Standard Methods and Procedures (SMPs) for Control of Brucellosis in the Greater Horn of Africa
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# Table of Contents

1.0 **Introduction**  
1.1 Standard Methods and Procedures (SMP)  
1.2 Brucellosis  

2.0 **Definitions**  
2.1 Surveillance and Epidemiology  
2.2 Planning Documents  
2.3 Personnel  

3.0 **Surveillance and Epidemiology**  
3.1 Case Definition for Brucellosis  
3.2 Predisposing Factors  
3.3. The Objectives of Surveillance  
3.4 Administrative Preparations for Surveillance  
3.5. Types of Surveillance and Epidemiological Approaches  

4.0 **Brucellosis Laboratory Detection Diagnosis and Vaccine**  
4.1 Minimum Pre-requisite for Laboratory Diagnosis  
4.2 Laboratory Tests for National Disease Control Programmes and for Livestock Export  
4.3. Field Diagnosis, Collection, Storage and Analysis of Samples  

5.0 **Brucellosis Control**  
5.1 Considerations for Brucellosis Control in the GHoA  
5.2. Quarantine and Movement Control  
5.3. Vaccination  

6.0 **Disease Reporting and Information Management**  

7.0 **Brucellosis and Trade**  

8.0 **Risk Analysis and Risk Mapping**
**Foreword**

The arid and semi-arid lands of the Horn of Africa (HOA) are home to poor and vulnerable populations, the majority of whom rely on livestock to sustain livelihoods. However, the performance of livestock in the region remains low, given the widespread occurrence of transboundary animal diseases (TADs) that are responsible for production losses, and reduced performance of intra- and inter-regional trade in livestock and livestock products. Because of disease outbreaks, live animal exports have been severely constrained during the past two decades, by bans imposed by importing countries to reduce risks associated with these diseases.

To address the negative impact of TADs on livestock trade, AU-IBAR and ICPALD together with the participating countries in the region, with financial support from the United States Agency for International Development (USAID), have developed a framework to support harmonization and coordination of the control of the diseases, referred to as the Standard Methods and Procedures (SMP) Approach. The SMP approach involves strengthening capacities of member states for surveillance, epidemiology, laboratory diagnostics, disease control programmes, and communications. The fundamental aspect of the approach is the linking of disease prevention and control activities in a country, to a set of regional minimum standards and procedures for TADs prevention and control in line with the World Organization for Animal Health (OIE) standards.

The minimum standards, procedures, methods and goals for a particular disease are contained in an individual SMPs. It deals with subject areas of surveillance, laboratory procedures and disease control, and states minimum standards, procedures and goals that must be met for harmonized regional control of a disease.

This booklet presents the SMPs for Brucellosis and deals with the specific dynamics of Brucellosis prevention and control in the Greater Horn of Africa (GHoA).

The compilation of the materials in the SMPs for Brucellosis, taking into consideration the characteristics of the Greater Horn of Africa, was made possible by technical experts from the region with technical support from AU-IBAR, FAO, OIE and AU-PANVAC. AU-IBAR is indebted to many scientists who reviewed the document and especially to Dr. James Wabacha the coordinator of the SMP-AH project for coordinating the preparation of the SMPs.

The SMPs for Brucellosis targets field veterinary personnel, policy makers, laboratory personnel and veterinary students in the region.

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Standard Methods and Procedures for Control in the Greater Horn of Africa - Brucellosis

1.0 Introduction

1.1 Standard Methods and Procedures (SMP)

The Standard Methods and Procedures (SMP) approach is designed to guide and harmonize the work of Departments of Veterinary Services (DVSs) in the Greater Horn of Africa (GHoA) region in their approach to the control of trade-related Transboundary Animal Diseases (TADs).

Standard Methods and Procedures are operational protocols to create uniformity in animal disease detection and control procedures throughout the GHoA. An individual SMP is a protocol that outlines the measures to be undertaken for control of a given disease. The SMP deals with subject areas of surveillance, epidemiology, laboratory procedures, and disease control and specifies procedures, minimum standards, and goals that must be met for harmonized regional control of the disease. It is supported with details as specified in Standard Operating Procedures (SOPs) for each subject area that detail how the SMP goals are to be met, taking into consideration the structure and capabilities of the DVS of a given nation.

An SMP is a functional, action oriented management support document and is not intended to provide a detailed description of the disease. It is a live and flexible document that can be changed as new science and new techniques for control are discovered.

This SMP deals with the specific dynamics of Brucellosis and specifies standards, methods, and procedures for management, control of the disease.

