Guidelines for coordinated human and animal brucellosis surveillance

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FOREWORD

In any disease control or eradication programme, decisions have to be made on the basis of information. If this information is faulty or biased, there is a greater likelihood that incorrect decisions will be made. The key to any programme, therefore, is to develop an efficient surveillance system, from which summarized data provides the best information available on progress or lack thereof towards stated goals. These guidelines have been prepared with that aim. They have been developed from reviewing both programmes in countries that have successfully controlled and eradicated Brucellosis as well as those countries or regions where the disease is still not under control. A somewhat ‘generic’ approach has been taken and it is emphasized that what has been successful in one country may not necessarily be successful in another, unless it is modified to suit local conditions and animal management systems.
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CONTENTS

Foreword iii
Acknowledgements iv
Contents v

INTRODUCTION 1

REVIEW OF THE EPIDEMIOLOGY OF BRUCELLOSIS 3

CRITICAL ISSUES IN DESIGNING A BRUCELLOSIS SURVEILLANCE SYSTEM 5

1. Identify indicators of human and animal health events 5
2. Establish clearly defined objectives 6
3. Develop specific case definitions 6
4. Identify existing data sources, or develop new data collection systems, including a flow chart 6
5. Pilot test the methods in the field 7
6. Define role of the laboratory in brucellosis surveillance 7
7. Control validity of the system 7
8. Analyse and interpret surveillance data 7
9. Develop dissemination methods 8
10. Evaluate brucellosis surveillance systems 8

SURVEILLANCE OF HUMAN BRUCELLOSIS 11

Case definition for human brucellosis 11
Laboratory criteria 11
Case classification 11
Sources and types of human surveillance 12
Data analyses and presentation of reports 12
Surveillance indicators 12

SURVEILLANCE OF ANIMAL BRUCELLOSIS 15

General features 15

1. The major species of Brucella infecting animals and humans in the country 15
2. Estimates of current or baseline levels of infection in the primary animal reservoirs 15
3. Definitions 17
4. Type of livestock production, marketing and slaughter systems 18
5. Availability of information on livestock numbers and identification in herds, flocks and villages 18
6. Stage of brucellosis control programme 19
7. Laboratory support and testing strategies 19
8. Data recording systems for surveillance 20
9. Brucellosis surveillance indicators 22
10. Epidemiological analyses of surveillance data 22
11. Political and legal factors in surveillance 23
12. Financial and administration factors in surveillance 24
13. Culture, motivation and education factors in surveillance 24
SURVEILLANCE OF BOVINE BRUCELLOSIS

1. High or unknown prevalence phase with no control programmes
2. Mass vaccination phase
3. Test and removal, segregation or slaughter phase
4. Freedom phase: herds, regions and countries

SURVEILLANCE OF PORCINE BRUCELLOSIS

1. Identification of the agent
2. Serological tests
3. Requirements for Vaccines and Diagnostic Biologicals
   1. High or unknown prevalence phase with no control programmes
   2. Mass vaccination phase
   3. Test and removal, segregation or slaughter phase
   4. Freedom phase

SURVEILLANCE OF OVINE AND CAPRINE BRUCELLOSIS (EXCLUDING Brucella ovis INFECTION)

1. Identification of the agent
2. Serological and allergic skin tests
3. Requirements for vaccines and diagnostic biologicals
   1. High or unknown prevalence phase with no control programmes
   2. Mass vaccination phase
   3. Test and removal, segregation or slaughter phase
   4. Freedom phase
   4. Maintenance of officially free status
   4. Suspension and recovery of officially free status

SURVEILLANCE IN CAMELS, WILDLIFE AND OTHER SPECIES

INTERSECTORAL COLLABORATION AND COOPERATION IN BRUCELLOSIS SURVEILLANCE

SUMMARY

INFORMATION RESOURCES FOR BRUCELLOSIS

Internet resources
Printed resources
Chapter 1
Introduction

Although brucellosis has been, or is close to being, eradicated from a number of developed countries, it continues to be a major public and animal health problem in many regions of the world, particularly where livestock are a major source of food and income. There are many reasons why brucellosis remains endemic. These include expansion of livestock herds and flocks, with associated uncontrolled movements; lack of veterinary support services and vaccines; and husbandry practices favouring the spread of infection. Human cases continue to occur following international travel, traditional use of raw milk products and following close contact with infected animals.

The approach to control, prevention, or eradication of brucellosis in a country or region will depend on many factors, such as the level of infection in the herds or flocks, type of husbandry, economic resources, public health impacts, and potential international trade implications. Decision-making by those charged with policy making is likely to be intuitive unless accurate and current epidemiological information is available. The purpose of these general guidelines on surveillance in both human and animal populations is to provide a set of principles and techniques that can be used to develop and monitor new or existing brucellosis control programmes. The guidelines can also help in assessing the effectiveness of regulatory and advisory measures designed to safeguard public health.

In using these guidelines to develop new or to review existing surveillance systems, it is unlikely that adoption of all recommendations is possible, or even necessary. Rather, specific recommendations should be adapted to fit local or regional resources, needs, knowledge and practices, at least initially. As progress is made, changes can be incorporated to ensure that success is finally achieved. If progress is not made, then note should be taken of key epidemiological indicators, and corrections made on the basis of this information rather than for intuitive or political reasons.
Chapter 2

Review of the epidemiology of brucellosis

Brucellosis is a zoonotic disease occurring in humans and various species of domesticated and feral (wild) animals. The three species of *Brucella* of major concern here are:

- *Brucella abortus* (biovars 1–6), affecting primarily cattle, other bovidae, and cervidae;
- *Brucella suis* (biovars 1–5), affecting primarily swine; and
- *Brucella melitensis* (biovars 1–3), affecting primarily sheep and goats.

All the above *Brucella* spp. are not host-specific, and may transmit to other animal species under appropriate conditions.

Initial infection in the reservoir species is often followed by abortion and subsequent delayed or permanent infertility. Infection is usually chronic in animals, and treatment is rarely undertaken. Infected animals shed the organisms in uterine discharges following abortion and subsequent parturition, and also in the colostrum and milk.

Brucellosis is a herd or flock problem. It is spread within the herd primarily by ingestion of contaminated material. Venereal infections can also occur, but this is mainly seen with *B. suis* infections. Congenital (*in utero*) or perinatal infections may also occur, with the ensuing development of latent infections. Spread between herds usually occurs by the introduction of asymptomatic chronically-infected animals.

Human infections are characterized by a variable incubation period (from several days up to several months), and clinical signs and symptoms of continued, intermittent or irregular fever of variable duration, with headaches, weakness, profuse sweating, chills, depression and weight loss. Localized suppurative infections may also occur. The course of the disease can be variable, especially in persons either not or inadequately treated.

Diagnosis of clinical brucellosis in humans and animals is initially made by use of appropriate serological or other immunological tests, and confirmed by bacteriological isolation and identification of the agent.

Transmission of infection to humans occurs through breaks in the skin, following direct contact with tissues, blood, urine, vaginal discharges, aborted foetuses or placentas. Food-borne infection occurs following ingestion of raw milk and other dairy products, but rarely from eating raw meat from infected animals. Occupational airborne infection in laboratories and abattoirs has also been documented. Accidental inoculation of live vaccines (such as *B. abortus* Strain 19 and *B. melitensis* Rev.1) can also occur, resulting in human infections. There are also case reports of venereal and congenital infection in humans.

The disease occurs worldwide, except in those countries where bovine brucellosis (*B. abortus*) has been eradicated. This is usually defined as the absence of any reported cases for at least five years. These countries include Australia, Canada, Cyprus, Denmark, Finland, the Netherlands, New Zealand, Norway, Sweden and the United Kingdom (2002 OIE Reports). The Mediterranean countries of Europe, northern and eastern Africa, Near East countries, India, Central Asia, Mexico and Central and South America are especially affected. While *B. melitensis* has never been detected in some countries, there are no reliable reports that it has ever been eradicated from small ruminants.

The sources of infection for humans and the species of *Brucella* spp. found vary according to geographical region. It is usually either an occupational or a food-borne infection. Both sporadic cases and epidemics occur in humans, but often the disease or infection is either unrecognized, or, if diagnosed, not reported to the public health authorities.
Methods of prevention include health education to reduce occupational and food-borne risks, including pasteurization of all dairy products. However, education campaigns have never resulted in fully eliminating the risks of infection, and the ultimate prevention of human infection remains elimination of the infection among animals. This can be achieved by a combination of vaccination of all breeding animals to reduce the risks of abortion and raise herd immunity, followed by elimination of infected animals or herds by segregation and slaughter.
Chapter 3

Critical issues in designing a brucellosis surveillance system

Epidemiological surveillance is the ongoing and systematic collection, analysis and interpretation of health-related data. The process involves describing and monitoring health events in populations of humans or animals, or, in the case of a zoonosis such as brucellosis, both. For example, typical questions to which a surveillance system might be asked to provide answers include:

- How extensive is the infection, and when and where is it occurring?
- Which species of Brucella are involved?
- Which animal species are involved?
- Is the prevalence and incidence (human or animal) decreasing, increasing or static?
- When epidemics occur, what is the source, and how is the agent being transmitted?
- What strategies should be adopted to control, prevent and ultimately eradicate the infection?
- What are the knowledge, attitudes and practices of the populations affected?
- What laboratory or field research needs to be undertaken?

Because surveillance systems vary widely in methodology, scope and objectives, that which is important in one system may be less important in another. For example, it may be necessary to compromise the sensitivity (ability to detect infection) of surveillance in order to achieve other attributes, such as simplicity and timeliness. However, the surveillance tests should have adequate sensitivity and specificity and should be properly standardized. Thus, surveillance systems should be flexible, with the motto “adapt not adopt”.

Traditionally, a distinction is made between passive and active surveillance. Passive surveillance (or monitoring) is the routine, mandated reports that a Health or Veterinary Department receives on brucellosis, while active surveillance is where specific efforts are made to supplement the passive data by use of directed investigations, surveys or epidemiological studies. Passive surveillance is generally less costly than active surveillance, but its sensitivity and specificity are generally unknown. Active surveillance is more specific and sensitive, and the performance of the system should be measurable. These two systems of data collection are not interchangeable, but both are necessary and should be integrated wherever possible.

Brucellosis poses a number of challenges in designing an effective surveillance programme. The infection is chronic in both humans and animals, symptomatology and incubation periods are variable, and laboratory confirmations are essential. The human links to the animal reservoirs may be ill-defined. In areas where the disease is of greatest importance, animal populations may be poorly identified, not enumerated or even inaccessible for long periods.

There are ten basic steps in designing and operating a coordinated human and animal brucellosis surveillance system, and these are discussed below.

1. **Identify indicators of human and animal health events**

   Surveillance should always be outcome-oriented, and thus focused on events associated with the disease under surveillance. These include specific epidemiological indices such as the total number of cases, incidence and prevalence rates, and severity as measured by days hospitalized and economic impacts such as productive days of work lost for humans or reduced fertility in the case of animals. Indicators of surveillance may take several forms:
Critical issues in designing a brucellosis surveillance system

- **Numerical**, such as the number of known infected herds in an administrative unit;
- **Ratios**, such as the number of newly identified herds in a year compared to the same figure for the previous year; or
- **Rates** (percentages), where both a numerator and a denominator are available. For example, the herd incidence rate for a year would be the number of newly identified infected herds divided by the number of known (uninfected) herds at risk.

