farm in the same box. For the purpose of communal areas a diptank is considered as one farm – different owners or groups on same can be differentiated on CA5 form accompanying the blood samples.

d.) Milk samples

Milk samples collected in tubes (obtainable from the state veterinary laboratories) and containing either formalin (**can decolourise the antigen and is thus not preferred**) or bronopol preservative must reach the laboratory within 2days. These specimens must be kept at 4°C as elevated temperatures can affect test results markedly. Milk samples must be collected from the can or bulk milk tank after its contents have been well agitated, using a clean scoop or syringe. Under no circumstances must a tube containing preservative be used to scoop out a sample as there is a grave risk that the preservative will run out of the tube and contaminate the bulk milk.

Detailed information is available in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, available electronically at <http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/>

## 5.2 Tests to demonstrate *Brucella abortus* (Direct)

### 5.2.1 Microscopic

Smears may be made of the foetal stomach fluid or of the aborting cow's lochia by placing a small amount of the fluid on a clean, dry microscope glass slide. Another clean glass slide is then used to spread the material evenly over this slide. Allow to air dry and fix by moving the smear through a flame (heat fixation). Do not cover with a cover slip.

Impression smears may be taken from freshly cut and blotted tissue surfaces, e.g. cotyledons, by firmly pressing the slide surface against the tissue. Allow to air dry and heat fix. Smears may be made of foetal stomach fluid,

foetal lungs, cotyledons or cow lochia and stained with the Stamps modification of the Ziehl-Nielsen stain.

*Brucellae* stains red against a blue background (Stamp stain) whereas most other bacteria stain blue. Confusion with other pathogens such as *Coxiella burnetii* and *Chlamydophila psittaci* (*Chlamydia psittaci*) is possible, and culture of the pathogen should be carried out for confirmation. The stain cannot be used to distinguish field strains of *B. abortus* from the vaccine strains (S19 or RB51). Differentiation of *B. abortus* from *Nocardia* is relatively easy since there are marked morphological differences between species of these genera. *Brucella abortus* stains Gram-negative, but with this stain the pathogen is not easily discernible where cellular debris and other organic material are present.

### 5.2.2 Bacteriological culture

All strains are relatively slow growing, and because the specimens from which isolations are best attempted are frequently heavily contaminated, the use of a selective medium, e.g. Farrell's medium is advocated. Incubation normally continues for 72 hours, but a negative diagnosis can only be made after a week long incubation. Specimens which may be used for *B. abortus* isolation include: foetal stomach fluid, spleen, liver, placenta, lochia, milk (especially colostrum or milk within a week of calving), semen, udder tissue and lymph nodes. Supra-mammary lymphnodes are preferred for chronic and latent infections, whilst retropharyngeal lymphnodes are preferred for early infections. Iliac, prescapular and parotid lymphnodes may also be used and it is always best to sample all relevant sites. If serological reactions are thought to be caused by S19 vaccine strain then it is important to collect pre-scapular lymph nodes as well. All *B. abortus* isolates should be forwarded to laboratories capable of biotyping.

## 5.3 Tests to demonstrate *B. abortus* antibodies (Indirect)

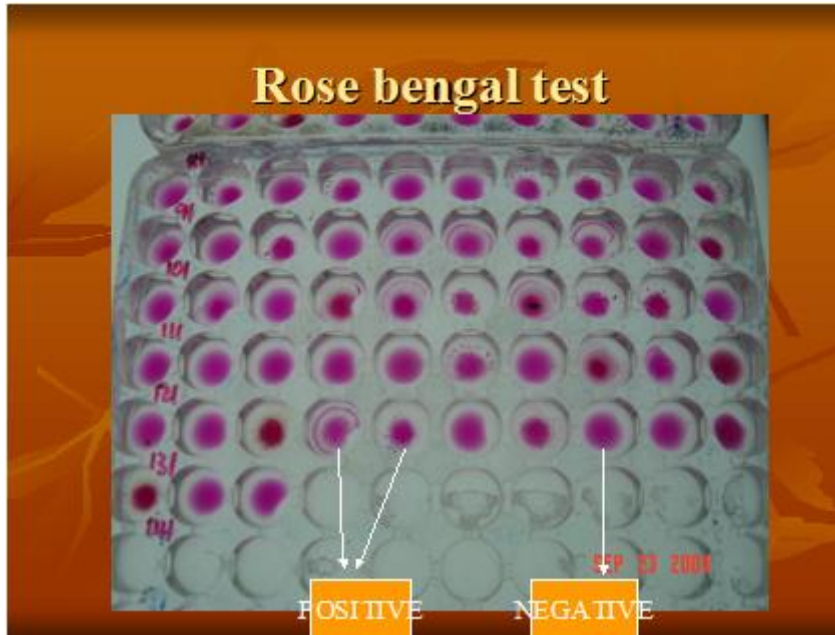
### 5.3.1 Bovine brucellosis antibodies and serological tests

In animals vaccinated with S19, IgM, IgG1 and IgG2 (humeral antibodies) are produced. After six months, IgG2 has usually disappeared, but very low levels of IgM and IgG1 may be present, often in concentrations which are too low to be detected by the CFT. In infected animals, higher levels of IgG1 are usually present and these are detected by the CFT. A brief description of the tests used in the various veterinary laboratories of Veterinary Services is given below. The actual techniques are described in detail in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. All serological tests must be carried out strictly in accordance with standard operating procedures (SOPs) and standardised control sera must be used as stipulated in the methods.

### 5.3.2 Rose Bengal test (RBT)

This very sensitive test is used to screen serum samples. It does not differentiate between field and S19 vaccine strain reactions, but is quick, inexpensive and easy to perform. False negative reactions are rare but may sometimes be due to excessive heating in storage or in transit. Positive reactions should be investigated using suitable confirmatory and/or complementary strategies (including the performance of other tests and an epidemiological investigation).





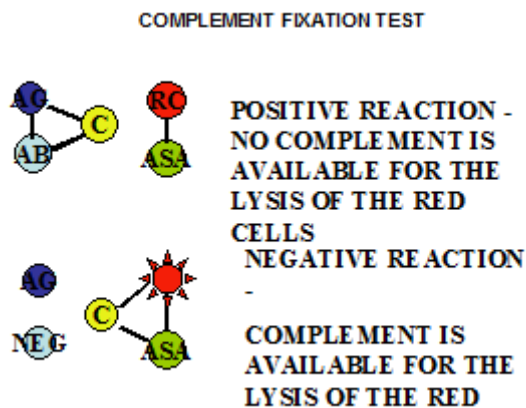
### 5.3.3 Complement fixation test (CFT)

Refer to the latest edition of the SAVLSF Harmonized Serology SOP for *Brucella abortus* Complement Fixation Test (CFT).

The CFT is both sensitive and specific, in the hands of experienced users, and is used as a definitive (confirmatory) blood serum test. In most cases, the CFT is used on RBT positive sera, but like the RBT, it is also affected to a large extent by the misuse of strain 19 vaccine, particularly when recent or repetitive vaccinations have been used in sexually mature heifers and cows. It is almost impossible to prescribe strict cut-off readings that indicate infection particularly when S19 vaccination reactions play a role due to its misuse.

The CFT is a relatively complex test to perform. The reagents include *B. abortus* CFT antigen, complement,amboceptor (haemolysin), ovine erythrocytes and test serum with CFT buffer as the diluent.

The tests are semi-automated and microtitre volumes (0,025 ml) are used. Repeatability with regard to results must be within two dilutions within a laboratory and within 4 dilutions between laboratories. This means, for instance, that within one laboratory, a serum known to have an end-point of 120 IU/ml could test anywhere between 86 and 172 IU/ml without any conclusions being drawn as to a real decrease or increase in antibody level. Inactivated serum (heating at 58 °C for 50 minutes) used for the CFT subsequently cannot be used for the RBT or SAT.





The CFT (for brucellosis) detects the presence of antibodies to *Brucella abortus* and possibly related organisms.

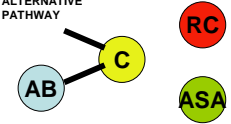
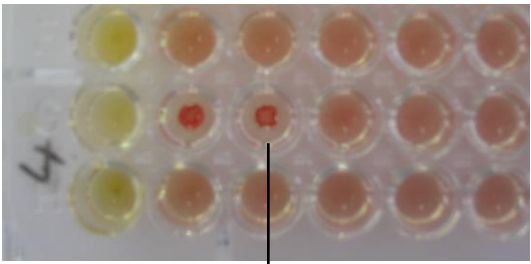

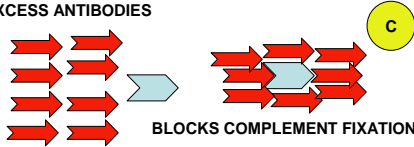
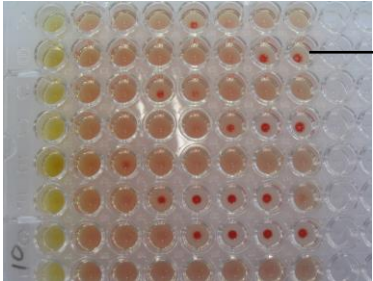
Factors which may cause 'positive' serological tests include:

- True infection - either acute or chronic
- Past exposure to true infection but no persistent infection
- Infection with or exposure to *B. abortus* S.19 organisms
- Exposure to antigenically related organisms, e.g., *Salmonella*, *Yersinia*, *Pasteurella*
- Laboratory test artifacts that affect the sensitivity and specificity of the test.

Factors which may cause no reaction to serological tests include:

- Absence of true infection
- Recent infection
- Delayed sero-conversion - especially in the case of heifers infected as calves (latent) infections.
- Non sero-conversion due to localisation of infection or immunological incompetence
- Laboratory artifacts which affect the sensitivity of the test.
- Testing within 14 days post calving

Also field and laboratory identification errors may result in 'reactors' losing their reaction suddenly or non-reactors suddenly reacting (human error).

<p style="text-align: center;"><b>CFT REACTIONS</b></p> <p><b>1. ANTICOMPLEMENTARY REACTIONS (AK)</b></p> <p>ALTERNATIVE PATHWAY</p>  <p><b>POSITIVE REACTION - NO COMPLEMENT IS AVAILABLE FOR THE LYSIS OF THE RED CELLS</b></p>	<p style="text-align: center;"><b>ANTI COMPLEMENTARY REACTION</b></p>  <p style="text-align: center;">↓ Positive</p>
<p style="text-align: center;"><b>CFT REACTIONS</b></p> <p><b>2. PROZONE REACTION</b></p> <p>ANTIBODY-ANTIGEN REACTION BINDING COMPLEMENT</p>  <p>EXCESS ANTIBODIES</p>  <p><b>BLOCKS COMPLEMENT FIXATION</b></p>	<p style="text-align: center;"><b>PROZONE REACTION</b></p>  <p style="text-align: center;">→ Prozone reaction</p>

### 1. *Anti-complementary reactions*

Anti-complementary reactions occur when the complement cascade is initiated in the absence of *B. abortus* antigen-antibody complexes. An anti-complementary control can be set up where the antigen is excluded.

Causes of anti-complementary reactions:

- Immune complexes present in the serum of infected animals.
- Activation of the complement cascade by bacterial contaminants through the alternative pathway.

The sample can be accepted as negative if it reads positive at lower dilutions, but negative at higher dilutions and the anti-complementary control is positive.

See the SAVLSF Harmonized SOP, section 10.3 for further testing of anti-complementary sera.