1.2 Brucellosis

Brucellosis is primarily a reproductive disease of zoonotic importance widely spread throughout the world. The species of concern for the GHoA are B. abortus (Ba), primarily in cattle and causing Undulating Fever in humans, and B. melitensis (Bm) in sheep and goats causing Malta Fever in humans. Brucellosis also occurs in swine, camels, dogs, equine and several species of wildlife. Clinically, the disease is characterized by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis, and arthritis (rarely), with excretion of the organisms in uterine discharges and in milk.

Exposure in livestock is primarily via oral contact with uterine materials (placenta, uterine fluids), milk of infected dams, and vaginal discharges. Human exposure is primarily via ingestion of unpasteurized milk from infected animals, and secondarily from exposure to uterine discharges and infected tissues.
Brucellosis or Malta Fever is one of the most serious zoonoses in the world. It is an occupational hazard of livestock farmers, dairy workers, veterinarians, slaughterhouse workers, and laboratory personnel, all of whom are considered to belong to the high-risk occupational group. Anyone working with diagnostic sampling and/or testing must take careful precautions and use proper biosafety and biosecurity. All infected tissues, cultures and potentially contaminated materials should therefore be handled at biocontainment level 3. When Brucellosis is diagnosed in animals, human health authorities should be immediately notified. Brucellosis is a disease in which humans and animals act as sentinels for each other, so when infection is confirmed in animals, human involvement should be presumed and dealt with, and vice versa. The importance of cooperation between livestock health and human health authorities cannot be overstated. Brucellosis is an excellent example of a disease with implications for the One Health concept of cooperation between the veterinary and human health professions.

Development of peri-urban dairy farms and production systems has introduced a new dimension in the epidemiology of Brucellosis with the promotion of the dairy industry, the intensification of livestock production through genetic improvement of local breeds, and the introduction of exotic breeds. Reproductive failure damages all three of these activities. The importance of the disease in dairy farming is a major concern because the milk and dairy products are important sources of contamination and spread. The risk of transmission is higher in developing countries where infection in animals is not yet under control and where the pasteurization of milk and milk products is not systematic. In rural areas, consumption of raw milk coupled with unsanitary conditions favour transmission to humans. There are compelling reasons to start an immediate concerted effort for comprehensive control of endemic livestock Brucellosis in the region in order to mitigate the economic impact as well as the public health importance. Owing to the complexity of the disease dynamics, Brucellosis is a very expensive and a very long-term disease to manage.

Studies on the profitability of Brucellosis control in Mongolia have shown that the most economic way to control Brucellosis is by mass vaccination of livestock with a societal benefit ratio of 3 to 1. Similar studies should be done in Africa. Once Brucellosis prevalence is below 1% a change to test-and-slaughter strategy could be contemplated. This however requires an enabling environment. If test-and-slaughter systems are implemented, there must be sufficient public funds to compensate farmers for culled stock. If farmers are not compensated they may sell infected animals illegally which in turn contributes to continued transmission of the disease both in susceptible livestock and humans. An additional factor is that efficient disease control, especially for Brucellosis, requires a system of livestock identification and traceability so that source herds can
be identified. Both vaccination and test-and-slaughter strategies require appropriate management, animal identification, and monitoring of the control programme, and especially the monitoring of vaccination coverage.

Brucellosis is a complex disease with many facets. If control programmes are to be initiated they must be approached with a great deal of well-considered planning and design, long-term commitment of personnel and time, and assurance of stable funding over many years. Excellent research has been done in recent decades and new options for control have been introduced, including greatly improved laboratory tests. For example, an option for small-stock mass vaccination has been introduced, a milk ELISA test has been developed for the dairy industries, and there has been significant improvement in efficacy and safety of vaccines.

Brucellosis in livestock in GHoA can be distinguished into two categories whose control programmes are fundamentally different – see section 5 on Brucellosis control. *Brucella abortus* is essentially a disease of cattle, both dairy and beef breeds, and should be approached in strong partnership with human health authorities. *Brucella melitensis* is a disease principally of goats, secondarily of sheep, and is a problem primarily in pastoral livestock production areas. *B. melitensis* is a major impediment in the export trade market to the Arabian Peninsula and Middle East and has historically caused a great deal of loss to producers and traders.

In the GHoA, a valid control programme must begin with an area-wide survey to determine the incidence and prevalence of the disease so that spatial distribution is understood and disease control response is well targeted. This should include a survey of technical literature, collection and analysis of diagnostic laboratory reports; access to the testing records of Export Quarantine laboratories for animals being exported; and determination of the possibility of doing any trace-back to source herds or at least source areas. The probable impossibility of accurate traceability is recognized, but at the very least there should be an attempt and Export Quarantine laboratories have probably done more Brucellosis testing than any other laboratories in the region and also may provide data relevant to determination of incidence and prevalence.

