

Epidemiology and surveillance of brucellosis

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Summary

Brucellosis is one of the most important infectious diseases worldwide. Nevertheless, since it is not regarded as a priority by national and international health systems in many endemic regions, it is considered to be a neglected zoonosis. Measures to prevent and control brucellosis rely upon direct approaches aimed at minimising the risk of spreading infection among animals. Collectively, these measures tend to reduce the exposure of animals to *Brucella* spp. and to increase resistance to infection in susceptible animals. To implement an effective disease control strategy, detailed information about the presence of the pathogen in a specific territory is of fundamental importance. For that reason, particular emphasis should be placed on active surveillance using serological methods. Serological surveillance provides useful information to aid in understanding epidemiological patterns and assess the impact of brucellosis in the targeted area, paving the way to define the most suitable approaches for confining the disease within acceptable limits.

Keywords

Animal health – *Brucella* – Brucellosis – Diagnosis – Serology – Surveillance – Zoonosis.

Background

Brucellosis is caused by Gram-negative bacteria of the genus *Brucella*. The species that infect livestock are *B. melitensis*, found mainly in sheep and goats; *B. abortus*, found mostly in cattle; *B. suis*, reported principally from pigs; and *B. ovis*, found mainly in sheep (8). In these natural hosts, the disease is generally represented by abortion, reduced fertility and, in ruminants, lowered milk production (7). Brucellosis is one of the most common zoonoses throughout the world (16). Despite its widespread distribution, it is considered to be a neglected zoonosis because, in many endemic areas, it is not regarded as a priority by national and international health systems (19). Humans can be infected by ingesting contaminated milk or through direct contact with infected animals. In clinical terms, brucellosis is a complex entity with the most common sign being an undulant fever, frequently associated with chronic debilitating disease, accompanied by a variety of other symptoms (15). It is obvious that brucellosis can have a dramatic impact on both human and animal health, and that vigorous attempts should be made to prevent or control it in livestock in

endemic areas, as well as in countries where the infection is emerging or re-emerging (17).

The most significant benefits that come from strategies to prevent and control brucellosis are financial, due to *i*) improving the productivity of livestock and *ii*) savings in health costs for human treatment and hospitalisation.

Additional benefits are derived from averting disability-adjusted life-years (DALYs – a measure of overall disease burden), which, with brucellosis, could be high.

Measures to prevent and control brucellosis rely upon direct approaches, aimed at minimising the risk of spreading infection among animals. Collectively, these measures reduce the exposure of animals to *Brucella* spp., and increase resistance to infection in susceptible animals. This multifaceted strategy, as with other infectious diseases, should be based on, but not limited to, health education, proper management of replacement animals, controls on animal movement, good farming management practices and test and removal procedures. In addition, particular

emphasis should be placed on active surveillance to identify any infected animals and remove them from the population (test and slaughter). Alternatively, if the prevailing epidemiological and socio-economic conditions do not allow an effective test-and-slaughter policy, a large-scale vaccination campaign should be carried out. The aim of this article is to review the importance of such surveillance strategies when evaluating the impacts of brucellosis and selecting the most suitable strategy for controlling the disease in a specific area.

Surveillance

Surveillance involves the systematic collection, analysis and interpretation of raw data to provide information (10). The surveillance system should be designed according to the following factors:

- the specific objectives of the surveillance
- the criteria required to assess infection status
- the types of data that must be collected
- the methods and protocols for collecting information
- the logistic, human and economic resources available
- the use to which the acquired information will be put
- the laws and regulations under which this surveillance will be carried out.

When planning the surveillance system, the approach taken should be evaluated periodically to identify crucial information gaps that could hinder the efficacy of the programme (6).

Since brucellosis is a zoonotic disease, it is important that surveillance is applied to both humans and animals. Surveillance of human brucellosis can give important insights into the source of infection and the dynamics of the disease in the at-risk human population. Combining these data with information about the infection in livestock, the numbers of infected animals and the specific species of *Brucella* involved will provide the basic knowledge needed to design the most suitable strategy for controlling brucellosis in this set of circumstances.

Surveillance should be directed towards acquiring information that characterises epidemiological patterns and assesses the impact of brucellosis in the targeted area. This information should help to define the most suitable approach for controlling or confining the disease.

Surveillance activities should be directed towards obtaining periodic information on specific epidemiological

indices, including, among other things, a detailed census of susceptible animals, market and slaughter data, and statistical estimates of incidence and prevalence rates within the targeted area.

Although passive or active surveillance can be conducted, information should be acquired through active surveillance systems. Passive surveillance systems rely upon information from abortion samples submitted to diagnostic laboratories, and thus are limited by intrinsic biases, including the experience, judgement and decisions of veterinary and farming personnel in the field, the capabilities of the diagnostic laboratories, and by logistical difficulties which may hinder the collection of samples.

Isolation and identification of *Brucella* spp. from samples obtained after abortions or from the lymph nodes of reactor animals at slaughter is greatly encouraged as it provides information about the aetiological agent circulating within the area of interest. This information contributes to the assessment of epidemiological patterns and identification of critical foci in the target area. More importantly, precise knowledge of the aetiological agent may well influence the choice of vaccine, since the vaccines available do not all have the same efficacy against the various *Brucella* species.