Ideally, rates are preferred, particularly if the population of herds at risk changes over time. **Incidence rates** (i.e. new cases) are more useful in general as they reflect better the dynamics of the disease or infection under surveillance, rather than do **prevalence** (i.e. all cases) rates.

Specific indicators of surveillance can be identified, such as:

- **Performance indicators**. These are key, quantifiable and objective measures that indicate whether surveillance is effective. In other words, measures of a country’s capacity to detect disease or infection.

- **Diagnostic indicators**. These are used to identify why one or more of the above performance indicators are below expectation, and suggest remedial actions.

- **Resource or workload indicators**. These are used to measure process events or productivity, such as number of doses of vaccine issued or number of hours worked. These may not necessarily relate to the health outcome and also may not be very accurate. For example, animals missed in a vaccination programme may well be missed from both the denominator and the numerator.

All indicators should be periodically evaluated to ensure they are still appropriate for their original intended purpose. There is a tendency in some surveillance programmes to collect too much data on the chance it might be useful, so always distinguish between “need to know” and “nice to know” information, where “need to know” information is that which is critical to make the system function as designed.

**2. Establish clearly defined objectives**

For brucellosis, objectives could include:

(i) Determination of the incidence and prevalence of infected humans, animals and herds or groups of animals, villages, states, regions, etc.

(ii) Detection of epidemics and sporadic or endemic cases.

(iii) Identification of vehicles and routes of transmission to humans, whether food-borne, airborne, through animal contact or between flocks or herds of animals.

(iv) Monitoring of short- and long-term trends by location and over time.

**3. Develop specific case definitions**

For human disease, a specific set of symptoms and signs, together with laboratory tests, are needed to describe possible, probable or confirmed cases. With animals, isolation of *Brucella* species is used, with or without serological evidence. Whatever system is chosen, it should be both comprehensive and mutually exclusive. In other words, it must be possible to place every herd or every animal into a category and only one category. For animals, this may be positive, negative or uncertain. There should be a time limit on how long an animal could remain in the uncertain category. For herds and regions, specific definitions are critical to measuring progress. Categorization is obviously needed for computer-based recording systems. Abortion, while often an end result of *Brucella* infection, is an unreliable case definition for infection, as it can be multi-causal, although it may be a very useful sentinel event in the later stages of an eradication programme, justifying laboratory investigation.

**4. Identify existing data sources, or develop new data collection systems, including a flow chart**

Always carefully review what systems are in use already to see if some, or all, can be adapted for *Brucella* surveillance. For example, if visits are being made to herds or livestock markets for routine vaccinations, it may be possible to draw blood samples for *Brucella* serology at the same time.
The following questions should always be answered in designing a surveillance programme:

(i) Will brucellosis be a notifiable (by law or regulation) infection for physicians, veterinarians, laboratories, etc? Or will it be voluntary?
(ii) Will the system be complete census based, sample (random or non-random) based, or sentinel based?
(iii) Identify both passive and active surveillance components.
(iv) Can the populations at risk be identified and enumerated, and how accessible are they?
(v) Will data collection be for a specific time, or open-ended?
(vi) What samples and data will be collected, by whom, and where?
(vii) Who will provide the data and how reliable is this source?
(viii) How will the information be transferred and stored?
(ix) How will laboratory results be linked to the human and animal databases?
(x) Who will carry out quality control checks?
(xi) Who will analyse the data, how, and how often?
(xii) What format should the summarized reports take, and with what frequency will they be disseminated?
(xiii) To whom will the reports be distributed?

A flow chart should be built up to include each step in the process. Consultation with an expert in computerized database development is strongly recommended at this stage.

5. Pilot test the methods in the field

There will always be unforeseen problems, especially in any new system, so a pilot test is always required. For example, pre-test questionnaires, forms and computer programs. Major errors can result in participants rapidly losing confidence. Veterinary data collection poses real challenges, especially where owners may be suspicious or uncooperative. Ingenuity and some incentives might well be needed.

6. Define role of the laboratory in brucellosis surveillance

The directors of medical and veterinary laboratories should always be involved in the planning stages, as their workloads will be increased. Identify current and future resources for both regional and central laboratories, including training, equipment, reagents and supplies. All tests should be documented by Standard Operating Procedures, and include quality control programmes. Many countries have now automated some laboratory tests, with computerized output. If field tests are to be used, adequate training should be provided, documented, and participants tested, together with regular proficiency testing, including the use of check samples.

7. Control validity of the system

Whether paper- or computer-based, errors can always occur. The person with primary responsibility for the surveillance data base should, in conjunction with the epidemiologists, develop a routine of checking for errors, say in 10% of case reports, including missing data, so that major errors can be avoided. Check digits can be incorporated in the records or crucial data can be entered twice in a row, thus confirming its value.

8. Analyse and interpret surveillance data

Exploratory data analysis involves using techniques to make the overall dataset more understandable. This may include using visual displays to summarize the main features of the data, simplify their distribution, and clarify the analyses to be undertaken, including evaluating the influence of outliers on the analysis. A wide range of computerized graphics and mapping techniques are now available to produce useful summaries of datasets.
The real art of conducting surveillance lies in interpreting what the data appear to show in relation to the known epidemiological features of brucellosis. By proceeding from the simple to the more complex, including comparisons with historical data, surveillance provides the basis for appropriate actions. A key issue, however, is to know the inherent limitations of the data and being clear in describing them. Be prepared to question constantly. For example, if there have been new cases in an area previously considered Brucella free, has the case definition changed? Why is one district reporting many infected herds while an adjoining district reports none?

While epidemiological studies to identify risk factors are usually designed to collect data separately, it is possible to carry out simple-case control studies using, say, high incidence versus low or zero incidence areas to identify potential herd or flock risk factors that could be followed up by more intensive studies. For example, are large herds at greater risk of being infected than small herds? At this stage, statistical assistance would be advisable.

9. Develop dissemination methods

Obviously, new surveillance information, conclusions and recommendations soon become redundant unless distributed promptly to those with a need to know. Also, unless the providers of the data are kept informed, they may well lose enthusiasm. Therefore a regular reporting system should be developed, whether it be a simple newsletter, posted, faxed or sent electronically to the district level, or a more complex set of analyses for decision-makers. Media such as newspapers, radio, television and websites can be used for public information, and especially for livestock producers.

10. Evaluate brucellosis surveillance systems

Ideally, an evaluation of a surveillance system should be undertaken at regular intervals by an independent individual or group, preferably with experience of brucellosis epidemiology. Those charged with responsibility for the system should be asked to document the following components:

(i) Describe the health events under surveillance in terms of number of cases, incidence and prevalence. Change over time and by area should be available. Performance, diagnostic and resource indicators relative to the objectives should also be developed.

(ii) Describe the system to be evaluated, including the objectives, and case definitions of health events under surveillance. A flow chart of the system should be available. Each component of the flow chart should be described in detail, together with an overview of how the system operates, as outlined in steps 4 to 9, above.

(iii) Indicate the usefulness of the system by describing actions taken by decision-makers and others as a result of information generated from the surveillance data.

(iv) Evaluate the overall system for each of the following attributes:
   - simplicity,
   - flexibility,
   - acceptability,
   - sensitivity,
   - predictive value,
   - positive results,
   - representativeness, and
   - timeliness.

(v) Describe the resources used to operate the system, and, if possible, estimate the direct costs.

(vi) List conclusions and recommendations. State whether the system is meeting its objectives, and assess the need to continue or modify the surveillance system, or both.
In summary, surveillance programmes must fulfil at least three major requirements to be considered effective.

- **Sensitivity.** They must be able to detect a high percentage of field events compatible with the symptomatology and epidemiology of brucellosis. Low sensitivity is a clear reflection of under-reporting.

- **Specificity.** After investigation, an effective surveillance system must be able to provide a definitive diagnosis for a high percentage of brucellosis-compatible field events.

- **Timeliness.** Current information must be provided in a timely fashion to enable prompt field responses to situations identified.

The needs must be met of both those conducting surveillance and those utilizing surveillance data in real-world settings. However, there is no perfect surveillance system and trade-offs must be made between sensitivity and simplicity. Each system is unique, and requires a balancing of the efforts and resources put into the system. These general guidelines are intended to make any system more objective, explicit, uniform and simple. Accurate and timely surveillance does not necessarily ensure that the right decisions are made, but it should reduce the chances of wrong decisions. Remember, if the information remains unused, organizing a surveillance system is a waste of resources: time, staff and money.
Human brucellosis can be a very debilitating disease, although the case fatality rate is generally low; it often becomes sub-clinical or chronic, especially if not recognized early and treated promptly. All ages are susceptible, and even congenital cases have been recorded. Few studies have attempted to measure infection in the general population, but a recent study in southern Saudi Arabia showed about 20% of the population had serological evidence of exposure. High-risk groups include those exposed through occupation in contexts where animal infection occurs, such as slaughterhouse workers, hunters, farmers and veterinarians.

Small common-source epidemics occur as a result of the ingestion of unpasteurized dairy products, especially soft fresh cheeses of goat or sheep origin. In temperate climates, human cases of occupational origin are more likely to be seen in the spring and summer months, corresponding with abortion, parturition and post-partum care of animals, especially small ruminants. Aerosol infections can occur, especially in abattoirs. Laboratory workers involved in diagnosis and vaccine production are also a high-risk group. Where the infection has been controlled and eventually eradicated in the livestock, there has been a very significant reduction in human cases. Therefore, in these countries, a recent history of overseas travel may be relevant.

The primary objectives of human surveillance should be to identify new human infections. This is usually reported as cases per 100 000 population. Another objective is to determine whether the infections are primarily of food-borne or occupational origin. If food-borne, are they from home produced or commercial foods? If the latter, should this be publicised and a recall made? The routine surveillance of high-risk foods is likely to be both expensive and not really provide the security that can be provided through Hazard Analysis Critical Control Point (HACCP) programmes, such as mandatory monitoring of heat treatments. A secondary objective is that human infections may lead to the identification of previously unrecognized infections in animals.

**Case definition for human brucellosis**

The recommended WHO Case Definition is:

- **Clinical**: An illness characterized by acute or insidious onset, continued, intermittent or irregular fever of variable duration, profuse sweating, particularly at night, fatigue, anorexia, weight loss, headache, arthralgia, and generalized aching. Local infection of organs may occur.

**Laboratory criteria**

- Isolation of *Brucella* spp. from clinical specimens (note that repeated attempts may be necessary); or
- *Brucella* agglutination titre, e.g. standard tube agglutination tests: SAT\(\geq\)160 in one or more specimens obtained after onset of symptoms; or
- ELISA (IgA, IgG, IgM), 2-Mercaptoethanol test, Complement fixation test, Combs, fluorescent antibody test.

In small laboratories or clinics, a Rose Bengal screening test may be used. Positive results should always be confirmed by the tests listed above.

**Case classification**

- **Suspected**: A case that is compatible with the clinical description and is epidemiologically linked to suspected or confirmed animal cases or contaminated foods of animal origin.
Surveillance of human brucellosis

- **Probable.** A suspected case that has symptoms compatible with disease and is positive in the Rose Bengal test, but negative in blood culture and showing low titres in the confirmatory tests.
- **Confirmed.** A suspected or probable case that is laboratory confirmed.