Mobility of pastoral livestock is acknowledged, and the mass vaccination of small ruminants for prevention of *B. melitensis* is becoming a feasible control measure and response. Pastoral cattle are not as mobile and may be possible to handle with a more targeted approach – and *B. abortus* is important to address as it has direct bearing on human exposure via widespread use of milk as a staple food.
The dairy industries differ in that they are typically more sedentary, including both small producer aggregation schemes and commercial production, and present a different set of circumstances and opportunities, in that identification and traceability is much easier and herd testing and vaccination programmes are greatly enhanced. It is strongly recommended that any Brucellosis investigation or control work done in the dairy industry be done in close cooperation with human health authorities.

As previously noted, a central facet of any Brucellosis control programme – or any other programme that promotes or involves culling of infected livestock – is provision of fair and adequate compensation for any livestock culled. Without such compensation the programme will be opposed in all possible ways by livestock owners, and will collapse. As an important prerequisite, if a programme is initiated it must have stable, long-term, adequate funding.
2.0 Definitions

2.1 Surveillance and Epidemiology

Surveillance
Continuous collection and analysis of animal health data to inform disease control programmes.

Passive surveillance
A method of surveillance that enables veterinary authorities to collect animal health data and information from disease reporting stakeholders.

Active surveillance
A method of surveillance in which epidemiological information is collected by purposeful and planned interventions.

Syndromic surveillance
A surveillance approach based on observing signs of disease and determining the type of disease without necessarily diagnosing the specific disease.

Targeted surveillance
A form of active surveillance based on probability of occurrence of disease in a given area.

Risk based surveillance
A form of active surveillance that focuses on a certain area or livestock population based on perceived level of threat, risk and/or consequences.

Participatory disease surveillance
A form of active surveillance that uses participatory approaches in search of disease, including input from local livestock producers and others in the livestock value chain.

Epidemiological unit
A group of animals with a defined relationship sharing common likelihood of exposure to a disease.

Risk mapping
A tool used for identification, assessment, communication and mitigation of a disease in a certain geographical area.
Zero reporting
Periodic standard reports indicating that surveillance in any form for a given disease has been carried out and no disease occurrence has been encountered. Zero reports are a valuable tool to indicate negative results of constant and ongoing passive and/or active surveillance.

2.2 Planning Documents
Standard operating procedures (SOPs)
A plan of action for a particular undertaking that stipulates exact details of what must be done to accomplish the task.

Preparedness plans
Preparedness planning involves capacity building, equipment procurement, personnel responsibility allocation, and training in all the disciplines that support effective disease control, e.g. epidemiology, laboratory, disease management, etc.

Rapid response plan
A pre-programmed plan for immediate response to a report of an outbreak of a TAD or other emergency disease with the goal of eliminating the index case and preventing an epidemic spread. The rapid response plan includes three components: the Epidemiology Section for disease investigation; the Laboratory Section for confirmation sampling; and the Disease Control Section for immediate disease control interventions as need be.

Contingency plan
An operational plan designed for immediate control of a disease outbreak, typically composed by the Department of Veterinary Services for use within that country.

2.3 Personnel
Veterinary officer
Government employed veterinarians and field staff.

Veterinary personnel
All people associated with veterinary work including public veterinary staff (government at any administrative level) and private veterinarians and their staff members.
3.0 **Surveillance and Epidemiology**

3.1 **Case Definition for Brucellosis**
Suspected Brucellosis in sheep, goats, cattle, camel and other susceptible animals will be based either on a positive serological result or clinical signs if any one or more of the following are encountered: abortion, retained placenta, stillborns, orchitis, epididymitis, reproductive failure and arthritis (hygroma).

A positive case of Brucellosis will however be based on laboratory confirmation of the disease.

Important: A tentative diagnosis of Brucellosis can be arrived at based on clinical signs, but laboratory confirmation is required for differential diagnosis with other diseases with similar signs such as Rift valley Fever (RVF), Q fever, leptospirosis, listeriosis, campylobacteriosis, chlamydia infection and blue tongue for small ruminants.

3.2 **Predisposing Factors**
For Brucellosis, animals are infected for life. In general, the potential predisposing factors include:

3.2.1 Breeding system, herd size, management practices, insemination methods, source of replacement stock and other management practices like exchange of males for breeding which foster spread of infection.

3.2.2 In the GHoA region, uncontrolled breeding, communal grazing and watering areas, in the pastoral production systems are major predisposing factors.