2. *Prozone* = A prozone reaction occurs in the CFT when lower dilution of the test serum test negative but higher dilution test positive. It usually occurs in serum from highly positive animals with an abundance of IgG2 antibodies. These are non-complement fixing antibodies and at lower dilutions block IgG1 antibodies from binding to the antigen. At higher dilutions the concentration of IgG2 antibodies is lower allowing IgG1 antibodies to bind. When it is seen the result is still regarded as positive. It may however give rise to false negative reactions.

### 5.3.4 Enzyme-linked immunoabsorbent assay (ELISA)

Indirect ELISA - Depending on which kit is used the i-ELISA is generally more sensitive for detecting antibodies to *Brucella* spp than are the RBT, and CFT, but great care must be taken in animals vaccinated with S19 vaccine. The i-ELISA also gives good results when used to test milk.

Competitive ELISA – depending on which kit is used, it may have the potential to eliminate some, but not all, FPSR (False Positive Serological Reactions) due to cross-reacting bacteria. It may also be able to eliminate most S19 vaccine reactions. Sensitivity should be more or less similar to RBT and i-ELISA

### 5.3.5 Fluorescence polarization assay (FPA)

The FPA is a simple and rapid technique for measuring antigen/antibody interaction and may be performed in a laboratory setting or in the field. The mechanism of the assay is based on random rotation of molecules in solution. A fluorescently-labelled antigen of small molecular weight is added to serum or other fluid to be tested. If antibody is present, attachment to the labelled antigen will cause its rotational rate to decrease and this decrease can be measured.

### 5.3.6 Milk ring test (MRT)

#### a.) Principle

The MRT is a very sensitive test which may be used to monitor the negative brucellosis status of dairy herds. The test volume of milk must be adjusted to compensate for the dilution factor from bulk milk samples from large herds. The samples are adjusted according to the following formula: herd size < 150 animals use 1ml bulk tank sample; 150-450 use 2ml bulk tank sample; 451-700 use 3ml bulk tank sample. Bulk milk samples, collected at regular intervals (e.g. every month), provide early warning of the disease, although false positive test results may also be obtained when many cows are in early or late lactation or have mastitis. Late (adult) vaccination may also affect the test results for some months. The MRT is used for screening and cannot be used as the sole criterion for the diagnosis of brucellosis. It is not a test used to identify individual animals. The test is carried out in test tubes in the laboratory.

A stained antigen (0,03 ml) is added to a defined volume of milk (1 - 3 ml depending on the herd size), the tube is shaken and then incubated for an hour at 37 °C. A further incubation at 4 °C overnight (18 – 20 hrs) increases the sensitivity of the test. Samples should not be more than 72 hours old.

Milk samples must be delivered on ice to the laboratory within two days of collection, even if they have been preserved with formalin (**can decolorize the antigen thus not recommended**) or bronopol. Adjustments for the number of cows represented by the bulk tank samples are made by increasing the volume of milk and using the same amount of antigen. It is thus important to ascertain the number of cows contributing to the bulk tank samples before commencing the test. Monthly bulk MR testing in closed herds may obviate the need to bleed herds for the renewal of brucellosis-free declarations if all the tests are negative, but this will mean there will be an absence of individual records for cows.

The MRT is a cheap and efficient means of monitoring the brucellosis status of dairy herds. The MRT detects the presence of *Brucella* antibodies in milk samples and, where bulk milk samples are tested, the presence of brucellosis in the herd. The expected sensitivity is 95% probability of detecting one infected cow in a 100 cow herd providing that:

- a. there are no sampling or recording errors
- b. the infected cow is contributing to the bulk milk supply at the time of sampling
- c. the infected cow is excreting antibodies.

Probability of detecting milk from one reactor cow in different-sized herds using a one ml test volume in the milk ring test (Roepke M.H. and Stiles F.C. 1970. Potential efficiency of milk ring test for detection of brucellosis. Am. J. Vet. Res., 31(12):2145 – 2149).		
No. of cows in production	Estimated probability	Lower 95% confidence limit
<b>1</b>	0.993	0.975
<b>2</b>	0.984	0.965
<b>4</b>	0.967	0.950
<b>8</b>	0.931	0.907
<b>12</b>	0.871	0.825
<b>25</b>	0.818	0.770
<b>50</b>	0.696	0.653
<b>100</b>	0.521	0.474
<b>200</b>	0.389	0.343
<b>400</b>	0.248	0.207

**Note:** The MRT must be adjusted to compensate for the dilution factor from bulk tank milk samples from large herds. The samples are adjusted according to the following formula: herd size < 150 animals use 1 ml bulk tank milk, 150 – 450 use 2 ml milk sample, 451 – 700 use 3 ml milk

\*Results depend on how many cows contribute to the bulk tank & the size of the bulk tank. Adjust milk sample volume according to the number of contributing cows.

Factors which result in positive reactions include:

- a. True infection with *B. abortus*
- b. *B. abortus* strain 19 infection
- c. Colostrum in milk - early in lactation
- d. Cells in high concentration in the milk - late lactation
- e. Mastitis
- f. Freezing or other mishandling of milk
- g. Variation in antigen between batches
- h. Variation in interpretation of results
- i. Fresh milk – must be kept for more than 24 hours at 4°C before testing

Factors which cause false negative reactions include:

- a. Infected cow not contributing to bulk sample e.g. dry or rearing calves
- b. Infected cow not secreting antibody in milk e.g. early infection
- c. Dilution of infected cow's milk by rest of herd
- d. Variation in antigen between batches
- e. Variation in interpretation of results
- f. Milk samples that were not kept cool
- g. Milk that was shaken excessively

Sampling errors and misidentification of the herd of origin of the milk sample may cause apparent false positive or false negative reactions.

b.) Procedures

i) Planning

- Identify all distributors that collect milk within the area.
- Identify contact at distributor and gain assistance to meet requirements.
- Obtain an updated accurate list of suppliers to each distributor at or before each sampling.

Plan a sampling schedule that is:

- Practical and convenient to distributor, field and laboratory staff.
- Ensure timing will enable a minimum of six MRT's for each herd spread approximately equally throughout the year (preferably each month).

ii) Sample collection

- Collect bulk milk samples by arrangement.
- Avoid cross contamination by factory personnel, field and/or laboratory staff. (The potential for cross contamination of samples increases when composite samples are used).
- Check samples against supplier list.
  - New samples
    - new supplier
    - old supplier recommencing supply
    - other
  - Missing samples - herd disbanded
    - herd dried off
    - sample missed
    - samples unsatisfactory
    - other

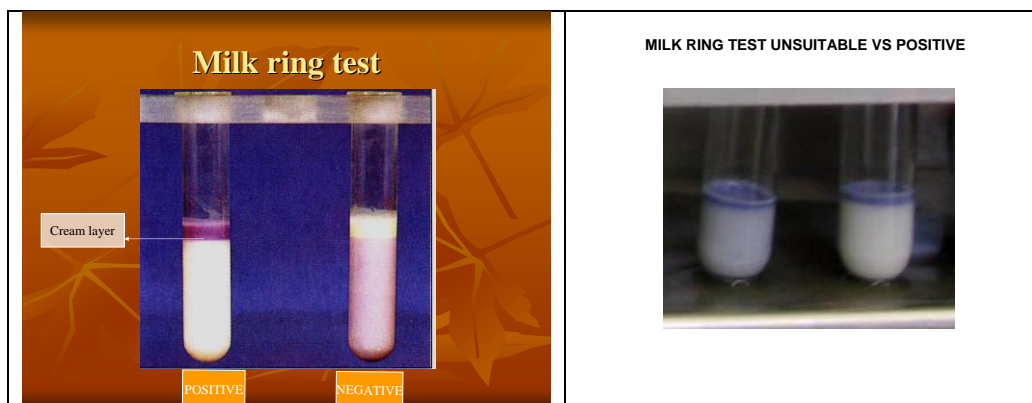
Note: Update supplier list with all corrections.

- Place samples in Rack (labelled 1 - 100). Have to be in numerical order.
- Prepare MRT identification/results sheet correlating supplier to sample.
- Forward samples and I.D. sheet to laboratory chilled but not frozen, preferably with preservative. The best preservative is Bronopol. Formalin should not be used as it decolourises the MRT – antigen.
- Return for missed samples.
- Return distributor/supplier sheet to State Veterinarian & copy to Deputy Director for updating of computer and manual records.

iii) Testing

- Receive samples and store chilled for 24 hours post-collection prior to testing.

- Test as per manual instructions.
- Record results on MRT Identification/Result sheet.
- Run concurrent controls.
- Forward MRT Identification/Result sheet to State Veterinarian & copy to Deputy Director.



#### Computer/ data Processing.

- Ensure that MRT positive herds are notified and followed up and bled by State Veterinarian staff a.s.a.p.
- State Veterinarian advises positive MRT results from other districts to appropriate State Veterinarian..
- Printout of lists of all results to be forwarded to Deputy Director.

## 6 Interpretation of serological results:

### 6.1 Calf-hood S19 vaccination

A definite diagnosis of brucellosis can be made only by isolation and identification of *B. abortus*. This approach is not practical on a large scale and heavy reliance is placed on the interpretation of serological test results. Normally the herd will be identified as a possible positive herd by means of a blood test. Samples will be taken for bacterial culture (proof of being positive for *B. abortus* - Golden Standard). Then the diagnosis will be made on serology – for individual animals. It is very difficult to be prescriptive with regard to strict cut-off points and a dogmatic approach can lead to incorrect diagnoses.

Recommended CFT positive levels have been set at 30 and 60 ICFTU/ml for correctly vaccinated and adult vaccinated animals respectively. These CFT levels should be accepted as indications only. It is almost impossible to create arbitrary positive levels if a live S19 vaccine is used irresponsibly. Such serological reactions may sometimes persist for many years, especially if the vaccine is used incorrectly.

*M. Laja*  
✓

**"INTERPRETATION" OF CFT TITRES**

Vaccination history	CFT IU/ml	Interpretation
Unvaccinated or calfhood vaccination or unknown	< 15	Negative
	18 - 24	Suspicious
	> 30	Positive
Adult vaccinated	≤ 24	Negative
	30 - 49	Suspicious
	≥ 60	Positive

**THIS TABLE MUST NOT BE APPLIED BEFORE THE INFECTION STATUS OF THE HERD HAS BEEN ESTABLISHED. There are few, if any, herds using strain 19 vaccine that not have a few uninfected reactors testing at 30 IU's or higher.**

The need to understand this difficult disease with all its oddities and exceptions to the rules is crucial in making the correct decisions. If it was as simple as deciding on a set of antibody levels which could be used to make the diagnosis with absolute certainty for all reactions, the disease would not be causing the problems it still does. A large proportion of South Africa's herds, many of whose owners closely adhere to the recommendations for the use of strain 19 vaccine, have the occasional animal which may test at 30 CFT IU/ml or even considerably higher. It may cause irreparable damage to pronounce such animals positive. The rigorous, unbending attitude of placing complete faith in cut-off points can do, and has done, almost as much damage to the objective of brucellosis eradication as have inaccurate laboratory tests, errors or too lax an approach in dealing with genuinely infected herds. The laboratory test can only be as good as the interpretation given to its result.