The above case definitions may require modification depending on the availability of medical services and laboratory resources.

**Sources and types of human surveillance**

Mandatory and immediate case-based reporting by all health-care providers should be required. In some countries, the provision of free treatments may provide an additional information source.

Mandatory reporting from laboratories of positive results, independent of physician reporting, can also be included and will usually increase the sensitivity of surveillance. Routine surveillance of high-risk occupational groups is also recommended, which may include collection of baseline samples for use in the case of future exposures.

Each human case should be investigated for surveillance purposes, and include demographic information as well as food history, animal contacts, type of work or activity at onset, and recent travel history. In addition, a joint investigation with veterinary colleagues is highly recommended (See section on Intersectoral Collaboration).

**Data analyses and presentation of reports**

These would typically include:
- **graphs** of number of susceptible, probable and confirmed cases, by month;
- **tables** of number of susceptible, probable and confirmed cases by age, sex, month and place; and
- **maps** showing distribution and number of suspected, probable and confirmed cases by place (e.g. county, province or state).

**Surveillance indicators**

**Performance-based indicators**
- number of new cases (confirmed) per 100 000 population compared with previous years or other equal time frames.

**Diagnostic-based indicators** include:
- proportional comparisons: suspected, probable and confirmed;
- number of epidemiological investigations in relation to number of confirmed cases;
- comparison of sources of reports, such as physicians, hospitals, laboratories and other; and
- comparison of probable sources, such as food-borne, animal contact and other.

**Resource-based indicators** include:
- number of bacteriological tests relative to number of serological tests; and
- number of culture-positive cases in relation to number of cultures attempted.

At the *peripheral level*, all possible sources should be included to improve the sensitivity of the system, including private physicians, other health workers, public clinics and hospitals, especially from patients presenting with “fevers of unknown origin”. Some countries have designated specialist physicians to review all suspected cases to improve specificity of surveillance data. Ideally a “zero reporting system” should be used and reports submitted at least weekly to the intermediate level.

At the *intermediate level*, considered here to be equivalent to district, county, province or other administrative unit, case reporting would be to both the central level and the equivalent veterinary office level. (See section on Intersectoral collaboration) All reports should be validated at this level, and epidemiological case reports completed and, if necessary, field investigations undertaken of suspected outbreaks. Data from all reporting areas should be compared and regular feedback provided to the peripheral level.
At the central level, the national epidemiological surveillance unit should:
➢ tabulate, check and entry all reports from districts and regions,
➢ develop at least quarterly reports,
➢ take action on outbreaks,
➢ produce educational material where necessary, and
➢ liaise with the Ministry of Agriculture and with other national entities such as Inter-Ministry Zoonoses Committees or Brucellosis Advisory Committees.

Periodically, the surveillance programme at the central level should be independently evaluated to determine performance efficiency.
Chapter 5
Surveillance of animal brucellosis

GENERAL FEATURES

The design of an effective surveillance system for animal brucellosis in a region or country depends on many factors, as discussed below.

1. The major species of Brucella infecting animals and humans in the country

In this document, we are primarily concerned with B. abortus, B. melitensis and B. suis infections. In some countries, all three species may be present, while other countries may have only a single species. Alternatively, it may not be known with certainty which Brucella species are present, unless bacteriological investigations have been undertaken.

2. Estimates of current or baseline levels of infection in the primary animal reservoirs

Traditionally, these estimates have been based on data passively acquired from the results of bacteriological and serological data from:

- abortion submissions to diagnostic laboratories,
- routine testing of on-farm samples, such as milk or blood,
- notifications from veterinarians if brucellosis is reportable to the authorities, and
- off-farm sampling from markets or slaughterhouses.

All of these results may be biased. For example, diagnostic samples may be representative of herds close to a laboratory or from larger herds where the owners or veterinarians are motivated to submit samples. Slaughterhouse and market samples are probably not truly representative. Most animals will be free from clinical disease, will be older animals, and also decisions to sell animals depends on many factors, often unrelated to disease considerations.

Therefore active surveillance should be undertaken to provide a more reliable estimate of Brucella infection in a region or country. There are three basic approaches to this task:

- Undertake a total (census) testing. This is usually impracticable because of cost.
- Carry out random (probability-based) sampling, where both groups and individual animals have an equal chance of being sampled.
- Carry out non-random (purposive) sampling of suspected high-risk groups of animals. Again, these are likely to be biased if “convenience” determines origin of samples, such as from bleeding herds, or only at vaccination sites, or close to veterinary clinics, or from cooperative owners.

Ideally, a random sampling programme should be undertaken to provide statistically reliable estimates of the prevalence of infection. This presupposes that a reliable and current sampling frame is available of villages or herds and flocks. If this is not available, it may be necessary to use alternate methods, such as random sampling based on geographical location.

The actual techniques used might be:

(i) Simple Random Sampling, using tables or computer-generated numbers. This requires that the animals or herds be identified.

(ii) Systematic Random Sampling, whereby every n^th animal, herd or village is selected for inclusion in the sample. Two advantages to this method are that the population size need not be known with certainty, and nor do individual animals have to be identified.
Surveillance of animal brucellosis

(iii) **Stratified Random Sampling**, involving dividing the population into sections (strata), although the actual number of strata should be kept to a minimum. Suitable strata include:
- administrative areas (districts),
- village or herd sizes,
- production systems, or
- ecological conditions.

(iv) **Multistage Sampling**, involving sampling in two or more stages. For example, randomly selects herds, then randomly select animals within those herd. An example of this technique is developed below.

**Example:** Assume you wish to estimate the baseline prevalence of brucellosis in a province, governorate or similar administrative unit.

**Step 1:** Primary Sampling Unit = village, herd or flock.

A random sample generated either from a complete listing, or on a geographical basis using map coordinates if a list is not available.

**Sample size:** If there is no prior knowledge, assume that 50% of the villages or herds or flocks are infected. From the table below, identify the approximate sample size required to estimate prevalence in a very large (infinite) population with the desired fixed-width confidence limits.

<table>
<thead>
<tr>
<th>Expected Prevalence</th>
<th>90% Desired Accuracy</th>
<th>95% Desired Accuracy</th>
<th>99% Desired Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9% 10%</td>
<td>5% 10%</td>
<td>1% 5% 10%</td>
</tr>
<tr>
<td>10%</td>
<td>24 25 138</td>
<td>35 36 173</td>
<td>60 60 239</td>
</tr>
<tr>
<td>20%</td>
<td>43 43 246</td>
<td>61 61 323</td>
<td>106 106 425</td>
</tr>
<tr>
<td>30%</td>
<td>57 57 323</td>
<td>81 81 432</td>
<td>139 139 557</td>
</tr>
<tr>
<td>40%</td>
<td>65 65 323</td>
<td>92 92 432</td>
<td>159 159 637</td>
</tr>
<tr>
<td>50%</td>
<td>68 68 323</td>
<td>96 96 432</td>
<td>166 166 663</td>
</tr>
<tr>
<td>60%</td>
<td>65 65 323</td>
<td>92 92 432</td>
<td>159 159 637</td>
</tr>
<tr>
<td>70%</td>
<td>57 57 323</td>
<td>81 81 432</td>
<td>139 139 557</td>
</tr>
<tr>
<td>80%</td>
<td>43 43 246</td>
<td>61 61 323</td>
<td>106 106 425</td>
</tr>
<tr>
<td>90%</td>
<td>24 24 138</td>
<td>35 35 173</td>
<td>60 60 239</td>
</tr>
</tbody>
</table>

From the above table, if the expected collective prevalence is 50%, then a sample of 96 villages or herds or flocks would be needed for 95% confidence at ±10% desired accuracy. For great accuracy, say ±1%, then the sample size increases considerably, to 9 604.

When sampling from a finite population of size $N$, an adjustment can be made to account for this using the formula:

$$1/n = 1/nX + 1/N$$

where $nX$ is the sample size calculated above.

Using the above example, assume the population of villages or herds ($N$) was 1 150

Then $1/n = 1/96 + 1/1150$, so $n = 89$.

Thus testing of 89 villages or herds would be sufficient.

**Step 2:** Secondary Sampling Units = individual animals

Obviously in the case of Brucellosis these would be sexually mature male and female animals, as we are attempting to detecting the number of infected villages or herds, i.e. those with at least one infected animal.
From the following table, assume that the expected individual prevalence within the village or herds or flocks is 15%, and the desired confidence level is 95%. Sample sizes for varying population sizes are as follows*:

<table>
<thead>
<tr>
<th>Eligible animals</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td>500</td>
<td>19</td>
</tr>
<tr>
<td>1000</td>
<td>19</td>
</tr>
</tbody>
</table>

* The reader is referred to standard epidemiological and statistical texts on different prevalence rates, desired confidence levels and sample size.

Additional villages or herds may be added to compensate for any refusals. All selections must be random, and the final sampling number will be calculated for each strata.

From this exercise it should be possible to estimate:
- the collective prevalence, i.e. number of infected villages or herds or flocks in the region, and
- the approximate levels of individual animal infection within villages or herds or flocks.

This information can then provide the basic framework needed to establish a surveillance programme to support control or eradication.

3. Definitions

Any surveillance system for brucellosis must include a set of specific definitions that clearly describe the terms used so that there is little room for doubt. These could include any or all of the following:

(i) **Units of observation**

Traditionally the infection status of individual animals has been measured in brucellosis surveillance, but, as stressed earlier, rather than being an individual animal problem, brucellosis is a herd, flock, village or regional epidemiological problem. Therefore, group data are a more accurate measurement of progress or lack thereof. For example, if the individual animal prevalence is gradually decreasing, but the herd or flock prevalence is increasing, then obviously the control programme should be investigated to determine the reasons for this anomaly.

Usually a herd or flock has been defined on the basis of species, ownership and location, such as “All animals of the same species or multiple susceptible species under common ownership or supervision that are a group on one or more parts of any single premise (lot, farm, ranch, etc.)” or “a group of animals that is maintained separate from other animals by an approved fence or natural barrier.”

These definitions require modification if animals are on two or more premises that are geographically separated but with interchange of animals between them, or if the animals have contact with animals from different premises. In other situations, animals of the same species may commingle on community pastures, or as part of a village flock or herd – a situation probably best described using geographical coordinates, i.e. by mapping.

Where there is a village system or extensive nomadic or transhumant systems, it becomes more difficult to define the observational unit unless sampling is confined to a specific time of the year where location is reasonably certain. Mixing of small ruminants (sheep and goats) is very common in many countries that rely on extensive grazing. Intensively managed herds or flocks may be self-contained and essentially closed to all other livestock except via the introduction of semen or embryos.

(ii) **Test eligible animals**

While latent infections (up to 10% of animals born to infected dams) occur in brucellosis, the clinical disease is confined to sexually mature animals. Therefore, age ranges and sex status for surveillance purposes should be clearly defined, such as the following:
 Surveillance of animal brucellosis

- **Cattle**: Test eligible animals, including unvaccinated cattle of 6 months of age and older, and official calfhood *B. abortus* vaccinates above 18-20 months of age. Note that some countries exclude spayed and castrated animals from testing.