3.2.3 Livestock mobility also favors contact between infected and susceptible herds.

3.2.4 Presence of naive populations is a major predisposing factor.

3.3. **The Objectives of Surveillance**
a. Improve knowledge of the epidemiology of brucellosis;
b. Determine the occurrence and the distribution of the disease or infection;
c. Monitor the disease trends and control interventions;
d. Detect as early as possible the Brucellosis emergence;
e. Provide data for use in risk analysis, risk mapping, trade and for targeted interventions;
f. As a support tool for test and slaughter policy of infected animals.
Note that since Brucellosis is a zoonotic disease, under a “one health” paradigm simultaneous surveillance of Brucellosis in animals and humans is recommended, because human Brucellosis cases can be considered as a sentinel for ongoing transmission in animals. This requires close collaboration between veterinarians and human doctors, and an institutional arrangement between the livestock and public health sectors.

3.4 **Administrative Preparations for Surveillance**

a. A functional surveillance system requires improvement and maintenance of capacity for Brucellosis control including the following:

b. Sensitize, create awareness and train livestock value chain actors, including producers, both farmers and pastoralists, middle men, traders and transporters, slaughter house attendants on disease recognition and reporting (using convenient methods such as mobile phones, digital pen and paper, etc);

c. Train field veterinary personnel (public and private) on Brucellosis recognition and reporting, use of appropriate standardized case definitions, a well-organized reporting system, and a good animal health data management system, e.g. ARIS 2;

d. Equip veterinary services with necessary logistical materials and provide adequate technical staff to undertake investigation of reported Brucellosis cases. The veterinary services itself should be equipped, at appropriate administrative levels, with necessary sample collection equipment, disease reporting tools and materials, including standardized reporting formats, mobile phones, digital pens, etc;

e. Policy/legal frame work supportive of surveillance.

3.5. **Types of Surveillance and Epidemiological Approaches**

Surveillance may involve one or more of the following activities: passive surveillance and active surveillance encompassing syndromic, sero surveillance; participatory epidemiology; and outbreak investigation of suspicious cases.

Given the high zoonotic importance of Brucellosis pathogens and the fact that index cases may be detected in humans, it is highly recommended that the animal and human health sectors work in close collaboration in the disease outbreak investigations and control.

3.5.1 **Passive surveillance field actions**

1. The national veterinary authorities will engage and sensitize livestock value chain actors, including producers, traders and transporters, and abattoir workers to report any disease events encountered to the nearest animal health facility, either public or private. This will include educational and informative materials on disease recognition and reporting and use of methods such as mobile phones, digital pens,
1. Pen and paper, radio and television programmes, posters, information leaflets, community meetings, etc.).

2. Veterinary personnel undertaking routine animal health activities e.g. market stock route inspection, vaccination campaigns, extension services, abattoir activities, etc. are expected to carry out syndromic surveillance during which they will inspect livestock for signs of clinical disease and collect data from livestock keepers;

3. Communication materials including field syndromic manuals shall be developed and disseminated to make it easier for Brucellosis-related syndromes to be easily recognized by value-chain actors, including producers, traders, transporters and wildlife actors.

4. Stakeholders along the value chains (livestock producers, traders, transporters, butchers, abattoir workers, veterinary personnel and private animal health providers, and others) will be sensitized all along the livestock value chain, i.e to report any suspected or confirmed cases of Brucellosis.

5. In case of reports (written or rumors) of suspected Brucellosis from the community and other stakeholders, the responsible veterinary personnel will conduct further investigation with sample collection and submission to the laboratory. The responsible veterinary personnel will immediately report to the CVO and make a record in the standard reporting format.

6. Secondary data from diagnostic and Export Quarantine laboratories (public, private research and teaching institutions) may be analyzed to determine any pattern in Brucellosis distribution and possible hotspots or sources, aiming at a time span of at least 10 years and more if possible.

7. If a disease outbreak is confirmed, veterinary authorities shall institute appropriate control measures as indicated under section five.

8. The records will also be submitted to the Central Epidemiology Unit by the 15th of the following month in the standard monthly report.

3.5.2 Active surveillance

For cost-effectiveness, targeted active surveillance will complement passive surveillance using the following methods:

3.5.2.1 Milk surveillance

Milk surveillance is a cost effective herd-based surveillance targeting milk collection centres or milk processing plants with well-planned trace back mechanism to farms and individual animals. For this, undertake milk ELISA testing at the milk collection centre as well as at farm level including individual animals. Then enter test results and the herd identification into a database. It is recommended that all member states of the GHoA undertake this type of surveillance in order to determine the current status of the
disease at both national and regional level and also to minimize the surveillance cost.