Considerable insight into the disease and its complexities is needed to make serological interpretations. A very strict and very controlled approach must be used with regard to S19 vaccination as it becomes an almost impossible task to differentiate S19 vaccine (when used haphazardly) reactions from field strain infections in an infected herd. It is not possible to be prescriptive with regard to "cut-off" points in all situations. When a herd has been proved to be infected, strict interpretations are made. It is thus difficult to be strict in approach if the actual disease status is in question. Therefore the first step is to establish the infection status of the herd.

When assessing the brucellosis status of each reactor and herd the following factors should be considered:

- **Reactor** Identification, serological titre, origin (bred or introduced and from where), vaccination status (S19 or RB51), when vaccinated, vaccination syringes re-used or not, current pregnancy status, date of calving/abortion, possible exposure to infection, age of animal, previous titre (previous test result).
- **Herd** Previous brucellosis history, fate of previous reactors, system and quality of management e.g. closed herds, speculation herds, restricted calving pattern or all year round calving. Level of monitoring, e.g. MRT, abortion investigation, herd tests.
- **Neighbours and other contacts** (both trace forward and trace back)  
Brucellosis history and current status of herd, degree of contact with other cattle and livestock (both direct and indirect).
- Previous serological test results or culture results

**Note:** The reliability of answers given by the owner/manager may be assessed and the information cross-checked as necessary. Discrepancies must be clarified and details verified.



A positive test result does not necessarily mean that the animal is infected, but because these two concepts are often held as synonymous, interpretation must be based on establishing whether the herd is infected or not. (It is equally true that a negative test result for an animal in an infected herd does not then automatically mean that the animal is not infected).

Bacteriological isolation of *B. abortus* from a foetus or from the milk, lochia, udder tissue, lymph nodes and joints of a serological reactor is indisputable evidence of infection (Golden Standard). This approach cannot be used in all cases, but it makes the initial decision much easier and it should be attempted if possible. Generally it is necessary, and indeed sufficiently reliable, to make a positive (=infected) diagnosis using serological results. It is sometimes difficult to make this decision on the basis of a single herd bleed. Any uncertainty should be clarified by repeated herd bleeds. By comparison of the results of the sequential herd bleeds (optimally at two month intervals) and by applying the following criteria, it is nearly always possible to arrive at a decision:

- An increase in the number of animals with relatively high reactions (eg. 30 CF IU/ml and higher) indicates spread and therefore infection (in the absence of proof of any vaccine involvement).
- A tendency for the titre of the known reactors to rise indicates probable infection. A decrease in the titres is strong evidence of non-specific reactions unless this phenomenon can be related to vaccine administration.
- An infected herd will nearly always have a few animals with extremely high CFT titres (392 IU/ml and higher). These animals will fall into all age groups and not just the younger groups.

Sometimes, however, it is still not possible to be decisive even after two herd bleeds. The repeat bleed may show the same number of animals reacting (some with high titres) without any evidence of spread. It is not correct to decide at this stage that the herd is clean. The reacting animals must be kept separate (especially if heavily pregnant) and remainder monitored. The suspect animals with high titres must be sampled for bacteriological culture (milk, foetuses, etc.). It may be expedient to sacrifice one or two suspect animals with high titres and collect lymph nodes and other appropriate samples for bacteriological culture.

Once the herd has been diagnosed positive (=infected), there is no going back. Frequent bleeds at two month intervals must be carried out and all reactions interpreted very strictly. **The temptation to distinguish infected animals from vaccine reactors must now be resisted.** All new reactors with CFT titres as low as 18 to 24 IU/ml must be regarded with deep suspicion. An "overkill" factor of 5-10% amongst the reactors in an infected herd must be accepted as unavoidable.

The principle of examining test results based on the herd's brucellosis status is a very reliable approach. Test results must be compared to calving /abortion history, vaccinations, ages of animals, etc. A summary of the criteria used in the interpretation of serological reactions is shown in table 1 below.

The guidelines in table 1 must be read in connection with the rest of the content of chapter 6.

TABLE 1 SUMMARY OF SOME DIAGNOSTIC CRITERIA AND SEROLOGICAL INTERPRETATION	
HERD (CLOSED) WITH SEROLOGICAL REACTORS	
<b>INFECTED</b>	<b>NOT INFECTED</b>
1. SPREAD (NEW REACTORS) ON REPEAT BLEEDING 2. LOW TITRES INCREASING ON REPEAT BLEEDING 3. CORRELATION BETWEEN ABORTERS AND REACTORS 4. REACTORS IN ALL AGE GROUPS 5. ISOLATION OF <i>B.abortus</i> biovar 1 & 2	1. ONLY STRAIN 19 ISOLATED 2. NO SPREAD ON REPEAT BLEEDING 3. TITRES DECREASE & NO NEW CASES 4. REACTORS CORRESPOND WITH VACCINATION 5. NO FIELD STRAIN ISOLATION
<b>APPROACH</b>	
1. STRICT INTERPRETATION 2. $\geq 30$ IU REGARDED POSITIVE 3. NEW REACTORS REGARDED POSITIVE	1. ALL TITRES REGARDED SUSPECT 2. CULTURE FROM HIGH REACTORS
<b>NB! This table must be read in conjunction the rest of the content of chapter 6.</b>	

### 6.1.1 Guides to decision-making

In general, reactors(s) which:

- have a CFT titre 49 IU/ml or lower;
- were bred on the property;
- were S19 vaccinated;
- and after full investigation can be shown to have had no exposure (direct or indirect) with herds or animals of suspect brucellosis status, may be declared 'aberrant' without further testing.

Other reactors will require further investigation and retesting.

In the case of suspect or positive herds the State Veterinarian must complete a SR1.

### 6.1.2 Retesting reactors

In general the following Reactors will be re-sampled. (*Please note "Vaccinates" refer to calfhood vaccination with S19.*)

CFT of 60 IU/ml or higher – S19 vaccinates

CFT of 24 IU/ml or higher - non S19 vaccinates

CFT of 24 IU/ml or higher – S19 vaccinates if CFT of 60 IU/ml or higher are present also

\*\*It is always better to retest the whole herd rather than just suspicious reactors, so that one can ascertain whether there is spread or not.

a.) Live animals

- Blood for RBT and CFT
- Milk (in case of lactating females) for culture (preferably colostrum, or as close after calving as possible) (**CFT titre 240 and higher – difficult to isolate below this figure**) and typing of *B. abortus*
- Bulk milk (in case of dairy herds) for MRT (MRT on individual animals are fairly meaningless)
- Vaginal mucus or discharge (if any) for culture and typing preferably within 7 days but up to 1 month post calving
- A negative results does not necessarily mean it is a negative animal.

b.) Dead animals. (refer to 6.1.3. Procedures for slaughtering reactors)

- Blood and milk as for live animals
- Tissues from both supra-mammary lymph nodes
- Tissues from both retropharyngeal lymph nodes
- Tissues from both pre-scapular lymph nodes (especially if suspect S19 could be causing reactions)
- Tissues from foetus and placenta if present (minimum = lung, abomasal content and spleen)

**Other samples: tissues from both iliac lymph nodes, tissues from both parotid lymph nodes, uterus samples, udder tissue samples and hygroma fluid if present.**

In special circumstances other specimens may be collected or the whole beast may be submitted to the Veterinary Laboratory. In these circumstances prior consultation with the laboratory should occur, specifically indicating that brucellosis is suspected. The State Veterinarian will liaise with the head of the laboratory.

Strict isolation of pregnant (>5 months), recently aborted or calved reactors should occur promptly as they are the greatest threat to the Programme by potential contamination of the environment with infective discharges.

Investigation of all reactors and reports (SR1) must be forwarded by the State Veterinarian to the Deputy Director within three weeks unless the Deputy Director approves extending the investigation in special circumstances.

### **6.1.3 Procedures for slaughtering reactors:**

Reactors that have recently aborted or calved (within 6 weeks) pose a significantly greater public health risk than do other reactors. Special consideration should be given to the disposal of these reactors to overcome this risk. They will of course be C-branded on the right side of the neck as will all other confirmed reactors but they should only be slaughtered with special precautions at the end of a day's slaughtering at a designated abattoir which must be disinfected immediately after the slaughter (**normal cleaning at end of day**) of any batch of C- branded animals. **These animals should be accompanied by a red cross permit to warn abattoir workers that the animals are positive for brucellosis.** Appropriate samples should **be collected from selected animals (e.g. on high titres etc.) to confirm that the animals are positive bacteriologically.**

Abattoir workers handling infected carcasses should wear shoulder length disposable plastic gloves. Waterproof aprons and boots as well as approved surgical masks and goggles are needed. Highly infectious material such as lymph nodes should not be roughly cut but handled cautiously to prevent the formation of aerosols.

All heifer calves of reactors must be treated as suspect animals and permanently identified and **blood tested regularly before and after their first calving.** NB - ideally keep these animals separate if they are not slaughtered.

## 7 Control of bovine brucellosis

### 7.1 Introduction

Brucellosis in cattle is difficult to control. This is confirmed by officials in countries that have succeeded in eradicating the disease after prolonged, difficult and expensive campaigns. This despite the fact that a good vaccine, *Brucella abortus* strain 19 (S19), has been in use for 50 years. The disease has been studied for the past 90 years but still presents problems in the field and laboratory.

Despite these difficulties the same vaccine, laboratory methods and field approach to the disease used in South Africa, have proved effective in the control and eradication of the disease in other countries. In all cases, however, co-operation between the farmer, animal health technician, veterinarian and laboratory has been necessary for success. National or regional eradication campaigns may take a long time (a number of years rather than one to two years). Individual herds can mostly be cleared of the disease within two years, but a percentage of problem herds which take longer to clear must be expected. Control is based on providing the animals with effective immunity and removing infected animals from the herd timeously to prevent spread of infection to clean stock.

### 7.2 Vaccination

Strain 19 & RB51 are the only *Brucella* vaccines currently approved for use in cattle in South Africa. Statutory use of S19 is limited to the single inoculation of heifers between the ages of four to eight months. The use of S19 more than once in the same animal is illegal because of the persistent vaccine reactions that it causes. Booster vaccinations with RB51 vaccine as set out below will ensure an improved and more persistent immunity.

No animal, except heifers between the ages of four to eight months of age, may be given S19 vaccine except with the written permission of the Provincial Director. The use of S19 in heifers under three months of age leads to poor development of immunity. The persistence of vaccination titres is linked to the age at inoculation and time of testing. In heifers inoculated before 6 months of age and tested after 18 months of age, usually less than 0,5% should have troublesome titres. It is ideal to mark S19 vaccinated heifers.

The older the heifer at inoculation and the younger she is tested, the greater the percentage with positive reactions to all tests. On an infected property heifers from positive dams have passive immunity for up to 5 months, which interferes with their active immunisation. **On infected properties heifers should be vaccinated as soon after five months as possible. On clean farms the heifers should be vaccinated as soon after four months as possible. Early maturing breeds such as Jersey and other Channel Island Breeds should be inoculated as close as possible to four months of age to avoid persistent titres as a result of S19.**

No bulls of any age may be inoculated with S19 or RB51 as it can cause orchitis and infertility in males.

#### 7.2.1 Handling of S19 & RB51 vaccines

Always handle with care as self-inoculation (by injection or contact with mucous membranes) can lead to brucellosis **as the S19 & RB51 vaccines are live vaccines**. If it occurs, contact a doctor immediately.

- Always keep vaccine cool and out of direct sunlight.
- Use a separate syringe for S19 vaccination. Disposable syringes are preferred. If this syringe is later used to vaccinate animals against other diseases the S19 residues can result in persistent vaccine reactions that will be very difficult to differentiate from wild strain (natural disease) infection. Disposable needles should be used
- Don't use the vaccine after the expiry date.