- **Sheep and goats**: Test eligible animals, including all unvaccinated animals over 6 months of age and *B. melitensis* Rev.1-vaccinated animals older than 18-20 months of age.

(iii) **Brucellosis-exposed animals**
For example, all cattle in a known infected herd or that have been in contact with known *Brucella* reactors in a market for at least 24 hours.

(iv) **Criteria for Brucella-free herds and regions**
Freedom may be defined on the basis of time and specific areas, as well as on the history of successful elimination of infected animals or herds or flocks.

(v) **Types of production system**
These would be dairy, beef, confinement, etc.

(vi) **Types of officially recognized identification systems**
This would include vaccinated and reactor animals

(vii) **Permits for movements**

(viii) **Quarantined areas**

(ix) **Types of tests their interpretation, and vaccines officially recognized**

4. **Type of livestock production, marketing and slaughter systems**

Great variation exists among livestock production systems worldwide, from the landless (total confinement) intensive systems of dairy cattle to the extensive husbandry of mixed species grazing with very low animal concentrations per unit area. Obviously, the type of system will affect the rate of spread of infection both within and between villages, herds and flocks. In the early stage of test and slaughter phases, on-farm village sampling is preferred, particularly as the owner can be actively involved in education.

Later, as the prevalence decreases, off-farm sampling at markets or abattoirs is generally more cost-effective, providing that ownership or identification is maintained.

In most livestock, parturition is seasonal, and knowledge of these patterns is important in determining probable times for the occurrence of abortions. Migratory herds or flocks may be more easily located during lambing or kidding periods.

The sale of animals for meat depends on many factors and is usually not random throughout the year. In some cases, long periods may occur with very few animals sold, so a slaughter-based surveillance programme could be inefficient. Markets, especially if terminal (i.e. animals destined for slaughter), are a very useful “public event” in the life of an animal, when it is accessible for sampling. If movement permits are required, this event can also be used for blood sampling.

Testing of bulk milk for brucellosis is a valuable screening test in cattle, and samples may be collected either on-farm where tanker collection is undertaken or at a milk plant where producers bring their milk for sale on a regular basis. Routine milk quality examination samples may also be used for brucellosis testing.

In summary, a careful study of all the livestock systems in relation to brucellosis epidemiology should be undertaken prior to commencing surveillance, facilitating the determination of the most convenient and economical sampling sites.

5. **Availability of information on livestock numbers and identification in herds, flocks and villages**

Most countries have an established system for collecting livestock data, varying from complete census at specified intervals to intermittent sampling programmes. Periodic census data may also be updated by estimates between censuses. As parturitions are often seasonal, the time of the year when sampling occurs should be specified.
Ideally, the number of flocks and herds and their distribution should be obtained. However, given the dynamics of livestock production, any statistical data soon becomes outdated. Also, if livestock data is related to any taxation system, there will probably be an under-reporting bias. Therefore it is recommended that those responsible for surveillance use complementary information sources, such as district veterinary lists, farmer or cooperative organizations memberships, and even aerial photography, to ensure that any sampling programme is as complete as possible. In some countries, all herds and flocks are registered with the Veterinary Department or another government agency. Where there is multiple ownership of herds or flocks, it may be necessary to keep two registers – one for the main owner who has more than one flock, and another for the direct owner who has only one flock. For grazing purposes, nomadic or transhumant flocks may be required to get permission, and this is given only to those owners that have properly vaccinated and registered their animals.

Many different systems of animal and herd identification are used for disease control and surveillance purposes. For brucellosis surveillance, the minimum should be a herd or flock identifier such as permanent ear or tail tags, ear notches, tattoos or branding. If individual animal identifiers are used, information on their vaccination status should also be included. In some countries, temporary identifiers are placed on animals prior to marketing or slaughter to enable trace-back to the herd of origin for positive test reactors. There is no perfect system of identification as losses occur by accident or intent – nevertheless, a reliable herd or flock identification system is integral to any surveillance system, especially where trace-back to the herd or flock of origin is to be attempted.

6. Stage of brucellosis control programme

In the control and eventual eradication of brucellosis, there are generally four overlapping phases:

(i) No or minimal efforts to control the infection. Sporadic testing of animals may have been done, but usually for diagnostic purposes following abortion. Some herds or flocks may have been vaccinated.

(ii) Intensive vaccination phase of herds and flocks, using either *B. abortus* (strain 19 or RB 51) or *B. melitensis* (strain Rev.1) to vaccinate either sexually immature or adult animals.

(iii) Test and removal, segregation or slaughter phase of infected animals, with the ultimate aim of developing *Brucella*-free herds, flocks or regions of a country. During this phase, vaccination is usually phased out towards the end of the eradication programme.

(iv) Freedom phase, where, once having eradicated the infection, intensive surveillance is maintained for at least five years to confirm that the agent is no longer present in the population.

The choice of sampling and types of herds or flocks to monitor will obviously depend on the phase of brucellosis control. For example, once the prevalence of infected herds has been reduced to a low level, it is usually uneconomic to continue testing all eligible animals and surveillance can focus on problem herds, abortion incidents, herds adjacent to known infected herds and off-farm testing, such as in markets and slaughterhouses.

7. Laboratory support and testing strategies

Two types of laboratory support are needed for Brucellosis control and surveillance: bacteriological testing, and serological testing.

*Bacteriological Tests*

Appropriate facilities are needed to isolate and identify all suspect *Brucella* spp. from abortion materials (foetal stomach contents and cotyledons), milk and vaginal discharges, as well as tissues from slaughtered reactor animals, such as supramammary lymph nodes. Ideally, isolates of *Brucella* spp. should be “fingerprinted” by biotyping. Periodically, isolates should be confirmed by submission to a WHO/FAO Collaborating Centre. As laboratory exposures to *Brucella* spp. have occurred frequently, any laboratory to be used for isolation of brucellae should have a primary biohazard containment facility, to minimize the risk of human infection.
Serological Tests

Many serological tests for brucellosis have been developed, or are under development. However, for serological surveillance to be successful, it is advisable to concentrate on a few tests only, and ensure that these are quality controlled and can be carried out with the facilities available. Given that no test is both 100% sensitive and specific, it is not advisable to use a “battery” test approach in the mistaken belief that if enough tests are done the results will become clear. Rather, what is needed is a simple and clearly defined testing strategy with defined endpoints, and a rigorous approach to borderline cases, which takes into account the epidemiological features of brucellosis. Note that only certain tests for surveillance purposes are recognized as official by the OIE Manual of Standard Diagnostic Tests and Vaccines (OIE, 2000). If sensitive and specific enough, any country may, however, declare other tests used for diagnosis, control and surveillance purposes in their programmes.

When individual animals are tested to ascertain if the herd is infected, the number of animals tested and the critical number of reactors used to decide the health status of the herd becomes very important in influencing the herd level sensitivity and specificity. If the test specificity is less than 100%, then as the number of animals tested increases the probability of at least one false-positive animal increases, and so the herd specificity decreases. The herd sensitivity, herd negative predictive value and herd apparent prevalence increase directly with the number of animals tested, but the herd positive predictive value decreases. Herd sensitivity can be increased by using a test that is less than 100% specific. These features should be borne in mind when interpreting the natural history of brucellosis, and particularly as it is recognized that larger herds are more likely to be infected than smaller herds.

Serological tests can be divided broadly into two groups:

- Screening tests used in the field clinics or in regional laboratories, such as the Rose Bengal or buffered plate agglutination. The Rose Bengal test has a very high sensitivity to ensure that infected animals are not missed. The milk ring test is also an excellent screening test for dairy cattle. Indirect ELISA tests are also being used to screen milk and serum.

- Confirmatory tests used in a central or regional laboratory, such as competitive ELISA, immunodiffusion or complement fixation tests, are very useful in distinguishing vaccinal antibody responses from those induced by field infections.

It is important to note that, during the course of a brucellosis programme, testing strategies will change. For example, when the prevalence of infection is high, a test of adequate sensitivity but high specificity is desirable to detect most of the truly diseased animals and herds, and to minimize the number of false-positive reactors. In contrast, as the prevalence decreases, a sufficiently specific but highly sensitive test is recommended. It is thus important to decide how to classify positive animals, such as single reactors in an otherwise negative herd. This may be the first sign of a herd breakdown, or it may be a false positive of no importance. In some countries, the problem of false-positive serological reactions due to cross-reactive bacteria (e.g. Yersinia enterocolitica 0:9) has complicated the eradication of brucellosis.

A number of commercial kit tests are also available and may be particularly useful for confirmatory purposes, but their costs often preclude their use in large surveillance programmes. Automation of some tests may also allow for economies of scale.

‘Banking’ of a representative sample of sera in a deep freeze is strongly recommended for retrospective investigations of problem herds.

8. Data recording systems for surveillance

The integrity and accuracy of any surveillance system is dependent on how data is recorded in the field and laboratory, how it is transcribed and summarized, and finally how it is interpreted. Computerized databases are now gradually replacing manual recording systems. A variety of paper records are required for primary data entry, including:

- **Herd, flock or village form**, containing information on:
  - district, province, region, day of visit, owner, address, latitude and longitude;
the species, number of animals, breed, production system (housing or grazing), and breeding policy;

- fertility and abortion rates, and other signs; and

- history of brucellosis, control measures and vaccinations.

A herd or locality identifier should be included.

(ii) **Individual animal sample** form, with information on sample number, sex, age, vaccination status, identification, laboratory number and laboratory test results.

(iii) **Reports from brucellosis sero analysis database**, so prevalence results can be analysed by geographical area, vaccination history, type of herd, housing or grazing.

(iv) **Serum bank search** form (if applicable).

Note: The above forms (i–iv) are available from the Animal Production and Health Section of the Joint FAO/IAEA Programme for Sero-Monitoring of Brucellosis, in the form of a software database.

(v) **Abortion outbreak/incident forms**

(vi) **Epidemiological investigation of reactor herds**, including origin of reactors, herd additions, animal removed from infected herds, and any area herds investigations.

(vii) **Records of off-farm testing** including market and slaughter animals and trace-back results to herds of origin.

Other forms may be developed depending on individual requirements. In terms of the amount of information to be recorded, a balance needs to be made between what is essential for epidemiological and surveillance analyses (“need to know”) and what could be termed “nice to know”, i.e. of minimal value for decision-makers. Regular validity checks should be carried out on a percentage of records (5%) to determine obvious errors and assess the extent of missing data.

It is important to distinguish between zero reporting and non-reporting of zero incidences. The former indicates that the reporting office is active, whereas the latter cannot be distinguished from failure to conduct surveillance or report.

Other computer software that could be used to develop a brucellosis database includes:

- **TAD info- FAO software Java Version.** Website: http://www.fao.org/ag/AGA/AGAH/EMPRES/tadinfo2/e_tadinf.htm

- **Epi Info (with or without Epi Map).** This is available in the USA from:
  - USD, Inc.,
  - 2075-A West Park Place,
  - Stone Mountain, GA 30087
  - Telephone (770) 469 4098
  - Fax (504) 469 0681
  - E-mail: usd@usd-inc.com
  - Website: http://www.usd-inc.com

It can also be downloaded from: www.cdc.gov/ > Publications and Products > Click on Epi Info and Epi Map. Instructions are provided for downloading. Free Technical Support for these Programmes can be obtained by e-mail at epinfo@cdc1.cdc.gov. Epi Info is now available in 12 non-English languages.