3.5.2.2 **Syndromic investigation of abortions**
This involves systematic abortion screening of animals that either abort or have other reproductive problems associated with Brucellosis infection, for example, weak calves or infertility. In this regard, conduct testing on maternal blood samples, maternal tissues (e.g., placenta or uterine discharge), or aborted foetal tissues. For laboratory-based surveillance, ensure that sexually mature animals are targeted, since due to their reproductive problems, they have a higher probability of Brucella infection than any other population segment. It will also provide great value for epidemiological investigation due to the direct link back to the herd-of-origin. For the successful implementation of this surveillance, perform standardized laboratory diagnosis as an important component for an integrated national laboratory-based surveillance stream in addition to the awareness of veterinarians and livestock farmers on the need for samples. In addition, educate and create awareness among veterinary personnel and livestock owners on disease recognition and reporting.

3.5.2.3 **Sero-surveillance field actions**
For cost-effectiveness, targeted sero surveillance will be implemented to provide baseline data that is currently inadequate in the GHoA.

a. Design a survey protocol outlining sample size determination, sampling method, target population, sampling units and sampling frame, taking into consideration livestock and wildlife;

b. Prepare data collection tools, including questionnaires for epidemiological interviews, forms, and data collection software;

c. Mobilize survey teams composed of properly trained personnel;

d. Develop a survey programme together with the survey teams;

e. Share the programme with relevant stakeholders in targeted areas;

f. Ensure that all necessary technical and logistical equipment is at hand;

g. Collect blood samples using appropriate tools and techniques such as vacutainers, filter paper, microbleeders, syringes, etc.; Sample mature animals, specifically cattle and sheep over 12 months. Note that Brucellosis monitoring using sero-surveillance may not be reliable if previous vaccinations have taken place in the herd/region.

h. Conduct sero-monitoring in Brucellosis vaccinated areas to check for presence of antibodies in a target population for purposes of assessing the level of herd immunity. This should be done 15-45 days post vaccination. This assesses the vaccination coverage and not effectiveness of vaccination.

i. Ensure proper environment and time for serum separation, and proper storage of sera;
j. Ensure accurate labeling of samples, maintenance of test and identification records, the samples cold chain, and proper laboratory submission procedures;
k. Data will be entered in the Central Epidemiology Unit database for analysis and reporting;
l. If laboratory testing detects a positive sample, the responsible veterinary personnel should conduct an investigation;
m. If a disease outbreak is confirmed, veterinary authorities should institute appropriate control measures;
n. Ensure that activity reports are compiled as soon as the exercise is completed in the standard monthly report.

3.5.2.4 Participatory disease surveillance (PDS)
The purpose of PDS is to identify disease foci, establish and map the history of Brucellosis in an area using the syndromic case definition of Brucellosis. PDS is a good tool to establish the disease history for “the syndrome” or the disease in an area. It can be used to map the history of the disease in an area. There may be areas where disease occurred in the last month or half a year ago or more than a year ago. Obtaining this information would help in understanding the epidemiology of the disease. PDS is based on communication and transfer of indigenous knowledge for animal diseases, using a variety of procedures. To implement PDS, follow the actions below:
1. Relevant veterinary authorities identify target to susceptible areas and communities concerned;
2. Prepare relevant questionnaire protocols specifying key issues that help map the history of Brucellosis in susceptible area;
3. Draw up a PDS programme and share it with the target communities;
4. Identify key contact people and if possible translators to be used;
5. Implement informal interviewing;
6. Undertake ranking and scoring in a participatory manner with the local communities;
7. Undertake visualization of data to achieve a common understanding with the communities;
8. Undertake data cross-checking by probing, triangulation and laboratory diagnosis for confirmation;
9. Complement information so far collected with secondary information sources, direct observation and laboratory diagnosis;
10. Submit data collected to the Central Epidemiology Unit by the 15th of the following month;
11. Share reports generated thereof promptly with the relevant stakeholders.
3.5.2.5 Surveillance in wildlife
Wildlife surveillance should be an integral part of the surveillance system in order to improve understanding of wildlife in the epidemiology of Brucellosis and its possible role in epidemics among livestock. Regional ecosystem approach in the wildlife Brucellosis surveillance may be considered. The methodologies of surveillance involves:

a. Passive surveillance and reporting involving all stakeholders including scientists, universities, national park managers/responsible staff. Whenever possible, laboratory-based surveillance should be considered.

b. Retrospective surveillance and opportunistic serum collection;

c. Wildlife serum in cryobanks may be tested to provide a baseline of the prevalence and geographical distribution of Brucellosis in wildlife. Serum should continue to be collected opportunistically from wildlife and banked at -70 °C (with proper labels and georeferencing) for future analysis.