- Take note of the number of doses per vial to prevent wastage (for example, don't use twenty doses for two heifers).
- Vaccinate the correct dosage subcutaneously according to the package insert.
- Do not smoke or eat while handling the vaccine (contact with mucous membranes can cause vaccine induced brucellosis.)

### 7.2.2 Immunity

Vaccine use can reduce the incidence of the disease by increasing the level of herd immunity. Vaccination can help reduce the number of infective organisms excreted during calving/abortions in an infected herd/animal.

The use of S19 vaccine has the possible disadvantage of persistent vaccination titres. It would be helpful if vaccination could be stopped to exclude false positive reactors. Expert overseas opinion, however, advises that vaccination should not be stopped before the prevalence in a country or area is reduced to below 0,2%. Since the advent of RB51 vaccine the problem of persistent vaccine reactions has been excluded (if RB51 vaccine is used).

The vaccines give a good immunity because they contain live organisms, which persist for a short interval in the animal. Initially the immune response to S19 is humoral with the production of antibodies that can be detected by serological tests. If such an animal is not further exposed to the organism or the vaccine adjuvant, the humoral response disappears and is replaced by a cellular immunity, which cannot be detected in the laboratory. Protective immunity to brucellosis is, to a large extent, cellular in nature (because *Brucella* organisms are intracellular). **Residues of S19 in syringes and needles are antigenic and have been shown to boost antibody titres which may cause false positive results on serological tests.**

At present a very low percentage of heifers are being inoculated and at this level little progress in controlling brucellosis can be expected.

Note that RB51 vaccine does not produce a humoral antibody response that is picked up by the normally used serological tests.

Once-off vaccination with either vaccine only gives about a 70% protection against the chances of the animal picking up the disease. Also the chances of a lifelong immunity with a once off vaccination are relatively slim.

Vaccine	Advantages	Disadvantages
RB 51	Does not cause vaccine reactions & can thus be repeated & given to adult cows	Relatively expensive
S 19	Relatively cheap	If used in animals older than 8 months of age, or repeated can cause vaccine reactions difficult to distinguish from wild strain

### SUGGESTED VACCINATION PROTOCOL FOR AN INFECTED HERD WITH MANY ABORTIONS DUE TO BRUCELLOSIS:

1<sup>st</sup> step to stop abortions

- Warn farmer that vaccine can cause abortions (typically 0.2 – 2% or higher). Get him to sign an indemnity form.
- Vaccinate all female cattle on farm > 8 months with RB 51 – repeat after 4 – 6 weeks
- Vaccinate all female cattle again with RB51 after they have calved

Young animals

- Vaccinate heifers 4- 5 months with S19 (or RB51)
- Vaccinate with RB51 at 8-9 months of age (not at weaning as weaning stress will reduce efficiency of vaccination)
- Vaccinate heifers 2-3 months before mating with RB 51

**SUGGESTED VACCINATION PROTOCOL FOR HEIFERS IN AREAS WITH HIGH BRUCELLOSIS PREVALENCE:**

- Vaccinate heifers 4- 5 months with S19 (or RB51)
- Vaccinate with RB51 at 8-9 months of age (not at weaning as weaning stress will reduce efficiency of vaccination)
- **Vaccinate heifers 2-3 months before mating with RB 51**

The rationale for repeated vaccinations is: (see table below)

1. Once off vaccination only gives about 70% immunity.
2. Booster vaccinations should improve level of immunity of herd as well as individuals, which is important in badly infected areas.

**Improve Herd Immunity**  
Routine vaccination with Brucellosis vaccines

	Naïve females exposed to vaccine	<u>Assumed no. obtaining life long ??? protection against Brucella infection</u> (becoming carriers)
1 <sup>st</sup>	100 (S19 or RB51)	70
2 <sup>nd</sup>	30 (RB51)	21 (91% may now have protection)
3 <sup>rd</sup>	9 (RB51)	6 (97% may now have protection)
4 <sup>th</sup>	3 (RB51)	2
5 <sup>th</sup>	1 (RB51)	0.7 (99.7% likely to be protected)

• Re-vaccination is NOT possible with S19 vaccine.  
 • Improved protection against infection can be obtained when RB51 is used in the re-vaccination schedule – 2 boosters  
 • If RB 51 use as initial vaccination can test heifers at much younger age

(No absolute protection is conferred by any of the brucellosis vaccines even if administered more than once. A single brucellosis vaccination only confers protection against abortion in approximately 70% of vaccinates. To address the remaining ~30%, heifers should be re-vaccinated with RB-51 to increase the level of protection against abortion in the population – without the risk of false positive serological test results. Protection against abortion is improved, yet vaccinated cattle will still mobilize an antibody response when challenged by the field strain.)

Effective control of brucellosis will not be achieved without the strict application of all related control measures. Note that vaccination efforts may be nullified if there is poor biosecurity as infection pressure may overcome the protective immunity and render the animal infected.

NEVER vaccinate pregnant animals in a negative herd (any stage of pregnancy) with RB51 vaccine if not previously exposed to vaccine or natural disease, as the vaccine could cause up to 30% abortions and even

resorptions in early pregnancy. Cases have been reported where pregnant animals were vaccinated at 2 months of pregnancy with RB51, but they aborted 5 months later with RB51 being isolated from the stillborn/aborted foetus.

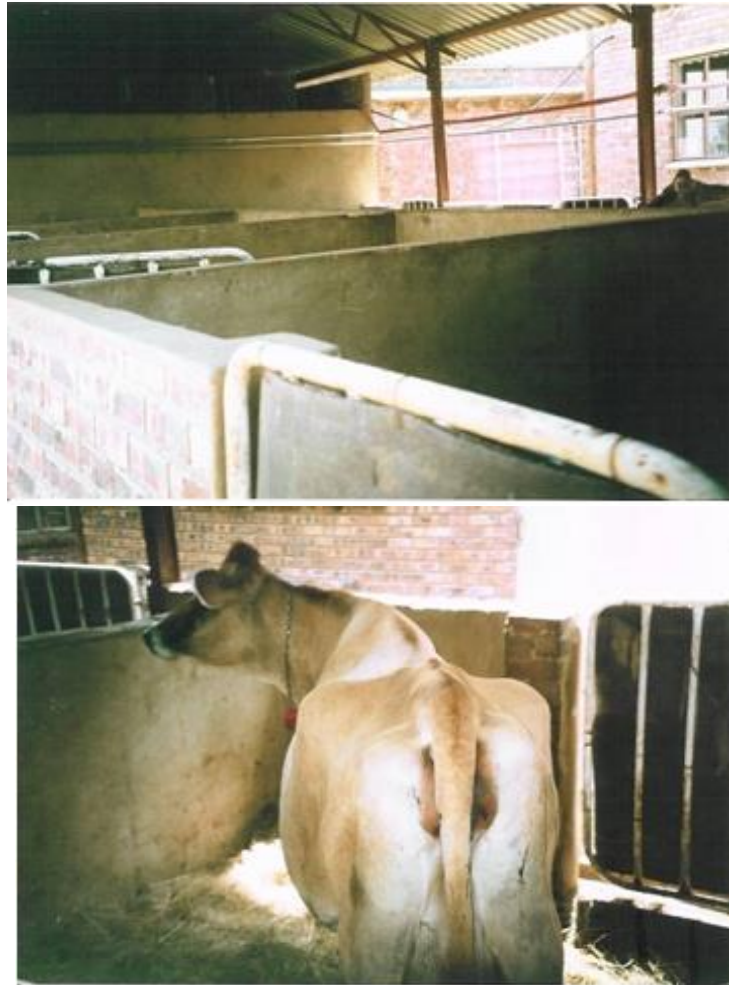
### 7.3 Economic factors and epidemiological considerations

The next most important consideration in controlling brucellosis is the economic consequences of the program to be introduced. The economic impact of a control program on a farming unit must be considered. No useful purpose will be served by bankrupting the farmer in an attempt to clear the property of the disease. Overseas experience suggests that a test-and-slaughter approach is only economically viable if the prevalence of infection is below 2%. In our experience other factors also play a role in such a decision:

- Value of the stock as opposed to slaughter value
- Genetic value of the stock
- The economic advantage of having a clean herd
- Whether intensive or extensive (easier to control) conditions prevail and
- The larger the herd, the more difficult it is to control the disease.
- Threat to human health.

With these provisions in mind a number of herds have been cleared of the infection where the results have been economically acceptable to the owners and where the initial prevalence was as high as 10-30 percent. **Because of the prolonged incubation period of the disease, success can only be assured if tests over a period of at least three years have proved negative.** The whole object of serological testing is to identify the infected animal before it has a chance to abort or give birth to a normal but infected calf and excrete bacteria into the environment. An infected animal may become serologically positive within eight days of becoming infected or this event may be delayed for as long as two years, or longer under exceptional circumstances, depending on when she becomes pregnant. Economics and practicalities again influence the decision and generally a two month test interval has proved effective.

The infected female does not abort (and therefore does not spread brucellosis) until she is at least five months pregnant. Ideally, on an infected property, pregnant cattle should be isolated from the time they are four months pregnant until they have tested negative at calving and again three weeks later. Effective isolation of animals under farming conditions is seldom achievable and the provision of such facilities is seldom economically feasible. Often the only practical approach is the very careful observation of all pregnant cattle so that impending abortions may be identified and isolated before the event, and normal calving scheduled for a dry, low-lying series of camps which are rotated so as to allow at least a month's rest. **Under no circumstances must kikuyu camps be used for calving as the organism will survive for long periods in cool damp kikuyu, which will assist in spreading the disease to negative animals. Ideally cemented separate calving facilities should be used in badly infected dairy herds as shown in the pictures below.** Run-off water management should be considered – fields should not be irrigated with run-off water



*Images showing separate pens for calving that can be subsequently cleaned and disinfected.*

Where a test-and-slaughter program is not economically viable, a number of alternatives may be implemented:

- The total slaughter of the herd with its replacement by clean, immunized stock should be considered after disinfection (with 2.5% formalin) of all installations (water cribs and buildings) and allowing all grazing one to three month's rest without stock. Although *Brucellae* may survive for as long as 10 months in cool, damp surroundings, the effectiveness of destocking a property for a month has proved sufficient to protect susceptible stock introduced thereafter in the cooler, moist climates of countries such as Ireland and Belgium. Under local conditions a rest period of three months should be more than adequate. Kikuyu pastures should be mown and not irrigated during the destocking period.
- The continuation of heifer inoculation with boosters as outlined above, as well as possible adult RB51 inoculation, will over a number of years lead to slow attrition of the older, infected animals with their replacement by younger, well-immunized stock. During this time cows may leave the farm for slaughter purposes only.
- The establishment of an infected herd isolated from the clean herd is sometimes a viable proposition, especially where a separate property is available for this purpose. An important principle in such a scheme is that a one-way movement of stock to the infected herd and eventually to slaughter is adhered to.
- In dairy herds positive animals must be isolated in low-lying dry portions of the property, not be rebred but kept for a final lactation provided that they are milked last. The milk derived from such a herd must be pasteurised before consumption. At the end of their lactation they should be slaughtered.
- All infected herds should be cleared of brucellosis by the methods outlined above and should be monitored for possible re-infection by monthly bulk-milk ring tests in the case of dairy cattle and annual serological tests



in beef herds for at least 5 years after clearing the herd of the disease. The cause of all abortions, stillbirths and weak or undersized calves must be reported by the stockowner to a state veterinarian/animal health technician for investigation.