- **Active Surveillance for Livestock Diseases - Practical Techniques for Developing Countries.** A manual and associated software is available from Dr Angus Cameron, Australia <angus@pnc.com.au>,

- **Database for the Surveillance of Rinderpest and Sero-monitoring of Brucellosis, Joint FAO/IAEA Programme, obtainable from IAEA, Vienna, Austria.** Website: http://www.fao.org/ag/age/d3/index.html

Note that computerized mapping programmes are also now used to identify brucellosis-infected and brucellosis-free zones or areas in countries. These are especially valuable if there is a unique farm identifier.
9. Brucellosis surveillance indicators

Performance, Diagnostic and Resource (Workload) indicators, as described earlier, will vary with both the species (bovine, ovine, caprine or porcine) affected and also the phase of the brucellosis programme, i.e. high or unknown prevalence; mass vaccination; test and removal, segregation or slaughter; and freedom.

Performance indicators

Ideally these are time-delimited rates with a numerator (infected animals, herds, flocks or villages or other administrative unit) and a denominator (“at risk” in the above categories). As mentioned, incidence rates based on groups of animals are the most sensitive indicators of success or failure of a programme. Alternatively, the ratio of newly identified herds for the current year compared to the previous year can be used to obtain the percentage reduction (or increase), calculated as:

\[
\frac{\text{Current year} - \text{previous year}}{\text{Previous year}}
\]

Similar prevalence rates for all known infected herds can be calculated.

Diagnostic indicators

Examples include:

- Number of complete herd tests at monthly intervals needed to clear herds of infection.
- Mean quarantined period of a herd or flock as an effectiveness of control.
- Relative sources of sero-positive animals as leading to infected herds or flocks.
- Efficiency of trace-back procedures.
- Number of epidemiological investigation carried out.
- Number of culture-positive animals in relation to the number sero-positive (slaughter examinations).
- Number of serological tests in relation to their classification of infected herds. The more serological tests, the higher the possibility of a better quality diagnostic system.
- Number of animals (or herds) vaccinated as a fraction of eligible animals (or herds).

Resource indicators

These can reflect the manager used, the budget spent or the resources used. Examples include:

- Costs per animal for vaccination.
- Costs for serology, bacteriology, etc., on a per-test basis.
- Costs for epidemiological investigations.

10. Epidemiological analyses of surveillance data

Surveillance data can be used in several general ways for decision-making in brucellosis control and eradication programmes, including to

- investigate sources of infection for individual herds or flocks;
- use aggregated descriptive data to monitor trends over time and space to detect problems as they occur; and
- identify risk factors using herds or flocks in a case-control study format, and these are discussed below.

Investigation of sources of infection for individual herds or flocks

All newly identified infected (or re-infected) herds or flocks should be carefully investigated using a standard form to record information such as:

- Owner’s full address or location, and date first positive tests recorded.
- Reason for testing, such as on- or off-farm, diagnostic, movement; area test; or following trace backs from other infected herds, adjacent or contact herds on common grazing.
Humans known to be infected.

Clinical signs present, such as abortion, retained placenta, difficult breeding, or reduced milk production.

Percentage vaccinated and type (young or adults).

Type of operation and total census of animals.

Breeding programme and usual timing of parturitions.

Origin of herd or flock.

Recent movements in or out, or both, associated with an infected herd or flock.

Names of adjacent owners of livestock.

Contact with other susceptible species.

Quarantine (if applicable) requirements.

Probable source of infection and date introduced.

In addition to the origin of reactor animals, other recent purchase or loan animals and animals removed should be recorded as carefully as possible. Finally, all the herds in the immediate area should be visited to determine if commingling is likely to occur with the infected herd(s) and whether they should also be tested.

Biotyping of the *Brucella* species may be used to assist in identifying potential sources of infection. This includes differentiation of wild from vaccine strains, which occasionally may cause abortion.

From this information it should be possible to determine if the source for the infected herd was either endemic infection – i.e. a prior history of brucellosis – or a recent infection by introduction of infected animal(s) or by direct contact with infected animals.

**Use of aggregated descriptive data to monitor trends to detect problems as they occur**

Aggregated herd and flock data should be examined for the whole country or regions within the country to determine trends over time (monthly and annually), and also by place, such as districts, provinces or governorates. Such information can be displayed by graphically using maps, graphs or tables using the appropriate software.

It is also useful to compare the “success rate” of the various surveillance sources, particularly in relation to costs. For example, routine on-farm testing of all herds or flocks compared with off-farm testing at markets, slaughter or through bulk milk testing.

**Identification of risk factors, using herds or flocks in a case-control study format**

Case-control studies can be carried out, using as a case definition an infected flock or herd with more than 5% of animals infected, with a known *Brucella*-free flock or herd as a control. Cases and controls should not be matched, except perhaps by region. From these studies, odds or ratios (or approximate relative risks) can be calculated to determine the effect of herd size, type of management, and vaccination status and production system as potential risk factors. Thus, “high risk herds” can be targeted for additional investigations.

**11. Political and legal factors in surveillance**

The costs of brucellosis control or eradication programmes are high, and involve considerable government resources, as well as contributions from the livestock industries. Without strong political support, programmes may be under-funded, particularly in the final stages, when the number of infected animals or herds detected is very low in relation to the inputs needed.

Legal factors are important in their effect on the enactment and enforcement of laws and regulations, such as for identification or compensation for infected animals. These may act to either encourage or discourage the detection and reporting of the health-related events. Ideally, representatives from livestock industries should have input into the development of government regulations so that they are not only scientifically defensible, but also perceived to be user friendly, and thus more likely to ensure compliance, and, ultimately, more accurate surveillance.
12. **Financial and administration factors in surveillance**

It is an administrative responsibility to ensure that any surveillance programme is adequately staffed with veterinarians, technicians and support staff so as to be able to carry out the programme within the planned time frame and budget. They should also be well trained and motivated to carry out sampling exactly as required by the epidemiologists.

Ideally, the veterinarian(s) responsible for the surveillance programme should have had advanced training in epidemiology and biostatistics, and be familiar with computer programs.

The surveillance activities preferably should be administratively separate from the actual control or eradication activities within government veterinary services. In effect, this acts to ensure that the epidemiologists present surveillance information in an unbiased format to the decision-makers. Budgets for surveillance are limited, and administrators must balance acceptable costs and acceptable risks.

13. **Culture, motivation and education factors in surveillance**

Cultural and social pressures often discourage reporting of animal diseases. In some societies, it may be necessary to convince the leader or village headman, for example, to facilitate sampling of animals. To overcome these barriers, educational efforts must be combined with changes in the reward system to provide some positive incentives for those persons or groups involved in farm surveillance. These rewards should vary to fit their desires and perceptions. For example, some may be rewarded by the timely investigation of abortion outbreaks and prompt return of results with interpretation and intervention whether the abortion is due to brucellosis or not. Others may need more tangible rewards to ensure cooperation, such as free medication for their livestock. Only through positive motivation will it be possible to obtain a high level of detection, accurate classification and timely reporting of brucellosis health-related events.

If current surveillance information shows that a brucellosis control programme is not achieving its desired objectives despite being technically sound, there may be significant problems in knowledge, attitudes or practices among either the public or the livestock producers. This is especially likely to be a problem in the final stages of an eradication campaign, when livestock producers may not have experienced brucellosis and no important economic losses due to the disease (abortion), and thus question its relevance. There are well-established techniques available using Participatory Rural Appraisal methods to determine knowledge, attitudes and practices associated with brucellosis in livestock owning communities. This is very clearly a legitimate component of brucellosis surveillance.
Chapter 6

Surveillance of bovine brucellosis

This section provides a summary of identification, serological and other tests, vaccines and diagnostic biologicals drawn from Chapter 2.3.1 of the OIE Manual of Standards for Diagnostic Tests and Vaccines (OIE, 2000).

Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and rarely by *B. suis*. It is usually manifested by abortion, with excretion of the organisms in uterine discharges and in milk. Diagnosis depends on the isolation of *Brucella* sp. from abortion material, udder secretion or from tissues removed at postmortem examination. Alternatively, specific cell-mediated or serological responses to *Brucella* antigens can be demonstrated.

**Identification of the agent**

The demonstration by modified acid-fast or immunospecific staining of organisms of *Brucella* morphology in abortion material or vaginal discharges provides presumptive evidence of brucellosis, especially if supported by serological tests. Whenever possible, the organism should be isolated, and the species and biovar should be isolated, and the species and biovar should be identified by phage lysis or oxidative metabolism tests, or both, and by cultural, biochemical and serological criteria. The recently developed polymerase chain reaction (PCR) and DNA-probe methods provide additional means of detection.

**Serological tests**

No serological test is appropriate for all epidemiological situations. The buffered *Brucella* antigen tests (Rose Bengal plate agglutination test and buffered plate agglutination test) are suitable for screening herds and individual animals. The reactivity of positive samples should be confirmed by the complement fixation test or by enzyme-linked immunosorbent assay (ELISA), both of which can also be used for both screening and confirmation. The serum agglutination test is inferior to other tests in specificity and sensitivity, and is not recommended if other procedures are available. The milk ring test and indirect ELISA performed on bulk milk samples are effective for screening and monitoring dairy cattle for brucellosis, but are less reliable in large herds and less sensitive with *B. melitensis*. Another immunological test is the brucellin skin test, which can be used for screening unvaccinated herds, provided that a standardized allergen preparation (e.g., brucellin INRA) is available.

**Requirements for vaccines and diagnostic biologicals**

*B. abortus* Strain 19 live vaccine should be prepared from USA-derived seed cultures\(^1\), and each batch must conform to minimum standards for viability, smoothness, pathogenicity and ability to immunize guinea pigs or mice against challenge with a virulent strain of *B. abortus*. Brucellin preparations for the intradermal test must be free of lipopolysaccharide and must not produce non-specific inflammatory reactions or interfere with serological tests. Diagnostic antigens must be prepared from an approved smooth strain of *B. abortus* and comply with minimum standards for identity, purity, sensitivity and specificity. Antigens for serological tests should be standardized against standard sera calibrated against the International Standard *B. abortus* serum.

Note: *B. abortus* infections, besides occurring in cattle (*Bos taurus* and *Bos indicus*), also occur in domestic buffalo (*Bubalus bubalis*), African Buffalo (*Syncerus cafer*), North American buffalo

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\(^1\) Obtainable from the United States Department of Agriculture (USDA), National Veterinary Services Laboratory (NVSL), 1800 Dayton Avenue, Ames, Iowa 50010, United States of America.
Surveillance of bovine brucellosis

(Bison bison), as well as cervidae and camelidae. Occasional infections in horses and dogs have also been reported.

*B. melitensis* and *B. suis* infections have been reported in cattle, especially if in contact with infected small ruminants and swine, respectively. It is important to recognize that disease may not always occur with these two *Brucella* species.

The following surveillance techniques are appropriate for the four phases of bovine brucellosis control and eradication.

Where calving is seasonal, actual testing periods might best be implemented to coincide with herd management practices.

1. **High or unknown prevalence phase with no control programmes**

During this phase, the magnitude and distribution of the problem should be determined, as discussed earlier.