Since wildlife is not vaccinated, trends and patterns in wildlife can be very useful in understanding the epidemiology of the disease.

3.5.3 Brucellosis outbreak/epidemiological investigation
Epidemiological investigation will be undertaken immediately after the first index case has been confirmed in a population. This is necessary for the determination of the extent of the disease outbreak and delineation of the outbreak area. Data collection forms for Brucellosis investigations should be pre-designed to be compatible with the database and analysis system being used.

The following activities must be carried out:

a. Mobilize an investigation team;

b. Use standardized Brucellosis outbreak investigation form;

c. Investigate factors related to livestock management and movement to determine source of outbreaks and spread of disease, and identify an appropriate intervention;

d. Collect blood, serum and tissue samples for laboratory analysis, using proper biosecurity in collection, storage, handling, and transport of samples;

e. All data collected should be managed in an appropriate database, analyzed, reported, and shared appropriately;

f. Following disease control interventions, surveillance is essential to confirm that the disease has been controlled;

g. Post outbreak surveillance may be required to confirm freedom from infection in the area, to detect infective agent activity in a vaccinated population, and to confirm effectiveness of a campaign.

h. It is strongly recommended to take-off the positive animals, which may require compensation payment to the livestock keeper.
4.0 Brucellosis Laboratory Detection Diagnosis and Vaccine

4.1 Minimum Pre-requisite for Laboratory Diagnosis
a. All countries in the GHoA should have a capacity to carry out basic diagnostic tests for Brucellosis.
b. Laboratory should have standard operating procedures for sample collection, handling, packaging, transportation and storage of the samples; Create a schedule for participation in proficiency testing programmes to improve laboratory standards and harmonization.
c. The basic assays which should be performed by Member States are: Rose Bengal Test (sensitive test), bacterial isolation and identification, Brucella milk ELISA, indirect ELISA.
d. Due to the significant zoonotic nature of Brucellosis, any agent detection procedures must be done only under proper biosafety and biosecurity conditions.

4.2 Laboratory Tests for National Disease Control Programmes and for Livestock Export
a. For national disease control programmes, the choice of what laboratory tests to use is at the discretion of the CVO and there is no requirement to use OIE approved protocols;
b. For livestock in export trade and any other animals moving internationally, all laboratories testing must use OIE approved tests or other tests as agreed to between exporting and importing nations.

4.3 Field Diagnosis, Collection, Storage and Analysis of Samples
4.3.1 Clinical diagnosis
Brucellosis will be suspected in sheep, goats, cattle and other susceptible animals if any one or more of the following clinical signs are encountered: abortion, retained placenta, still-births, orchitis, epididymitis, reproductive failure, rarely arthritis and hygroma.

4.3.2 Post mortem examination
It is important to note that appropriate biosecurity and biosafety measures must be observed, given that Brucellosis is a zoonotic disease.

4.3.3 Collection of samples in the field
Samples should be collected according to the expected laboratory assay to be performed but basically the following are required:
a. For antibody detection in suspect live animals, whole blood for serum collection and milk.
b. For antigen detection or isolation of the bacteria, samples must be kept chilled and transported under refrigeration or on ice to the laboratory. Suitable samples include aborted foetus stomach contents, spleen and lungs.

c. In live animals, whole blood in anticoagulant, placentomes, foetal membranes, vaginal swabs, uterine discharges, udder secretions, milk, semen and arthritis or hygroma fluids.

d. In dead animals, uterine discharges, aborted foetuses, udder secretions or selected tissues, medial and internal iliac, retropharyngeal, parotid and prescapular lymph nodes, spleen, mammary, uterus, testes and epididymis.

4.3.4 Storage of samples after arrival in the laboratory
All samples should be cooled immediately after they are collected, and transported to the laboratory in the most rapid way. On arrival, milk and tissue samples should be frozen (at least -20 °C) if they are for long storage. For sera, store at +4 °C, but for long storage at -20 °C.

4.3.5 Sample testing
All laboratory procedures described in this manual are as prescribed in the OIE Manual of Diagnostic.

4.3.5.1 Direct diagnostic assays – identification of the agents
a. Culture, isolation and identification
   i. Isolate and identify Brucella spp. using appropriate culture media and bacteriological techniques.
   ii. Use Polymerase chain reaction (PCR) recognition methods for identification.