## 7.4 The role of the bull

The infected bull is of minimal importance as a source of spread of brucellosis on the infected property, provided he is only used for natural service. **With artificial insemination he becomes an important source of infection. The use of an infected bull in a clean herd cannot be condoned under any circumstances.**

## 7.5 Latently infected animals

The occurrence of a latently infected heifer calf from an infected dam, which may only show the infection two years or even later, has been demonstrated experimentally and has long been demonstrated as a cause of the “two-year breakdown” syndrome in supposedly clean herds. The prevalence of this phenomenon may be between 2 – 20% or even higher. It can be excluded by slaughtering all heifers from positive dams.

Heifers of infected dams must be clearly marked so that they can be easily identified later. If these heifers are not slaughtered, isolate them after mating, test at 5 months pregnant and monthly thereafter up to one month after calving/abortion.

## 7.6 Stock introduction

The introduction of stock must comply with three criteria. They must:

- be correctly immunized
- derived from a brucellosis free herd
- permanently identified

If these criteria cannot be met, a less reliable control is to have the animal pass two serological tests spaced two months apart, the first on the farm of origin and the second in isolation on the buyer’s property before introduction into the herd. Pregnant stock should be kept in isolation until they pass a serological test three weeks after calving. Heifer calves should be kept separate until they can be serologically tested three weeks after calving (they can be latent carriers). In all cases the state veterinarian should be consulted on the brucellosis status of farms from which specific animals are to be purchased.

## 8 Test programmes

The objective of the Bovine Brucellosis Scheme is the detection, control, combating and eventual eradication of bovine brucellosis. In order to attain this goal various test programs have been initiated.

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

- (02) Maintenance herd programme
- (03) Surveillance herd programme
- (04) Diagnostic testing programme (individual animals)
- (09) Infected herd programme

Note that the review of this suggestion is still in progress.

The test programmes (with their computer allocated codes in brackets) which were used according to the Scheme, are suggested to be regrouped as follows:

- Accreditation (01) – not in use anymore
- Maintenance (old Annual Diagnostic) (02) – will fall under “Maintenance herd programme”
- Diagnostic herd test (03) – will fall under “Surveillance her programme”
- Ordinary diagnostic (04) - Will fall under “Diagnostic testing programme”
- Import (05) - Will fall under “Diagnostic testing programme”
- Export (06) - Will fall under “Diagnostic testing programme”
- Infected (09) – Will fall under “Infected herd programme”

Joining a programme is voluntary (except the infected and import programmes). 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)..

## 8.1 Accreditation (01)

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 brucellosis free. **Very few herds comply with these strict requirements and thus this program has been discontinued**

### 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, suspect or purchased 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 cow sheds floors, feeding and water troughs, etc. should be of such a nature that they can be thoroughly cleaned and disinfected when 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 dependent 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.

## 8.2 Maintenance (02)

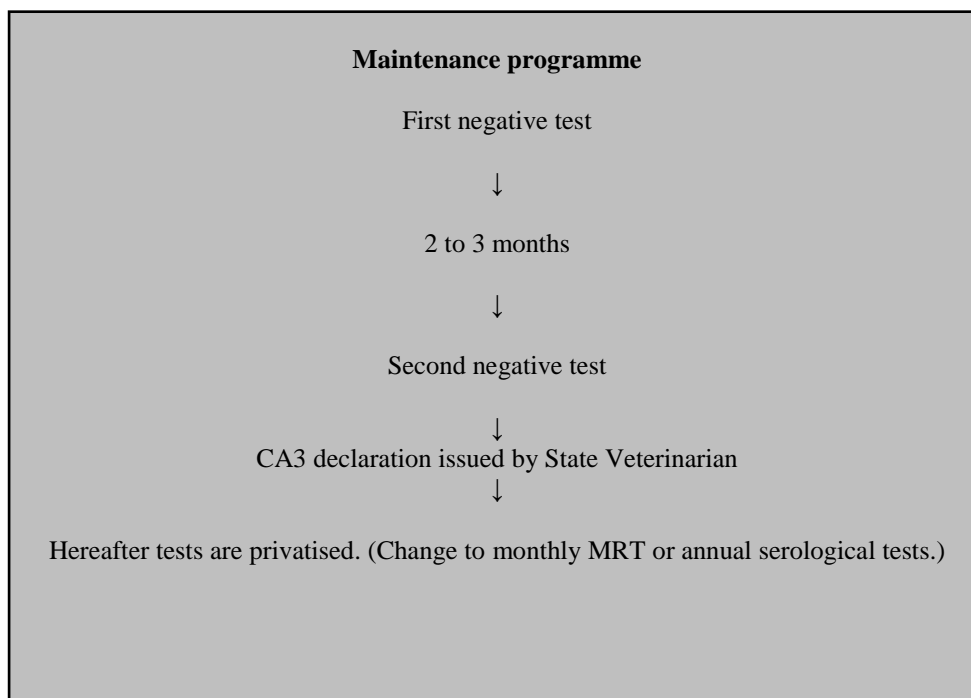
**All herds should preferably be incorporated into the maintenance programme.**

The purpose of this programme is to accommodate herds that do not comply with the requirements for accreditation, but require annual negative certification. Herds admitted to this programme complete a TB/CA1 agreement form in advance. Animals must be identified individually and at the owner's own cost. All animals (cows, heifers and bulls) over 18 months must be bled. Should any cattle react positively, the herd is included in the "infected programme". New herds that enter the programme must undergo two negative tests within an interval of not less than two months and not more than 5/6 months before a CA3 declaration is issued by the State Veterinarian.

The relevant **declaration** only indicates that the animals reacted negatively for brucellosis on the date of the second negative test and must not be confused with the official bovine brucellosis-free **certificate** (CA4) which was issued in respect of accreditation herds.

After a BR CA3 has been issued, the herd will be retested annually. These tests are for the owner's account. A BR CA3 declaration is issued by the State Veterinarian after every annual negative serological test or after completion of regular monthly bulk tank MRT specimens over a period of 12 months. (See schematic presentation of test intervals.).

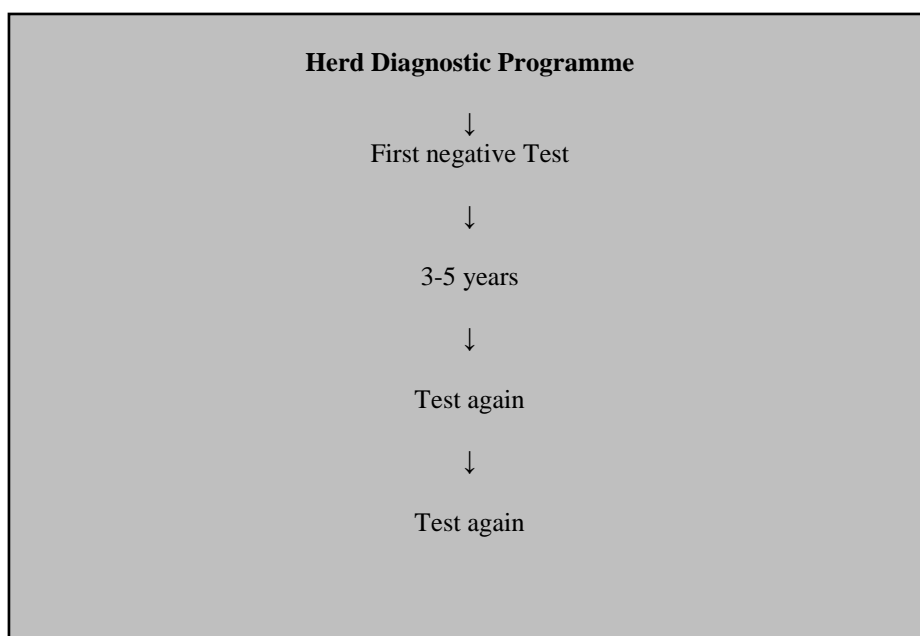
As in the case of the accredited programme, the onus rests with the stock owner to keep his herd free from brucellosis during the interim period by not infecting his herd, for example through purchases or through contact with infected cattle. It is not compulsory to keep records in respect of reductions and increases. For each herd a separate file is opened and a reference number allocated at the state vet office. All heifers and cows should ideally be vaccinated against Brucellosis.



### 8.3 Diagnostic herd (03) - (Herd Surveillance Programme)

Some stock owners are not prepared to subject their herds to the conditions of the accredited or maintenance programmes, but are nevertheless anxious to determine the brucellosis status of their herds. Such owners can be accommodated under this programme in order to establish reasonable quickly what the prevalence of brucellosis is in a herd in a certain area or local municipal area. Tests in accordance with this programme should preferably be undertaken on an organised basis by for example testing a whole local municipal area systematically from a certain point until the brucellosis status of the whole local municipality is eventually established (survey test).

The tests are mainly executed at the State's expense by officials. The agreement TB/BR must be completed in advance. Individual identification of animals is preferred but not essential and all animals (cows, heifers and bulls) over 18 months are tested. Should positive reactors be found, the herd is incorporated in the "infected programme", all animals tagged (if not tagged at time of bleeding) and re-bled immediately to identify and remove the positive reactors. A herd is normally only tested once every three to five years under this scheme.



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

A separate file is not kept for each individual herd tested under this scheme, except where the test has positive reactors. In such cases the herd is treated further as described under the "infected programme". Test results of negatively tested herds are placed on one local municipality file in numerical order and the following reference number is allocated to the file: Province/local municipality

## 8.4 Diagnostic (04) – (Diagnostic Testing Programme)

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 cattle in a herd, but not the whole herd, for example when one or more cows in a herd have aborted.

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 possible, the test can be conducted by State Veterinarians, but the owner must pay the costs of the test.

The agreement form (TB/CA1) should be completed in advance by the stock owner. Permanent individual identification of cattle tested in this programme is essential. Positive reactors found during the test obviously result in the herd being tested in accordance with the requirements of the “infected programme”.

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. The reference number on the file is as follows: -Provincial reference number.

## 8.5 Import (05) - (Diagnostic Testing Programme)

Cattle that are imported and kept at one of the quarantine stations are submitted to a brucellosis test at the specific centre. Because the test is compulsory for all imported cattle in accordance with the import permit, no TB/CA1 agreement is completed.

BR test reports are not placed in a separate file for each owner but in a joint import file with reference number Provincial Reference

## 8.6 Export (06) - (Diagnostic Testing Programme)

Most importing countries demand that cattle be subjected to brucellosis testing. The tests are conducted at the cattle owner's expense. A TB/BR is completed in advance. A joint file is opened for each province in respect of such test with the following reference number: Province reference number.

In terms of any legal aspects the import and export tests are incorporated under the “Diagnostic testing programme”

## 8.7 Infected herd (09)

A herd is regarded as infected when infection has been determined by either serological tests or isolation of *Brucella* organisms from lochia specimens, placenta tissue, foetuses and lymph nodes (culture is the golden standard). Such a herd is placed under quarantine and 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 No. 35 of 1984). This means that in all the mentioned cases where infection has been detected, the herd concerned will come under supervision and steps will be taken to eradicate infection in the herd.

Brucellosis is an infectious disease which is primarily spread between herds by movement of infected animals and within herds by contact between susceptible animals and infective material - generally at the time of calving or abortion of infected females. Infected animals usually develop serological titres, although delayed sero-conversion is not uncommon. The most common stimulus for sero-conversion is parturition, hence peri-parturient titre changes are not uncommon.