**On-farm surveillance**
- Voluntary investigation of abortion incidents and submission of tissues to a veterinary diagnostic laboratory for culture (passive, and frequently biased).
- Sero-surveillance (active).

**Off-farm surveillance**
- Percentage of abortion incidents confirmed by laboratory as brucellosis (passive and frequently biased).
- Bacteriological and serological examination of tissues and blood from cattle of breeding age at markets or slaughter (active).

2. **Mass vaccination phase**

*B. abortus* strain 19 is still considered the standard vaccine, although some countries are now using strain RB51 (rough) vaccines. Strain RB51 apparently does not result in antibodies detectable by current serological tests. The dose, route of inoculation, and age or sex of vaccination may vary from country to country.

**On-farm surveillance**
- Testing random samples of animals or herds within 2-3 weeks after vaccination using buffered *Brucella* antigen tests to evaluate strain 19 vaccine coverage. Over 80% of vaccinated animals should be seropositive. Also check the identification of vaccinated animals.
- Monitor abortion incidents as in the previous phase, or carry out surveillance on selected or sentinel herds to monitor abortion rates (active).
- Monitor randomly selected herds periodically using tests capable of distinguishing between serological responses due to vaccination and those due to infection, such as the complement fixation, immunodiffusion and competitive ELISA tests (Active).

**Off-farm surveillance**

Active surveillance of tissues and blood samples from randomly selected slaughter animals of breeding age, using bacteriological and serological tests as above.

3. **Test and removal, segregation or slaughter phase**

Note that, as the incidence or prevalence of infected animals and herds decreases to low levels, vaccination (especially with strain 19) may be phased out to reduce the problem of false-positive reactions. However, the premature ban of strain 19 vaccination is a common source of problems in the later stages. Combined conjunctival vaccination of 3–4-month-old replacement animals and eradication by test and slaughter are compatible.
**On-farm surveillance**

**Dairy cattle.** The milk ring test (MRT) and the indirect ELISA tests are recognized as low cost and efficient methods to identify herds with infected animals, even if the within-herd prevalence is low. If positive results are detected, then all animals in the herd should be serologically tested (see below). Note that in large herds the MRT test should be modified by either increasing the volume of milk tested or by taking segmented samples throughout the milking period. The milk ring test is usually carried out at intervals of 4–6 months, except in known infected herds, where monthly testing is advised. The MRT test may yield false-negative results in the early stages of *B. melitensis*-infected herds.

**Beef cattle.** The brucellin test is a low cost alternative to other screening tests, especially where the herd rate of infection is low. Although not recommended in vaccinated herds, it has been successfully used in New Zealand. The test is carried out by intradermal injection either in the cervical or caudal fold regions. It is examined by palpation 72 hours after injection, and a swelling of 2 mm or greater considered to be positive.

The sensitivity of the test is approximately 60–75% and the specificity 95–100%. An important practical advantage of this test is that it can be carried out concurrently with the tuberculin test. It may also be used to distinguish false-positive brucellosis serological reactions. The table gives the probability that a Brucellin skin test will identify a herd as infected with brucellos

<table>
<thead>
<tr>
<th>Number of infected animals in herd</th>
<th>Probability of classifying the herd as infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Clean: 0.4, Infected: 0.6</td>
</tr>
<tr>
<td>2</td>
<td>Clean: 0.16, Infected: 0.84</td>
</tr>
<tr>
<td>3</td>
<td>Clean: 0.06, Infected: 0.94</td>
</tr>
<tr>
<td>4</td>
<td>Clean: 0.03, Infected: 0.97</td>
</tr>
</tbody>
</table>

The prevalence of infection (*P*) in a herd may be estimated from the following formula:

\[ P = (t + S_p -1) \times (S_e + S_p^{-1}) \]

where *t* = positive test frequency, *S_p* = test specificity, and *S_e* = test sensitivity.

**Investigation of abortions** If abortion is made a notifiable event, this can be used as a supplementary tool, especially in herds that calve seasonally before the MRT is used. However, it may not be cost effective as a programme progresses.

A specific set of samples from each abortion (vaginal swabs, foetal membranes, foetal lung, and stomach contents, and serum from each animal) must be submitted to a diagnostic laboratory.

**Movement controls** If there are controls on the movement of cattle during a control programme, testing of eligible animals for brucellosis may be undertaken both before and after movement and quarantine on the farm of destination. The second test is particularly critical, especially given the variable incubation period of brucellosis and if the tested animals originate from a herd of unknown status. Movement testing can be a very useful surveillance tool, especially for those owners or dealers who regularly buy and sell cattle.

**Epidemiological investigations** When infected herds are identified and subsequently investigated (see section on surveillance), this may lead to testing of adjacent herds and the source herds of recent introductions.

**Individual animal tests** Once an infected herd has been identified, all test-eligible animals (sexually intact animals 18 months of age and over, or, if vaccinated as calves with strain 19, this may be increased to 20–24 months), are bled at intervals, usually 3–6 months, and reactor animals identified and removed from the herd.
The sequence of testing most often used has been:

(i) Use a buffered *Brucella* antigen test, such as the Rose Bengal or *Brucella* plate agglutination test. All positives are then examined with the complement fixation or ELISA tests and the positive animals removed.

(ii) If these tests can be automated it may be more economical to test all samples initially with the complement fixation or indirect ELISA tests. There are many advantages to centralizing these two tests to ensure quality control and consistency.

Serum agglutination tests are not recommended for routine testing and surveillance because they are inferior to complement fixation and ELISA tests in terms of sensitivity, specificity and practicability.

**Off-farm surveillance**

As the incidence or prevalence of infected herds decreases, it may be more cost effective to commence testing at markets and slaughterhouses if a reliable system can be developed of trace-back to the herd of origin. This may be accomplished by using permanent identifiers on the farm, such as ear or tail tags, or supplementing these with temporary identifiers such as bar-coded ‘back tags’ applied at entry to the market or slaughterhouse.

The probability of detecting infection in an individual herd is influenced by the herd size, rate of culling (removal) from the herd, and the prevalence of infection in the herd. In general, larger herds have a better chance of being located by market or slaughter surveillance than smaller herds. The percentage of ‘successful’ trace-backs to the herd of origin should be carefully monitored during this phase. If it falls below 50% then this is an indication that problems exist and should be corrected. In general, the MRT is more efficient in detecting infected herds than market or slaughter testing, but a combination of strategies is ideal.

4. Freedom phase: herds, regions and countries

According to the OIE International Animal Health Code (see Chapter 2.3.1, Bovine Brucellosis), for a country or region to be considered as officially free from bovine brucellosis it must satisfy the following requirements:

(i) Bovine brucellosis or any suspicion thereof is compulsorily notifiable in the country.

(ii) The entire cattle population of a country or part of the territory of a country is under official veterinary control and it has been ascertained that the rate of brucellosis infection does not exceed 0.2% of the cattle herds in the country or area under consideration.

(iii) The serological tests for bovine brucellosis are periodically conducted in each herd, with or without the milk ring test.

(iv) No animal has been vaccinated against bovine brucellosis for at least the past three years.

(v) All reactors are slaughtered.

(vi) Animals introduced into a free country of part of the territory of a country shall only come from herds officially free from bovine brucellosis.

In a country where all herds of cattle have qualified as officially free from bovine brucellosis and where no reactor has been found for the past five years, the system for further control may be decided by the country concerned.

For herds to be considered *officially free*, they must satisfy the following requirements:

(i) Be under official veterinary control.

(ii) Contain no animal that has been vaccinated against bovine brucellosis during at least the past three years.

(iii) Only contain animals that have not showed evidence of bovine brucellosis infection during the past six months. Some authorities would argue that a longer period of several years is necessary to reduce the risk still further. All suspect cases (such as animals that have prematurely calved) should have been subjected to the necessary laboratory investigations.

(iv) All cattle over the age of one year were subjected to serological tests, with negative results, performed twice at an interval of 12 months. This requirement is maintained even if the
entire herd is normally tested every year or testing is conducted in accordance with other requirements established by the veterinary administration of the country concerned.

(v) Additions to the herd shall only come from herds officially free from bovine brucellosis. This condition may be waived for animals that have not been vaccinated, but come from a herd free from bovine brucellosis, provided negative results were shown following a buffered Brucella antigen test and the complement fixation test during 30 days prior to entry into the herd. Any recently calved or calving animal should be retested after 14 days, as tests are not considered valid in female animals that have calved during the past 14 days.

For herds that may still be under a vaccination programme, a separate category of herds free from brucellosis is recognized, namely:

(i) Be under official veterinary control.

(ii) Be subjected to either a vaccination or a non-vaccination regime.

(iii) If a live vaccine is used in female cattle, vaccination must be carried out between three and six months of age, in which case these female cattle must be identified with a permanent mark.

(iv) All cattle over the age of one year are controlled as provided in paragraph 4) of the definition of a herd of cattle officially free from bovine brucellosis; however, cattle under 30 months of age that have been vaccinated using a live vaccine before reaching six months of age, may be subjected to a buffered Brucella antigen test with a positive result, with the complement fixation test giving a negative result.

(v) All cattle introduced into the herd come from a herd officially free from bovine brucellosis, or from a country or part of the territory of a country free from bovine brucellosis. This condition may be waived for animals which have been isolated and which, prior to entry into the herd, were subjected to the serological tests for bovine brucellosis with negative results on two occasions, with an interval of 30 days between each test. These tests are not considered valid in female animals that have calved during the past 14 days.

(vi) Risk assessment procedures have been utilized to evaluate the appropriateness of changes in surveillance in the transition from test-and-removal to maintaining freedom from brucellosis.

The current OIE Animal Health Code (2002) does not recognize off-farm surveillance, such as market or slaughter testing. The probability of locating infected herds by market or slaughter testing is dependent on the cull rate from the herd, the prevalence of infection in the herds, as well as the probability that an infected animal will pass through the surveillance system and the herd of origin is located. This in turn will depend on the percentage of identified animals, the percentage of samples collected at slaughter and the diligence of tracing activity.

The MRT and indirect ELISA are primary methods of surveillance for dairy herds once freedom has been established. Their efficiency depends upon the percentage of herds from which samples are taken, the number of times each herd is tested within a year, and the accuracy of identification of the herd.

Other surveillance activities in Brucella-free countries or regions should include:

- Testing prior to movement.
- Testing after movement and quarantine.
- Testing areas adjacent to borders where illegal introductions may occur.
- Testing imported animals of breeding age.
- Testing imported semen, embryos and ova.

Finally, there have been a number of instances recorded where a human case or outbreak of brucellosis has lead to a trace-back to an infected herd in a region hitherto considered free of brucellosis.
Chapter 7

Surveillance of porcine brucellosis

This section is a summary of Chapter 2.6.2, on Identification, Serological and other Tests, Vaccines and Diagnostic Biologicals, in OIE Manual of Standards for Diagnostic Tests and Vaccines (2000).