4.3.5.2 Indirect diagnostic assays – antibody detection tests
There are currently no available assays that can differentiate vaccinated animals from infected animals.
   i. Perform Brucella milk ELISA tests at milk collection centre in order to trace back infected herds or animals to farm level.
   ii. Brucella-buffered antigen test (BBAT) - it includes buffered plate agglutination test and Rose Bengal test.
   iii. For rapid screening of samples (presumptive testing), use RBT, a test recommended due to its high sensitivity. In addition, use RBT under field conditions, especially in mobile laboratories.
   iv. For confirmation of RBT positives, perform the recommended tests: the Fluorescence Polarisation Assay (FPA) and CFT on serum samples for examining both control programmes and in export quarantines.
   v. Perform enzyme-linked immunosorbent assay (Indirect ELISA; Competitive ELISA), a test prescribed for international trade and for sero-surveillance.
   vi. Perform the polymerase chain reaction (PCR) methods in regional support laboratories to provide additional means of detection.
5.0 Brucellosis Control

5.1 Considerations for Brucellosis control in the GHoA

a. Control of Brucellosis is a long-term programme with many potential variations depending on the circumstances of husbandry and socio-cultural practices. An adequate understanding of the epidemiology of Brucellosis is critical to its successful control. This disease is not homogeneous in a given country or region because it occurs in different epidemiological situations and farming methods, therefore requires adapted control measures, which may differ between and within countries.

b. Most of the countries of the GHoA do not have a Brucellosis control programme. In addition, there is no policy for slaughter of infected animals or compensations including the management of the disease. Strong political support over an extended period is necessary to prevent underfunding and disruption of any control programme.

c. Livestock farming mainly cattle, camels, sheep and goats in the GHoA present an enormous variety of breeding systems and environmental conditions. This diversity affects the distribution and the evolution of the disease in the region. As a consequence, the primary steps of a control programme are: to acquire knowledge of the situation and to define the epidemiological units of intervention. The primary unit of concern or epidemiological unit for Brucellosis is the herd, the flock or the group/holding including all epidemiologically-related susceptible animals.

d. There are a variety of options for control of Brucellosis and all should be considered so that the control programme can be tailored to the circumstances. There is no one-size-fits-all approach for this disease. To choose the best option of control, detailed information is needed on the distribution and the number of new cases in a country. However, if the disease is present (irrespective of its prevalence in animals), it is recommended to start annual mass vaccination campaigns of animals (covering over 80% of animals every year) for 5–10 years, before moving on to vaccinating young replacement stock along with testing and slaughtering of adults. The test-and-slaughter strategy can be advised only if public funds are available to compensate farmers for culled stock and if other enabling conditions are in place. Both interventions require well-functioning veterinary field and laboratory capacity. The epidemiological situation and the capacity of veterinary services should drive the choice of measures for Brucellosis control and results should be evaluated continuously.

In any case, for control programmes to be effective, the following characteristics of Brucellosis must be taken into consideration:

i. Disease spreads easily, particularly at the time of calving, between and within herds and flocks;
ii. Clinical signs are not pathognomonic and may be inapparent;
iii. Infected females do not always abort;
iv. Latent carriers usually occur;
v. Involvement of wildlife species;
vi. Transmission occurs both horizontally and vertically; through direct or indirect contact;
vii. No diagnostic tool correctly identifies all infected or non-infected animals;
viii. Available vaccines dramatically reduce the spread of Brucellosis but do not fully protect against infection.

The common control measures of Brucellosis include the following:
i. Vaccination (targeted vaccination, mass vaccination, and young replacement vaccination)
ii. Test and slaughter/stamping out in infected herds
iii. Frequent herd testing and removal of infected animals (depopulation)
iv. Animal identification and animal movement management
v. Appraisal of compensation scheme

5.2. Quarantine and Movement Control
Depending on the disease control options chosen, quarantine of affected animals and their source flocks and/or wider area, and/or stop-movement orders may be considered in the programme design. If the area of concern involves mobile pastoralism, movement controls of any sort may not be feasible. In this case, movement of animals from endemic areas to others should be restricted.

Identification of animals is a key tool for Brucellosis control. Animals dealt with in vaccination campaigns and/or other disease control intervention should be marked (branding, tagging, tattooing, RFID) so that their status is known.