The approach to investigating an infected herd centers on addressing the four questions:

1. Where did it come from?
2. Where has it spread in the herd?
3. Where has it gone to outside the herd?
4. What are you going to do about eradicating it promptly and effectively?

There is no recipe which will ensure that the correct assessment and decisions are made.

**Investigating officers must exercise sound professional judgement.**

### 8.7.1 Procedures

Once the herd is declared 'infected' the following procedures will be implemented:

- a. *Reporting:* Infection or suspected infection must be reported to the local State Veterinarian. State veterinarian will complete a SR1 form;
- b. *Branding of reactors:* Positive reactors are branded as soon as possible after the results have been made available (exception would be where animals are immediately moved to an abattoir under cover of a red cross permit, so as to avoid unnecessary bruising). This is done by or under the supervision of a State Veterinarian, Animal Health Technician or a contracted Private Practitioner authorised by the Director: Animal Health. Positive reactors are branded with a hot C-branding iron supplied by the State on the right hand side of the neck, approximately 15 cm below the junction of the head and neck. Prospective buyers should always be on the lookout for C-branded cows and should not buy such animals;
- c. *Quarantine:* According to the Animal Diseases Act (Act 35 of 1984) the onus rests with the stock owner to keep, in cases where a controlled disease has been determined or is suspected, such animals in quarantine until a State veterinarian authorises their release. The issuing of a written quarantine notice is therefore not obligatory, but to obviate any doubt it is advisable to issue a written quarantine notice in the prescribed manner. Currently, in terms of Animal Diseases Act (Act 35 of 1984), it states that the owner must notify any prospective buyer of cattle from his herd, that the herd is infected with Brucellosis and even though the cattle being sold have tested negative, they could be incubating the disease and spread it at a later stage. The owner is also compelled to notify all his/her neighbours. All neighbouring farms should be notified by State Veterinarian staff and tested as soon as possible.

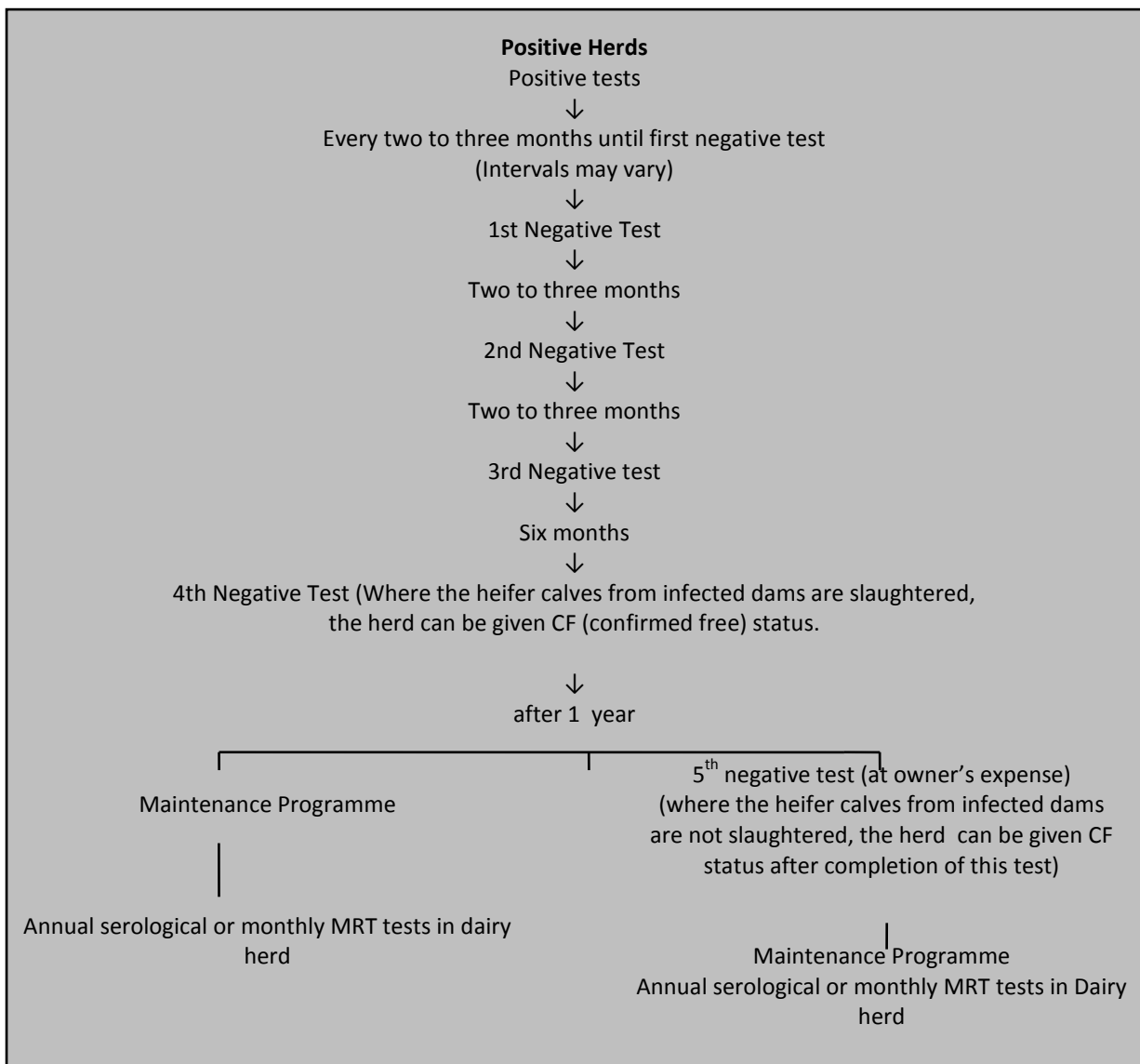
**Note that**

**brucellosis is a herd disease – to prevent dissemination, no animals (including negative animals from positive herds) should be sold until the farm is cleared of disease and quarantine subsequently lifted. Brucellosis may be incubated for extended periods of time, hence the sale of negative animals from a positive herd is seen as a high risk activity and should strongly be discouraged.**
- d. *Permits:* All cattle from an infected herd must be accompanied by a red cross permit for transportation to an abattoir, and may only leave the farm under cover of such a permit. Prior consultation with the abattoir must be carried out to ascertain whether the abattoir will receive *Brucella*-positive cattle.
- e. All sexually entire cattle over 18 months of age will be *individually identified* and their identification recorded on a stock register **(this is the responsibility of the owner);**
- f. All sexually entire cattle over 18 months of age will have their identification, and blood test results, from initial infected herd test, *recorded; (the State Veterinarian should keep a register of all the titres of all animals in a positive herd);*
- g. The *reactor report* must be completed by the State Veterinarian and handed to the Deputy Director;

- h. An *Eradication Plan* for infected herds will be developed and implemented, including action in relation to contact cattle outside the infected herd. This plan must be fully discussed with the owner and his co-operation obtained. Alternative strategies such as herd depopulation should be mentioned. S19 must be applied to all heifers between 4-8 months of age and RB51 vaccination to all cows that currently test negative;
- i. *Surveillance* will be maintained and reported on; infected herds & suspect herds must be treated as top priority;
- j. The Eradication Plan will be *reviewed* at least after each herd test to determine whether previous conclusions remain valid and/or circumstances changed and be revised as appropriate;
- k. After the positive reactors have been removed from the herd, be it through slaughtering or total separation, to the satisfaction of the State Veterinarian, the cattle of the remaining part of the herd must undergo bleeding at two monthly intervals until four consecutive negative tests have been reached;

\*\*The herd is re-bled six months later and in the case where the heifer calves of infected dams are slaughtered the herd can be granted confirmed free (CF) status. Where the heifer calves of infected dams are not slaughtered the herd has to undergo a further bleed 12 months later (fifth negative test).

\*\*see flowchart below.



*Arrangements 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 spread of the disease is eliminated as far as possible and that infection will eventually be eradicated from the herd.

Positive branded cattle may only be removed from a farm for slaughter at an abattoir. One of the following can be applied:

- immediate slaughter (can be done on the farm)
- postponed slaughter only under exceptional circumstances should this be allowed)

*Immediate slaughtering*

Positive animals should be slaughtered to prevent the spread of disease and to eradicate it. In the case of brucellosis, positive branded animals are placed under quarantine with instruction that these animals may only be transported to an abattoir for immediate slaughter and may not be moved to any other destination. Extension service and advice should be given to the farmer in respect of the advantages of immediate slaughter, and he should be persuaded to follow the advice.



The abattoir receiving the cattle must ensure that:

- The identification of the cattle correlates with information on the permit.
- The permit is stamped by the abattoir authorities and returned to the office where the permit was issued for record purposes.

#### *Postponement of slaughter*

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

Postponement of slaughter may be considered (provided that the milk from such animals is pasteurized or boiled before human consumption, and may NOT be sold or distributed raw to consumers) when:

- Animals of good quality such as high milk producers are involved;
  - The percentage of animals infected is high, e.g. 20 to 30%;
  - and there is a specific reason for postponement in respect of specific animals, such as cows at the peak of their lactation or cows with small calves at foot.
- *Isolation:* Postponement can only be granted when the positive animals are C branded (so they can immediately be identified should they in some way escape from isolation) and separated in such a way that they pose no danger to susceptible, uninfected animals.
  - *Duration of the period:* The cow must be slaughtered after the current lactation period has passed, or the calf has been weaned. This period will therefore not exceed 12 months. The purpose of postponing slaughtering is to enable the owner to make alternative arrangements, and not to keep on farming indefinitely with an infected herd. Such an animal/s may not be permitted to become pregnant again (danger of spread of infection) or calve on the farm.
  - *Milk for human consumption:* Milk derived from an infected herd 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.

#### **Files**

When a herd is tested under this programme, a separate file is opened and a reference number allocated.

For efficient interpretation it is essential that the State Veterinary office keeps sequential records of every animal's results. Proper records of all additions to and losses from the herd must be kept.

#### **8.7.2 Epidemiology report**

To be completed in own style under the following suggested headings, after thorough investigation of all the circumstances and verification of infection, where possible. ***This is for guidance purposes and represents a thorough approach.***

a.) Where did the disease come from?

List possible sources in order of descending priority with dates of possible introduction of disease. Explain and justify.

Consider the following:



- Was the disease introduced? i.e. with purchased cattle, spread from neighbours, or a resurgence of previous infection in the herd. (If due to resurgence - reasons will be required)
- Type of enterprise? (including other properties under same ownership)
- All stock purchases.
- All stock movements (hired grazing, shows, strays, trekking, loans, etc.).
- Previous brucellosis history for that herd (including fate of previous reactors), familial connection with previous reactors, date of birth in relation to last active infection on property - if applicable.
- Origins and movements of current reactor(s).
- Neighbours' current herd status and past brucellosis history – list all neighbours' names, farm names & contact details.

b.) What spread has occurred in the herd?

When did reactor become infected and infective and what animals in the herd may have been exposed to infection? Explain and justify.

Consider the following:

- Calving management (time of calving, calving pattern, age at first calving).
- Vaccination history (S19/RB51 coverage, over age vaccination with S19).
- Topography of farm versus surrounding area.
- Herd permanency.
- Movements of the reactors within herd.
- Reproductive history of reactor(s).
- Farm management practices.

c.) Where has the disease spread to?