Brucellosis in pigs is a bacterial infection that, after an initial bacteraemia, causes chronic inflammatory lesions localized in the reproductive organs of both sexes, and in the bones. The infection in pigs is caused by *Brucella suis* biovar 1, 2 or 3. The disease caused by biovars 1 and 3 is similar, while that caused by biovar 2 differs from 1 and 3 in its host range, its limited geographical distribution and its pathology. Biovar 2 is rarely pathogenic for humans, whereas biovars 1 and 3 are highly pathogenic for humans. Porcine brucellosis is of widespread occurrence; generally, however, the prevalence is low, with the exception of South America and Southeast Asia, where the prevalence is higher. In some areas, *B. suis* infection has become established in wild or feral pigs. Diagnostic methods recommended for wild and feral pigs are the same as for domestic pigs. Various biovars of *B. suis* cause infections in animals other than pigs, such as reindeer, caribou, hares and various murine species. These are dealt with in an appendix at the end of this chapter.

Signs in sows include abortion at any stage of gestation and birth of dead or weakling piglets. In boars, the most prominent symptom is orchitis, and the secondary sex organs may be affected. There may be *B. suis* in the semen, sometimes in the absence of clinical signs. Transmission during copulation is more common than is the case with brucellosis in ruminants. In both sexes, bones, and especially joints and tendon sheaths, may be affected, causing lameness and sometimes paralysis. Pigs are susceptible to artificial infection with *B. abortus* and *B. melitensis*, but there are no reports of natural disease in pigs being caused by either of these organisms. In humans, the infection is usually confined to those who are occupationally exposed to pigs, and to laboratory workers. However, *B. suis* is capable of colonizing the bovine udder, causing serious human epidemics.

**Identification of the agent**

*B. suis* is readily isolated from live pigs by culture of birth products, and from carcasses by culture of lymph nodes and organs. Selective media are available for culture of contaminated specimens. In nature, *B. suis* occurs invariably in the smooth phase – the appearance on solid medium is typical of smooth *Brucellae*. Biovars of porcine origin agglutinate with monospecific anti-A serum, and not with anti-M serum. Definite identification of species and biovars may be effected by phage lysis and biochemical tests, preferably carried out in specialized laboratories.

**Serological tests**

To date, no serological test has been shown to be reliable in routine diagnosis in individual pigs. For the identification of infected herds, the buffered *Brucella* antigen tests (BBAT), i.e. the buffered plate agglutination test and Rose Bengal plate agglutination test are more reliable in practice than are other conventional tests, such as complement fixation and standard agglutination tests, and are therefore recommended. The procedures for the BBAT are the same as those described in Chapter 2.3.1 of the OIE Manual, for bovine brucellosis. Other serological tests have also been developed, including indirect ELISA and competitive ELISA. The allergic skin test is also useful for identifying infected herds. In summary, it has been difficult to eliminate brucellosis in swine by test and removal, and a policy of slaughtering infected herds is preferred.
REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

*B. suis* (biovar 2) vaccine has been used in China, with apparent good results in pigs and also in small ruminants and cattle for the prophylaxis of brucellosis. It is considered that confirmation of the results obtained in China is required before strain 2 vaccine can be recommended for general use.

1. **High or unknown prevalence phase with no control programmes**

During this phase, the magnitude and distribution of the problem should be determined, as described earlier.

**On-farm surveillance**
- Voluntary investigation of abortion and weak piglets incidents, and submission to a diagnostic laboratory for culture (passive).
- Inspection of pigs for clinical signs, including especially orchitis (passive).
- Sero-surveillance using buffered *Brucella* antigen tests as herd tests only (active).
- Brucellin tests also used to identify infected herds (active).
- Sampling of in-contact feral swine (active).

**Off-farm surveillance**
- Percentage of abortion and other tissues from which *B. suis* was isolated (passive).
- Bacteriological examination of tissues (mandibular, gastrohepatic, internal iliac andinguinal lymph nodes) and blood for serological examination from breeding age swine at slaughter (active).

2. **Mass vaccination phase**

No data is available from countries using vaccines against swine brucellosis to support any specific sero-surveillance programme. Off-farm surveillance as in Phase 1. It could be that vaccines have demonstrated good efficacy, but they have never been used extensively in swine.

3. **Test and removal, segregation or slaughter phase**

Because none of the existing serological tests are reliable in individual pigs, buffered *Brucella* antigen tests (including the Card Test) are used to diagnose herd infections. Nevertheless, some countries do attempt herd eradication by testing all eligible animals (usually >6 months of age) every 30 days, and remove reactors for slaughter, continuing until the entire swine herd is negative. If this option fails, then depopulation (sell to slaughter) followed by repopulation with animals from brucellosis-free herds is carried out 30 days after the buildings have been cleaned and disinfected. An alternative option is to carry out offspring segregation, where female pigs (gilts) are separated from dams at approximately one month of age and reared separately. These animals should be tested 30 days prior to breeding.

Abortion incidents, movement tests, trace backs, adjacent herds, and epidemiological investigation of infected herds can be monitored as in bovine brucellosis.

**Off-farm surveillance**
- Providing it is possible to trace back to the herd of origin from markets or slaughter using temporary or permanent identifiers, all swine of breeding age should be routinely tested.
- If feral pigs are in contact, then continue random testing.
- Periodic bacteriological surveillance of reactor pigs from infected herds or randomly selected pigs or herds at slaughter monitored for isolation of *B. suis* as in Phase 1.

4. **Freedom phase**

The OIE International Animal Health Code does not prescribe conditions for country freedom for swine brucellosis, but a number of countries are free or are in the process of attaining freedom.
On-farm testing

OIE defines a herd as free from porcine brucellosis if it can satisfy the following requirements:

(i) Be under official veterinary control.
(ii) Contain no animal found to be infected with porcine brucellosis during the past three years and all suspected cases are subjected to laboratory investigation.
(iii) All cattle kept in the same establishment are officially free from brucellosis.

Although not stated, these herds should not have any direct contact with feral swine.

Breeding swine herds (all animals >6 months) can be validated as brucellosis free if:

(i) the entire herd is tested and found sero-negative; or
(ii) incremental testing is carried out as follows:
  ➢ Randomly test 25% of the swine every 3 months or 10% every month and all are sero-negative.
  ➢ No swine may be tested twice in a year.
  ➢ To maintain freedom status, herds should be retested every 12 months.
  ➢ Surveillance should be maintained for clinical signs.
  ➢ All movements into Brucella-free herds should either be from Brucella-free herds or, if not, the animals should be sero-negative 30 days prior to movement, isolated on arrival and retested 30–60 days later. If artificial breeding is used, all semen should come from boars in Brucella-free herds.

Off-farm testing

Periodic bacteriological and serological testing of any reactor or suspect swine sent for slaughter.
Chapter 8
Surveillance of ovine and caprine brucellosis
(excluding Brucella ovis infection)

This section summarizes Identification, Serological and other tests, Vaccines and Diagnostic Biologicals, based on Chapter 2.4.2 of the OIE Manual of Standards for Diagnostic Tests and Vaccines (2000).

Brucella melitensis (biovars 1, 2 or 3) is the main causative agent of caprine and ovine brucellosis. Sporadic cases caused by B. abortus have been observed, but clinical disease is uncommon. B. melitensis occurs in the Mediterranean region, but infection is also widespread, especially where small ruminants are the major livestock species. Canada and the USA are believed to be free, as are northern Europe, Southeast Asia, Australia and New Zealand.

Clinically, the disease is characterized by one or more of the following signs: abortion, retained placenta, orchitis, epididymitis and, rarely, arthritis.

B. melitensis is highly pathogenic for humans, causing one of the most serious zoonoses in the world. All infected tissues, cultures and potentially contaminated materials should be handled under conditions for biohazard containment.

Identification of the agent

Presumptive evidence of Brucella is provided by the demonstration, by modified acid-fast staining of organisms, of Brucella morphology in abortion material or vaginal discharge, especially if supported by serological tests. The recently developed polymerase chain reaction methods provide additional means of detection. Whenever possible, Brucella sp. should be isolated by culture – using selective media – from uterine discharges, aborted foetuses, udder secretions or selected tissues, such as lymph nodes, testes or epididymides. Species and biovars should be identified by phage lysis, and by cultural, biochemical and serological criteria.

Serological and allergic skin tests

The Rose Bengal plate agglutination, complement fixation and indirect ELISA tests are usually recommended for screening flocks and individual animals. The complement fixation test is the only test prescribed for confirmation and international trade, but other tests, such as the immunodiffusion and competitive ELISA, are useful for confirmation purposes. The serum agglutination test (SAT) is not considered reliable for use in small ruminants. For pooled samples, there are no useful tests equivalent to the milk ring test in cattle. The brucellin allergic skin test can be used as a screening or complementary test in unvaccinated flocks, provided that a lipopolysaccharide-free and standardized antigen preparation is used. Results must then be interpreted in relation to the clinical signs, history, and the results of serological or cultural examination.

Requirements for vaccines and diagnostic biologicals

B. melitensis strain Rev.1\(^2\) live vaccine is recommended to immunize sheep and goats at risk of infection from Brucella. Production of Brucella antigens of Rev.1 vaccine is based on a seed-lot system. Seed cultures to be used for antigens for serological and allergic skin tests and for vaccines should originate from reference centres. They must conform to minimum standards for viability, smoothness, residual infectivity and immunogenicity, if applicable. Antigens for serological tests

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2. Obtainable from: Institut National de la Recherche Agronomique (INRA), Laboratoire de pathologie infectieuse et immunologie, 37380 Nouzilly, France.
should be standardized against reference sera calibrated against the International Standard Anti-
*Brucella abortus* Serum, and the procedures and interpretations should conform to international
recommendations. Allergen preparations should be free of lipopolysaccharide to prevent anti-
lipopolysaccharide antibody production and local inflammatory reactions.

1. **High or unknown prevalence phase with no control programmes**

During this phase, the magnitude and distribution of the problem should be determined (as discussed
earlier).

**On-farm surveillance**

- Voluntary investigation of abortion incidents and orchitis/epididymitis lesions, and submission
to a diagnostic laboratory for culture (passive).
- Sero-surveillance (active).

**Off-farm surveillance**

- Percentage of abortion incidents confirmed as brucellosis (passive).
- Bacteriological and serological examination of tissues and blood from breeding age sheep and
goats at slaughter (active).

Note that in some countries, home or illegal slaughter may constitute a high percentage of all
animals killed so that legal-slaughter-based samples may not always be representative.

2. **Mass vaccination phase**

Where brucellosis is present at high rates, especially in developing countries, sheep and goats are
usually managed under extensive transhumant or nomadic systems. Under these conditions,
*B. melitensis* cannot be eradicated by test and slaughter alone, and a vaccination programme has to be
applied to reduce the risk of spread of the disease. Live Rev.1 *B. melitensis* vaccine is considered to
be the best available for use in small ruminants. Originally it was believed that exclusive vaccination
of the young replacement animals annually for 5–6 years (the usual productive life-span of these
species) would be sufficient, based on the assumption that the Rev.1 vaccine resulted in lifelong
immunity. However, this strategy has failed in both developed and developing countries. Possible
explanations for these failures included (i) a low vaccination coverage because owners keep
introducing replacements continuously throughout the year, (ii) vaccine quality; or (iii) decreased
immunity with age. Accordingly, whole flock or herd vaccination every two years is an alternative to
control *B. melitensis* infection under extensive management conditions. The major risk with the use
of Rev.1 at standard doses (1–2 × 10⁹ CFU/dose) administered subcutaneously to adult sheep and
goats is that some pregnant animals may abort. While parturition is seasonal, a few animals may be
pregnant at any one time. Reduction of the dose to 10⁴–10⁷ CFU/dose given subcutaneously has been
used in an effort to reduce the risk of abortion, but overall the results of experimental and field studies
have shown that the resulting immunity is inadequate. Conjunctival vaccination with the standard
dose of Rev.1 is safer than subcutaneous vaccination, but is not safe enough to be applied regardless
of the pregnancy status of the animals, and should be used at a time of the year when the majority of
the sheep are at least risk, i.e. during late lambing or kidding season, or during lactation.