5.3. Vaccination
5.3.1 Considerations in vaccination
a. Vaccination is often the first step in the control of Brucellosis. Effective vaccination requires coverage of over 80% of the eligible animal population, and vaccinations carried out for a period greater than twice the average production life (over 10 years in sheep and goats).
b. Under conditions of high to moderate prevalence, inadequate movement control or limited diagnostic capabilities, targeted mass vaccination of all animals (including adults) is the optimal tool for reducing the level of infection. When used exhaustively in the whole flock, the incidence greatly decreases. It is, however, critical to monitor
the effectiveness of vaccination.

c. Once the herd prevalence has been reduced, more effective control of the disease may be achieved through the implementation of a programme based on vaccination of young replacement animals combined with test-and-slaughter of adults. These programmes should be planned in the light of the area status, reflecting the risk of infection, instead of the holding status.

d. It should be stressed that having a population well immunized against Brucellosis makes the implementation of other sanitary measures more effective. In this way, the cost effectiveness of the eradication programme can be greatly increased.

e. In the context of the region, it may be possible to combine vaccination campaigns for Brucellosis and PPR or other disease. If a PPR or CBPP campaign is being planned as a primary activity, including Brucellosis should be a major consideration. Significant cost reduction could be attained with a combined campaign.

5.3.2 Different vaccination scenarios in endemic areas of the GHoA, that may be considered:

a. In a high-prevalence, endemic disease situation in small ruminants (over 5% herd prevalence, depending on the epidemiological situation, where a test-and-slaughter programme cannot be properly implemented and/or progress is not observed), mass vaccination is recommended as an emergency measure, which should be carried out in as short a time as possible. This should be reinforced with parallel measures such as movement restrictions and control of common grazing and later be followed by vaccination of replacement animals. The transition from mass vaccination towards young replacement vaccination should be planned in advance.

b. In a high-prevalence situation in cattle, a vaccination programme of replacement females combined with test-and-slaughter (or not) could be applied. The chosen vaccine strain should avoid interference with conventional serological diagnosis (RBT and/or CFT). Cattle can be re-vaccinated twice if required. Mass vaccination should be followed by vaccination of replacements, with the transition being planned in advance. Additional measures such as depopulation, movement restrictions and control of common grazing should also be considered.

c. In case of moderate to low herd/flock prevalence (for example, less than 5%, depending on the epidemiological situation) and the presence of important risk factors (movements, outbreaks in adjoining areas), a programme combining vaccination of young replacement animals (cattle, sheep, and goats) with test and slaughter in adults is recommended. Where risk factors cannot be controlled (for example, under conditions of transhumance), vaccination is recommended even when the prevalence is lower. The subsequent test and slaughter policy has to be based on the chosen vaccination strategy, taking into account the persistence of
residual antibodies due to the vaccine. As a general rule, animals should be tested six to twelve months after vaccination, depending on the route of administration of the vaccine. For sheep and goats, the use of the conjunctival route minimizes interference with serological testing. Tests interpreted in parallel to increase sensitivity will hasten eradication, but will increase costs due to the use of multiple tests and an associated reduction in specificity.

6.0 Disease Reporting and Information Management

Brucellosis is an important production-linked disease of both economic and zoonotic impact; and any positive cases should be reported to the Chief Veterinary Officer and through the CVO to human health authorities and other relevant stakeholders. All countries within the region should have a follow-up plan for managing positive cases in animal flocks/herds.

A Brucellosis information management database should be initiated in all countries of the region as well as a regional Brucellosis database using the continental ARIS-2 including other existing systems. This database should be carefully maintained so that if-and-when a programme for control/eradication is considered, all historical information is immediately available. The Brucellosis information could be shared/exchanged with human health authorities so that spatial visualizations of both human and animal Brucellosis can be undertaken. Brucellosis is a disease that firmly fits into the context of the “One Health” approach.

7.0 Brucellosis and Trade

There is a need for exporting and importing nations to have prior agreements on how to interpret and handle positive tests, both for the individual animal and the cohort group, for all of the diseases tested.

Regional Economic Communities (COMESA, EAC and IGAD) need to agree on standards to which the exporting and importing nations subscribe and to which the quarantine stations and veterinary authorities on both sides of the trading equation agree and adhere. Disease control and import decision making must be uniform and science based.

At export and import quarantine stations, a standardized regimen of response to presumptive screening tests and a standardized regimen of responses to subsequent supplemental confirmatory tests are suggested for Brucellosis.
Laboratory tests for national disease control programmes and for livestock export:

- For national disease control programmes, the choice of what laboratory tests to use is at the discretion of programme managers and there is no requirement to use OIE approved protocols.
- For livestock in export trade and any other animals moving internationally, all laboratory testing must use OIE approved tests or other tests as agreed to between exporting and importing nations.

8.0 Risk Analysis and Risk Mapping

Risk assessment, risk mapping, risk management, risk communication:

- A risk assessment should be carried out in cooperation with the surveillance and epidemiological investigation programme to determine extent and effects of the disease outbreak, and assist with designing the risk management and disease control strategies/programme.
- Spatial epidemiology and risk mapping are valuable tools and the database in Section 6 above is an important matter of consideration, and should be done in cooperation with human health authorities.