List all possible dangerous contact, direct and indirect, in order of descending priority. Explain and justify.

Consider the following:

- Type of enterprise (including other properties, same ownership) & management.
- All movements of reactor(s).
- All cattle movement off property since infection was introduced (sales, loans, strays, trekking, shows, hired grazing on and off the property).
- Contact with neighbouring herds (loans, strays, shared facilities, drainage, infective material).

d.) What are you doing about it? - Eradication Plan

### 8.7.3 Infected Herd Programme

Develop individual plans in consultation with owner taking into account -

1. Early eradication - specify target date. Ensure vaccination schedule is up to date.
2. (i) Definition of high risk groups - from epidemiological analysis.  
(ii) Depopulation of whole or part of herd. Pay attention to contacts at calving, calves at foot, non-pregnant dry cows, non-vaccinates when defining high risk groups.
3. (i) Identify all breeding stock - ear tags; institute a stock register where necessary e.g. problem herd.  
(ii) Blood test and slaughter regimen and rationale especially post-calving test.
4. Prevention of spread -
  - Isolation of reactors (positive and inconclusive) and surveillance,
  - fencing and property security,

- calving practices and management,
- vaccination of negative animals,
- isolation of high risk groups (pregnant animals), and
- pregnancy test and split herds.

5. Provide for review and modification of plans in the light of subsequent developments.

6. **N.B.** to get owner agreement and commitment from the start. An initial plan should be developed and discussed with the owner, a final plan agreed and provided to the owner in writing.

**Note:** In those cases where the problem is due to earlier poor eradication procedures every attempt must be made to implement standard procedures before resorting to more radical procedures

#### 8.7.4 High risk contacts

High risk contact herds must be identified -

##### *Possible source of infection*

Trace back herds that:

- Had cattle movements from infected property,
- came from possibly infected neighbouring herds,
- have had other cattle contacts, e.g. hired grazing/shows/loans/strays, and
- are from other properties under the same ownership.

**N.B.** Do not overlook the possibility that the infected herd may have been infected from more than one source.

##### *High risk Contact Herds possibly exposed to infection from infected herd*

Trace forward herds that:

- Received cattle movements off the infected property,
- have cattle that have had contact with cattle from the ~~4N~~ infected herd, e.g., sales, shows, hired grazing, strays, loans;
- have neighbour contact, and
- other properties under the same ownership or other management connection.

The period to be checked back on will depend on when the infection entered the infected herd (or more precisely when the index case(s) became infective and how well this date can be determined).

The possible dangerous contact herds will be subdivided into **trace-back** and **trace-forward** and ranked and listed in descending order of risk.

All listed herds will be contacted and blood tested immediately and again after the first complete calving after last possible exposure, unless the State Veterinarian can justify otherwise (see Herd Test Procedures).

#### 8.7.5 Disease Control Measures

a.) Movement Restrictions

Positive and inconclusive (until final diagnosis is made) reactors will be quarantined immediately **when** the herd is declared infected or within 21 days of detection of CFT titre of 60 IU/ml or greater, whichever is sooner. One needs to quarantine the entire epidemiological unit - the whole herd/farm and related herds/farms.



Neighbours of herds infected with brucellosis shall be notified & tested as a matter of priority. Investigation and testing of neighbouring herds are necessary as part of the breakdown investigation. Advice about measures appropriate to prevent or limit infection in the neighbour's herd should be provided.

Keeping in mind that brucellosis is a herd disease; it is regarded as a high risk activity to sell animals from an infected (positive) herd. Brucellosis may be incubated for extended periods of time, hence the sale of negative animals from a positive herd should strongly be discouraged. Notify previous buyers of animals and current milk buyers. The owner/farmer of dairy cattle may sell the milk if it is pasteurised or to "companies" that pasteurise the milk – selling of fresh milk is prohibited in a positive brucellosis herd.

A red cross permit is required for any animal movement off the farm (irrespective of test result) and movement is only allowed to an abattoir.

Salvage procedure/ subdivision of herd: If a farmer wishes to salvage herd members through subdivision of a herd, there needs to be an application to the DAH (Director Animal Health) through the local State Veterinarian and Provincial Director to move the test negative herd members to a "clean" farm. The farm has to be fenced, adequate biosecurity and management procedures be in place and all movements controlled through red cross permits. Testing will continue for a satisfactory period (depends on herd dynamics and structure) until the herd can be declared negative.

Owners/Farmers should be encouraged to introduce animals from a clean property (brucellosis free) and which have been adequately vaccinated. Ensure that the property/area and herd are clean before buying in new animals.

b.) Surveillance

Infected herds should be kept under regular surveillance (minimum requirement – farm visit and bleeding every two months). Property security and stock numbers will be checked and enquiries made regarding potential movements (or sales), purchases, calvings and abortions. Surveillance visits will be reported to State Veterinarian and noted on the Herd Brucellosis Summary on the Computer.

Monitoring of stock sales by AHT for C branded animals and animals from known infected herds will be carried out regularly.

Where the State Veterinarian considers a warning letter is appropriate the State Veterinarian will send the warning letter to the owner. Where prosecution is considered necessary the State Veterinarian will institute such prosecution.

### **8.7.6 Strain 19/RB51 vaccination (also see 7.2)**

The vaccine used routinely will be the standard dose of S19 in heifers 4-8 months of age or RB51. Ideally S19 vaccinated animals should be permanently marked.

However, RB51 will be considered in adult cows in high risk herds (i.e. many abortions in herd due to wild strain brucellosis infection, spread despite regular testing and elimination of reactors) when approved by the State Veterinarian in consultation with the Deputy Director and after having discussed the advantages and disadvantages with the owner and getting him/her to sign indemnity form.



## 9 Administration of the scheme

### 9.1 Forms in use

#### Application for brucellosis testing (TB/ BR)

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 purpose 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 Deputy Director: Animal Health.

#### Brucellosis test declaration (TB/BR)

(Refer also to the Bovine Brucellosis Scheme Regulations)

A declaration by means of a TB/BR) 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 annually following the serological test or after the prescribed number of monthly MRT's.

*Infected programme:* issued after the fourth negative test, if heifers of positive dams have been slaughtered, otherwise after the 5<sup>th</sup> negative test (See schematic representation under 8.7 "Infected Herd").

Copy of BR8 and declaration to the following

- Person who did bleeding of herd
- Deputy Director for control purposes

#### Official brucellosis-free certificate for accredited herds (BR4) - *no longer applies*

Refer to the Bovine Brucellosis Scheme Regulations

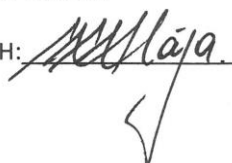
#### Bovine brucellosis test result (BR5)

This form is completed correctly and comprehensively by the sampler and must accompany all serum and milk specimens to the laboratory. The bleeder completes the form comprehensively, as well as the columns allocated to serum tube number and the columns allocated to the animal's ear-tag/identification number. The laboratory completes the rest of the relevant columns. The veterinarian who does the interpretation completes the relevant columns. The BR5 form should be accurately and fully completed. Cow numbers must correspond to sample numbers.

It is particularly important to indicate the purpose of the test clearly. Certain countries also require CFT tests on animals being exported, others CFT and SAT – this must be clearly stated on the form!

**NB:** REQUESTS FOR "CFT ALL" - The CFT will only be carried out on all sera for export tests where the importing country specifically requests this test. In general, the request for "CFT-all" will seldom be necessary - request must be in red at top of CA5 and signed by State Veterinarian.

The correct completion of the vaccination history is especially important for the correct interpretation of the



brucellosis status of a herd. The letter "h" must be filled in front of the specimen number for all heifers so that the veterinarian responsible for interpretation can see that the animal is young and that vaccine reactions could be playing a role. Should the sampler be of the opinion that specific information in respect of particular animals is necessary to arrive at the correct interpretation of the brucellosis status, it must also be indicated on the form. (i.e. animals that have aborted or have hygromas, etc.)

The form is completed in black ink in duplicate by the person taking the blood samples. The original is submitted together with the specimens to the laboratory and the copy kept for record purposes. After testing the specimens the laboratory completes the form and submits it to the State Veterinarian of the area. When the State Veterinarian interprets the results he submits **a copy to the sampler for reference purposes. The State Veterinarian must inform the owner of the result as soon as possible per telephone. The State Veterinary office will send a written result to the farmer on a CA8 form together with an advice form if there are any reactors (the BR5 forms are not normally sent to the owner). The original BR5 is kept on the herd's file.**

### **Monthly report-laboratory tests (BR6)**

Laboratories that conduct brucellosis tests, should complete this form monthly in triplicate, retain one copy and submit a second copy to their Deputy Director and the original copy directly to the Provincial Director of Veterinary Services.

### **Monthly return of tests (TB/BR7)**

This form should be completed at the end of every month by the State Veterinarian for tests performed in his area and is sent to the Deputy Director: Animal Health of the District. The Deputy Director: Animal Health of the district will summarise the tests for his district and submit to Provincial Director: Animal Health. The Provincial Director will submit a combined report for the province to the National Director: Animal Health

A collective report compiled by the National Director: Animal Health will be submitted monthly to all Provinces for cognizance.

### **Bovine brucellosis test report (BR8)**

The back of the CA8 form is only used in the case of accredited herds. The form is completed in duplicate. The original goes to the State Veterinarian and the copy is for the records of the sampler. Once he has done the interpretation of the results, the State Veterinarian submits the original CA8 form together with advice form (where there are any reactors etc) to the owner and copies as follows:

- Person who did bleeding of herd
- Deputy Director for control purposes

Infected and suspect herds - the owner must sign receipt of the result (advice) form and a signed copy must be kept on the herd file i.e. individual acknowledgement by owner after each test.

### **Brucellosis branding certificate (BR10)**

This form is completed in duplicate by the Official or Private Veterinarian. One copy is kept and the original submitted to the State Veterinarian, who must send a copy to Deputy Director: Animal Health. Once the animals have been branded, the owner or authorised representative must countersign the certificate.



### **Instruction (order) form (TB/BR11)**

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 - 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 Deputy Director: Animal Health.

### **File record chart (BR16)**

This form is used in the Deputy Director: Animal Health and State Veterinarian Offices for record keeping of tests on specific herds as well as positive reactors branded, etc. This form is attached to the inside cover of every file.

### **Notice to Private Veterinarians and Animal Health Technicians to test herds due for retest (TB/BR17)**

The form enables the State Veterinarian to remind a Private Veterinarian of the tests of his client's 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 Deputy Director: Animal Health.

### **Epidemiology Report on Brucellosis**

This form must be completed by the Animal Health Technician or State Veterinarian when investigating new brucellosis infected herds as explained in 8.7.2 "Epidemiology report".

### **Advice on control of brucellosis (Reactor Report)**

This form is completed by the State Veterinarian to notify the owner of the results of reactors and is sent together with the BR8 form to the owner and copies as follows:

- Person who bled herd
- Deputy Director for control purposes

Infected and suspect herds - the owner must sign receipt of the result (advice) form and a signed copy must be kept on the herd file i.e. individual acknowledgement by owner after each test.

### **Red Cross permit**

Female cattle from a positive herd may only be removed from the farm directly to an abattoir under cover of a red cross permit.