**On-farm surveillance**

- Test random samples of animals and herds 2–3 weeks after vaccination using the Rose Bengal
test to evaluate vaccine response and coverage. Also check identification (active).
- Monitor abortion incidents as in the previous phase, or carry out active surveillance on selected
or sentinel flocks and herds to determine abortion rates. Confirm using bacteriology whether
abortion is due to *B. melitensis*. As the vaccine strain (Rev.1) may cause abortion also, all
isolates should be typed especially to differentiate Rev.1 from field strains of *B. melitensis*
biovar 1.
- Monitor randomly selected flocks or herds periodically, using tests capable of distinguishing
serological responses due to vaccination from those due to infection. These include
complement fixation, immunodiffusion and competitive ELISA tests (active).
Off-farm surveillance

- Active surveillance of tissues and blood samples from randomly selected animals of breeding age from randomly selected flocks and herds for bacteriology and serology.

3. Test and removal, segregation or slaughter phase

Where the flock and individual animal prevalences are moderate to low and there are sufficient economic resources, a test and slaughter programme can be instituted. The conjunctival route of vaccination is compatible with this phase, as the bacteria are mainly restricted to the cranial lymph nodes, and although the immunity is similar to the subcutaneous route, the serological response is significantly reduced. Accordingly, when total eradication is the final objective, conjunctival vaccination of replacement animals with Rev.1 is the ideal tool for prophylaxis against *B. melitensis* infection in small ruminants.

On-farm surveillance

- In flocks or herds in which replacements have been continuously vaccinated for several years; there will obviously be a mixture of both infected and uninfected immune animals. Therefore the Rose Bengal or the indirect ELISA tests should be carried out periodically on all, or at least a representative random sample of, animals. Positive reactors can then be tested with the complement fixation, immunodiffusion or cELISA tests, and removed if positive.
- Currently, there are no equivalents of the milk ring test for use in dairy animals. The brucellin test may also be used to screen unvaccinated flocks to determine their infection status.
- Abortion incidents, movement tests, adjacent herds or flocks, and epidemiological investigation of infected flocks and herds can be monitored as in bovine brucellosis.

Off-farm surveillance

Because of their size and mobility, the marketing channels and slaughter locations of small ruminants, particularly in heavily infected regions, are so diverse that off-farm surveillance is problematical. If routes from to slaughter can be clearly defined, and trace-back possible, it may be practical to monitor long-terms trends by this method.

4. Freedom phase

According to the OIE International Animal Health Code (Chapter 2.4.2 – Caprine and Ovine Brucellosis excluding *B. ovis* infections), for a country to be considered as officially free from brucellosis the following must apply:

(i) the occurrence or suspected occurrence of caprine and ovine brucellosis has been compulsorily notifiable for at least five years; and
(ii) all flocks of sheep and goats in the country or part of the territory of the country are under official veterinary control; and either
(iii) 99.8% of these flocks are qualified as officially free from caprine and ovine brucellosis; or
(iv) no case of brucellosis in sheep or goats has been reported for at least five years, and no sheep or goats have been vaccinated against the disease for at least three years.

4. Maintenance of officially free status

For a country or part of the territory of a country to maintain its status as officially free from caprine and ovine brucellosis, a serological survey should be carried out every year on farms or in abattoirs on a representative sample of the caprine and ovine flocks of the country or part of the territory of the country sufficient to provide at least a 99% level of confidence of detecting caprine and ovine brucellosis if it is present at a prevalence rate exceeding 0.2% of the flocks.

Qualification

For OIE purposes, a sheep flock or goat herd officially free from caprine and ovine brucellosis must satisfy the following requirements:

(i) It is under official veterinary control.
No clinical, bacteriological or immunological evidence of caprine and ovine brucellosis has been found for at least one year.

It contains only sheep or goats not vaccinated against brucellosis or permanently identified animals which were vaccinated more than two years ago.

All sheep and goats over six months of age on the day of sampling have been subjected to a diagnostic test for brucellosis with negative results on two occasions, at an interval of not more than 12 months and not less than 6 months.

However, for flocks situated in a country or part of the territory of a country that qualifies as officially free under this paragraph, maintenance testing is not required.

When qualified, it contains only sheep and goats born therein or introduced in accordance with the appropriate requirements.

For a flock to maintain its status as officially free from caprine and ovine brucellosis, a sample of the animals in the flock must be subjected each year to a diagnostic test for brucellosis, with negative results:

- For a flock containing up to 1000 animals, the sample must include:
  - all males over six months of age;
  - all the animals introduced into the flock since the previous control; and
  - 25% of the pubescent females; the number of females included in the sample should not be less than 50, unless the flock contains fewer than 50 females, in which case all pubescent females should be included.

- For a flock containing more than 1000 animals, a serological survey should be carried out every year on a representative sample of the animals in the flock, sufficient to provide a 99% level of confidence of detecting caprine and ovine brucellosis if it is present at a prevalence rate exceeding 0.2%.

Controls must be carried out at up to three-year intervals, if the flock is situated in a part of the territory of the country where 99% of flocks are officially free from caprine and ovine brucellosis and the remainder are submitted to an eradication programme.

However, for flocks situated in a country or part of the territory of a country qualified as officially free (as noted above) maintenance testing is not required.

Whatever the periodicity of controls and the ways the status has been obtained, sheep and goats must only be introduced into the flocks in accordance with the stated requirements.

4. Suspension and recovery of officially free status

If a sheep or goat reacts positively to a diagnostic test for caprine and ovine brucellosis, the status of “flock officially free from brucellosis” shall be suspended and may not be recovered unless the following requirements have been fulfilled:

- all infected and in-contact animals have been eliminated from the flock as soon as the result of the diagnostic test was known;
- all the remaining sheep and goats in the flock over six months of age on the day of sampling have been subjected to a diagnostic test for caprine and ovine brucellosis, with negative results, on two occasions, at an interval of not less than three months; and
- for the purpose of the OIE Code, sheep flocks or goat herds free from caprine and ovine brucellosis.
Cross-transmission of brucellosis can occur between cattle, swine, sheep and goats and other species, including dogs, horses, feral swine, bison, reindeer, caribou and camels. In some instances, the infection is dead-end, e.g. dogs eating placentas from infected animals following abortion, while in other situations, such as feral swine, onward transmission to cattle or domestic swine occurs readily. Therefore, in any brucellosis surveillance programme where there is epidemiological or bacteriological evidence that wildlife or feral species are a source of new or re-infections in cattle, swine, sheep and goats, these species should be routinely monitored, either by a defined capture programme or using routine hunting activities to obtain tissues and blood for examination.

Camel husbandry is vital for numerous pastoralist groups in Asia and Africa. Infection in camels is caused by different biotypes of \textit{B. abortus} and \textit{B. melitensis}. Currently, many gaps exists in knowledge of the epidemiology of the disease in camels. Many countries with long standing traditions of camel keeping and usage do not have clear-cut policies regarding the control of camel brucellosis. Seroprevalence of brucellosis in camels is low in beasts kept under extensive pastoralist husbandry, whilst it is rather high in more camels maintained under intensive conditions. An important aspect of the epidemiology of brucellosis in camels is the role of the intercalving interval in the transmission of infection between camels within a herd. Nomadic camels usually have a lengthy intercalving interval, estimated to be between 2 and 3 years. Since most brucellosis contamination occurs following an abortion or delivery by an infected female, then the long intercalving interval might contribute to a lower incidence of brucellosis in nomadic or extensively raised camels. The use of Strain 19 and Rev.1 vaccines have been recommended for use in controlling camel brucellosis. In countries where camels are kept extensively and where seroprevalence of the disease is low, whole-herd vaccination is recommended, preceded by blood testing using the Rose Bengal test. Seropositive animals could be identified by branding or earmarking and retested. In high prevalence countries with intensive management system of camel production, test and slaughter followed by vaccination is recommended. In such countries, the economy is generally strong to support such control measures. Public education and sensitization is essential to the success of control programmes in both management systems of camels.
Chapter 10
Intersectoral collaboration and cooperation in brucellosis surveillance

As brucellosis is clearly one of the more important zoonoses in many areas of the world, and control or eradication efforts are primarily focused on reducing human exposure, it should be obvious that close and continued collaboration between Health (medical) and Agriculture (veterinary) staff should occur at all administrative levels if the ultimate goals are to be achieved. Provided surveillance data on human brucellosis are reasonably accurate, i.e. unbiased and timely, it can be a sensitive indicator of the status of animal infection in the country or region. Human epidemics, whether food-borne or animal-contact related, may direct veterinary epidemiologist to foci of animal infections.

At the central level, it is assumed that both Health and Agriculture Departments will have a legal basis for the mandatory reporting of suspected or confirmed brucellosis cases to their respective Ministries or Departments. Many Health Departments employ veterinarians in various roles, but ideally a veterinary epidemiologist in the human diseases surveillance unit can play a key role in intersectoral collaboration. In some countries, there may also be a legal requirement for all veterinarians or veterinary laboratories to report *Brucella*-infected herds or flocks directly by telephone to the Health Department for further investigation. Some countries have also established either national Zoonoses Committees or specific Brucellosis Committees, with multi-departmental representation, including livestock producers and representatives of the medical and veterinary professions to ensure better communication of surveillance information. As problems develop, they are more likely to be resolved if all parties are actively involved and their suggestions taken into consideration. At the central level, need for better agricultural extension of public health education can also be addressed.

At intermediate levels (state, provincial or governate), similar communication between medical and veterinary authorities is very desirable. At this level, joint epidemiological investigations should be carried out, especially of suspected outbreaks and individual human cases to determine the route of transmission and animal sources of infection. Joint meetings of medical and veterinary associations are also a useful means of exchanging information on brucellosis, as well as on other zoonoses.

At the peripheral or local level, personal contacts between physicians and veterinarians working in both the public and private sectors should be are strongly promoted, to ensure that both are made aware of the situation in their areas to ensure efficient collaboration.
Chapter 11

Summary

There are many factors involved in human and animal brucellosis surveillance, such that no one system can fit all countries or regions. Therefore, all of the factors discussed above should be considered carefully in the design or evaluation stage. Also, as a control or eradication progresses successfully, a surveillance programme will almost certainly require modification to take into account decreasing incidence rates and to focus on new or re-infections, which may be more difficult to identify.

Once eradication has been achieved, then it is critical to maintain surveillance, especially for international trade purposes.
Chapter 12

Information resources for brucellosis

INTERNET RESOURCES

2. OIE Paris, France. See http://www.oie.int/

PRINTED RESOURCES

The following publications and documents were used in preparing these guidelines


CDC [Centers for Disease Control, USA]. 1988. Guidelines for evaluating surveillance systems MMWR 37 (Suppl S-5)

CIDE [Centre for International Development and Environment]/WRI [World Resources Institute]. No date. Participatory Rural Appraisal Handbook.


Information resources