## 9.2 Records of individual animal reactions in infected herds

State Veterinarian office must have a herd record of every infected herd involving every animal in the herd in alphabetical/numerical order with test results etc. as shown below (State Veterinarian Clerk to record on computer and print out)

**Example: Farm name, owner, GPS coordinates, contacts.**

Animal No	03/08/99				22/09/99				25/10/99			
	RBT	SAT	CFT	Result	RBT	SAT	CFT	Result	RBT	SAT	CFT	Result
C1	+	134	172	Pos					+	424	784	Pos
2	-				+		0		-			
U9	-				-				-			
U10	-				-				-			
E11	+	424	784	Pos					+	424	784	Pos
12	+		0		-							
E12	-				-				-			
E14	-				-				-			
U14	-				-				-			
T16	-				-				-			
T17	+	40	24	Susp					+	53	30	Susp
U17	-				-				-			
T18									+	424	784	Pos
U18	-				-				-			
U19	-				+		0		-			
22	-				-				-			
R 22					+	134	120	Pos	+	424	784	Pos
R 25	-											
29									+	424	784	Pos
31	-				-				-			
35	+	160	98	Pos					+	106	98	Pos
37	-				-							
R 42					-							
44	-				-				-			
B45									+	424	784	Pos
50B	+	53	98	Pos					+	424	784	Pos
55	-								-			
9534	-				-				-			
9616	-				+	212	392	Pos	+	424	784	Pos
9638	+		0		+	53	AK15	Susp	+		0	Neg
9647	+		0						+	424	784	Pos
9650	-				-				-			
9657	-				-				-			
9659	-				-				-			
9670	-				-				-			
9682					-				-			
9683	-											
96109	+	424	784	Pos					+	424	784	Pos



This assists to ensure that:

- Duplicate numbers are picked up (otherwise incorrect animal branded)
- In previous positive herds - get person writing on BR5 to check off animals on individual cow list which must accompany sampler to the farm. Prevents chance of re-bleeding positives & allows branding positives not previously branded and prevents incorrect reading of tags, replacement of duplicate tags
- Enables official to show farmer that previous positives are not being isolated and to assist in convincing farmer of need to improve on his management.
- Follow the tendency of reactions especially where S19 incorrectly used

### 9.3 File reference system for various test programmes

#### Reference numbers in respect of Accredited/Maintenance/Infected herds

The general reference number is:

Province/Municipal area/Sequential herd number

See list of numbers for various provinces and their municipal areas at the end of document.

E.g. The reference number for the 12<sup>th</sup> herd in the Msukaligwa Municipal area in Mpumalanga province which is an infected herd will thus be 01/151/12.

The number is attached to a specific property in a municipal area, and should the farm or property change ownership, the number of the herd is kept if the new owner wishes to go ahead with the test scheme. A new TB/BR1 agreement must be completed in respect of the new owner.

For each herd a new file is opened.

#### Reference numbers in respect of diagnostic tests

The general reference number is:

Province/Municipal area

I.e. all filed on one file per municipal area.

## 10 Legislation applicable to bovine brucellosis

**Bovine Brucellosis is a controlled disease in accordance with the Animal Diseases Act (Act 35 of 1984) and the Animal Disease Regulations published in Govt. notice R2026 of 26 September 1986, as well as the Bovine Brucellosis Scheme Regulations published in Govt. notice R2483 of 9 December 1988.**

- **Notification of infection to neighbours and prospective buyers**

The owner or manager of land on which brucellosis infection occurs or an owner of animals infected with brucellosis must:

- a) Notify each owner or manager of adjacent land and each owner of susceptible animals on the same land as well as adjacent land.
- b) Notify each prospective buyer of his susceptible animals (i.e. animals presently testing negative) as well as any person who has bought susceptible animals from him during the immediate preceding period (i.e. before



the disease was established or suspected of being in the herd). **Note that this practice is strongly discouraged.**

- c) Refrain from removing “negative” animals from the farm without a permit as long as the herd is infected. The owner, or agent at an auction or, if neither of them do this, the State Veterinarian or Animal Health Technician, will announce that the cattle come from an infected herd and may therefore still be incubating the disease. The purpose of this is to prevent such animals from being purchased for breeding purposes by unsuspecting buyers.
- **Vaccination of heifers is compulsory between the ages of 4 and 8 months.**
  - **Reporting, branding of reactors, quarantine, permits.**
  - **An owner can be compelled by the Director of Veterinary Services to test his/her animals in situations where his/her herd could pose a possible threat to his/her neighbours.**
  - **Positive animals may be moved to an abattoir only under cover of a red cross permit.**
  - **Positive animals must be C-branded on the right side of the neck.**
  - **Animals may not be removed from the farm between the bleeding of the herd and the determining of the results.**
  - **If an owner of cattle becomes aware that, or suspects that his/her cattle are infected with brucellosis, he has a legal obligation to isolate these animals and to inform his/her State Veterinarian. No animal on his property may thereafter be removed there from unless authorized in writing by the State Veterinarian.**

The measures for combating brucellosis can be summarised as follows, mention being made of the relevant Section 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. BRUCELLOSIS
9 (2) (a) 11 (1) (b) (i) 31	11, table 2	Control measures Duties of owners/managers	Heifer vaccination Adult vaccination Testing Isolation Marking Slaughtering
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) (c) 11 (1) (a) 31	13	Isolation Duties of owners/managers	Isolation of contact or infected animal
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	Certificate, document, sworn declaration, containers and invoices pertaining to controlled veterinary acts

		acts	performed in terms of regulation 11.
9 (2) (c) 31	20 (1) (a) (xiii)	Movement restrictions	Prohibition of movement to accredited herds
9 (2) (c) 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 "C" branding on the right 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

### BOVINE BRUCELLOSIS SCHEME

(R2483 of 9 December 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 Brucellosis Scheme
3	Object of scheme	(1) Eradication of bovine brucellosis (2) by testing, identification, slaughtering, isolation, prevention of contact and information.
4	Application and scope of scheme	(1) <i>Brucella abortus</i> , cattle (2) Objects of five programs; accredited herd, annual diagnostic (maintenance), diagnostic herd, infected herd and diagnostic testing.
5	Manner of infection	(1) Excretion of <i>Brucella abortus</i> (2) Infection by artificial insemination, licking, grazing or consuming fodder, water or milk.
6	Characteristics of infection	(1) Abortion (2) Mostly normal second and later calvings post infection (3) Retained placenta (4) Low fertility, sterility
7	Tests for bovine brucellosis	(1) manner determined by director (2) Interpretation (3) Other tests
8	Requirements relating to a brucellosis test	(1) Only by an officer, authorized person or veterinarian (2) Prohibition of removal of cattle during test (3) 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 (as in regulation 13 (1)) (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) "C" brand on right side of neck, separation, prohibition on 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 after forfeiting/for his own account (2) Compensation (as in section 19 of Act 35 of 1984) (3) Valuation of cattle to be slaughtered (4) Time of slaughtering (5) Slaughtering of entire herd
14	Requirements for joining scheme	(1) – (5) Requirements for joining each program (see table below)
15	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 program in case of infected cattle herds, order to comply with requirements
16	Refusal of applications	Refusal of application due to non-compliance with requirements or inability to render services
17	Register of responsible persons and herds	State Veterinarian to keep a register with particulars of admitted responsible persons
18	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 program
19	Switching from one program to another	Conditions for switching from one program to another
20	Measures relating to participating herd	Measures pertaining to contact, sharing, introduction of cattle to herd, making cattle available for testing, applying control measures, heifer vaccination, recordkeeping and marking
21	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 program (4) Issuing of declaration i.r.o. cattle in diagnostic testing program (5) contents of declaration
22	Renewal of certificates	(1) Application for renewal of certificate (2) Brucellosis testing (3) Conditions for renewal of certificate (4) Duration of validity of certificate
23	Lapsing of certificates	(1) Conditions for lapsing (2) Conditions for issuing of new certificates in case of lapsing
24	Return of certificates	Return of lapsed certificates
25	Restrictions on the use of certificates and declarations	(1) Prohibition of misuse of declarations (2) Conditions for use of certificate
26	Tariffs for services	(1) Fees for services rendered as in regulation 27



	rendered	(2) Free services (3) Disinfectants and remedies at expense of responsible person
27	Commencement of scheme	1988-12-01

### REQUIREMENTS FOR JOINING BOVINE BRUCELLOSIS SCHEME

(Section 15 of Bovine Brucellosis Scheme)

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

## 11 Collection and dispatch of brucellosis samples

The following must be kept in mind when blood samples for brucellosis are collected and dispatched.

- Identification of the samples.
- The CA5 form must be completed fully and clearly and accurately.
- The sample tubes must be individually marked with a waterproof pen before starting to bleed the animals.

The cattle's identification numbers are written on the CA5, and on the tube the sequential number e.g. 1-100, etc. Before bleeding each animal ensure that the bottle number corresponds with the animal number on the CA5 form and before opening the crush pen to let a group of animals out ensure that the number of animals bled corresponds with the number of tubes used on the CA5. If at any stage they do not correspond immediately bleed that group over again. Preferably get owner/manager to write cattle numbers on CA5 and to correlate with your bleeding tube number.

- In previous positive herds, get person writing on CA 5 to check off animals on individual cow list which must accompany sampler to the farm. Prevents chance of re-bleeding positives, allows branding of positives not previously branded and incorrect reading of tags, replacement of duplicate tags.
- The tubes must be packed in the polystyrene container in the following way:
  - The tubes must be packed in sequence from left to right, filling the polystyrene box from the back to the front (see diagrammatic representation below).
  - The tube must be at least 2/3rds full.
  - Any blood on the outside of the stopper tube must be washed off.
  - Only clean dry tubes may be packed in the rack.
  - Keep the rack in the shade.
  - Any dust or foreign material must be removed from the rack before the lid is put on.
  - Under no circumstance may the rack be cut.
  - Write the particulars of the owner and the farm on the bottom part of the rack and on the lid
  - Do not put samples from more than one owner in one box.
  - After the serum has separated, place samples as quickly as possible in the refrigerator. Samples should not be frozen.

*M. Maja*

- If the samples cannot be delivered at the laboratory within three days, the clots must be removed.
- Clean serum can be kept in the refrigerator for 14 days.

#### PACKING OF BLOOD SAMPLE TUBES IN THE POLYSTYRENE RACK

When packing blood sample tubes into the polystyrene rack, the following must be adhered to:

- The polystyrene rack must be placed in front of you with the red label facing you.
- The blood sample tubes must be placed into the rack in numerical order and in the sequence as indicated in the diagram.

1	2	3	4	5	51	52	53	54	55
6	7	8	9	10	56	57	58	59	60
11	12	13	14	15	61	62	63	64	65
16	17	18	19	20	66	67	68	69	70
21	22	23	24	25	71	72	73	74	75
26	27	28	29	30	76	77	78	79	80
31	32	33	34	35	81	82	83	84	85
36	37	38	39	40	86	87	88	89	90
41	42	43	44	45	91	92	93	94	95
46	47	48	49	50	96	97	98	99	100



#### DISPATCH OF SAMPLES

- Pack the racks tightly so that the lids cannot move.
- Never send samples over a weekend or on a holiday.
- If possible contact the laboratory to confirm that the samples are on their way and to find out when they will arrive.
- If there is an opportunity for road transport, make use of it.
- If no results are received back, enquire in time.
- Ensure samples are packaged and transported in terms of the National Road Traffic Act, 1996 (Act No. 93 of 1996).

Samples that are received without the correctly completed CA5 form and/or properly marked tubes and holders will be destroyed as the incorrect results could reach the farmer resulting in the incorrect animals being culled with a possible civil claim against the Department for negligence.