Bacteriological isolation provides a definitive diagnosis. However, positive isolation cannot be carried out for all infected animals. The procedures for microbiological isolation are time consuming and require biohazard containment facilities to minimise the risk of human infection. As a result of its efficiency and reliability, indirect diagnosis, consisting mainly of the detection of specific antibodies or evaluation of hypersensitivity reactions, is more widely used than direct diagnosis.

Serological surveillance

For large-scale surveillance and eradication purposes, serological methods based on the detection of antibodies against specific antigens of *Brucella* make up the most widely used technology.

The O-polysaccharide (O-PS) of *B. abortus* lipopolysaccharide is of particular diagnostic importance, as the majority of detectable antibodies are directed against it (4). Only internationally recognised serological tests, standardised against international anti-*B. abortus* serum, should be used (4, 18). An immune response, detected by serological tests, is influenced by many factors, including the incubation period of the disease, the mechanism of infection, the age of the animal, its pregnancy status and infections caused by cross-reactive bacteria (12).

The incubation period of brucellosis varies a good deal, with some infected animals having a very long incubation period, during which they remain seronegative.

Humoral responses of *Brucella*-infected animals can be influenced by physiological conditions. For example, the humoral response can be subdued and transitory in pre-pubertal animals and long-lasting in adults, with persistence titres influenced by pregnancy and lactation. Uterine infection in pregnant animals causes a large and persistent rise of antibodies but this may be delayed until abortion or parturition. Invasion of the lactating udder causes a reduced serological response, whereas localisation confined within the lymph nodes may induce a minimal or no serological response. Up to 10% of calves born to infected dams may become 'latent carriers', with the infection remaining serologically latent until the animal matures or becomes pregnant. Disease spread between herds usually occurs through the introduction of asymptomatic, chronically infected animals.

Another important influence on humoral response is vaccination status. Production of antibodies after vaccination with smooth strains of *Brucella*, such as *B. abortus* S19 or *B. melitensis* Rev.1, have been widely described. These antibodies, mainly directed towards O-PS, interfere with serological screening of animals and make the interpretation of surveillance tests difficult, since the vaccination titres cannot be differentiated from those observed in animals infected with field strains (5, 13).

Attempts have been made to develop new vaccines based on 'rough' phenotypes (see below). Those vaccines await further evaluation in field experiments.

The magnitude and duration of humoral responses after vaccination are related to many factors, including the age at which vaccination takes place, the dosage, the route of administration and the pregnancy status of the animal. Vaccination of adults leads to a persistent antibody response whereas vaccinating animals under six months of age is usually associated with interfering antibodies that have less persistence. Vaccination of pregnant animals is associated with a slower return to seronegative status. Therefore, vaccination of young animals is recommended.

Antibodies due to vaccination are more reactive in some serological tests. At present, there is no widely available test capable of distinguishing vaccinated from infected animals.

To circumvent the limitations of vaccination with smooth (S) strains, new vaccines based on rough phenotypes have been developed. In some countries, *B. abortus* S19 has been replaced by the rough strain *B. abortus* RB51 (14, 18). Other experimental vaccine candidates, such as *B. melitensis*

B115, have been evaluated in laboratory animals, but not in field studies (2, 3). As a result of their rough phenotype, these vaccines do not induce cross-reacting antibodies to O-PS which interfere with serological surveillance tests. Monitoring humoral responses after vaccination with these rough strains required the development of specific serological tests, using homologous rough strains as antigens (1).

False-positive serological reactions can also occur through natural infection with cross-reactive Gram-negative bacteria, such as *Yersinia enterocolitica* O:9, Group N *Salmonella* (O:30), *Escherichia coli* (O:157 and O:116), *Pseudomonas maltophilia*, and *Vibrio cholerae*, which all have antigenic similarity with *B. abortus*. These bacteria can induce antibodies which cross-react with S-LPS antigens in diagnostic tests. In particular, serological interference induced by *Y. enterocolitica* O:9 has complicated the eradication of brucellosis in some countries (11).

Many attempts have been made to develop a serological test which accurately determines the infection status of an animal, distinguishing between the immune response of a vaccinated, latently infected or clinically infected animal, or a combination of two or more of these events. At this time, no test is appropriate for all epidemiological situations, with those tests that are currently available having considerable limitations, especially when it comes to screening individual animals.

When evaluating the diagnostic efficiency of each serological test, its specificity, sensitivity and predictive values should be considered. Specificity indicates the probability that the test will identify all non-infected animals. In contrast, sensitivity indicates the probability that the test will identify all infected animals within a given population.

The positive and negative predictive values indicate the probability that positive or negative results will correspond to truly infected or healthy animals, respectively. The prevalence of a disease within the population has an important influence on the positive predictive value of a diagnostic test. When the prevalence decreases, the predictive value of a positive test also declines, indicating increased potential for false-positive reactions. In contrast, at low prevalence, the number of false positives may exceed the number of true positives.

Depending on their sensibility and/or specificity, serological tests can be used in a surveillance system as screening or confirmatory tests. Screening tests are rapid and inexpensive methods with high sensitivity to ensure that infected animals are not missed. Confirmatory tests are more specific methods, useful for minimising the number of false-positive reactors, and able to distinguish between vaccinated and infected animals.

Since no single test has both 100% sensitivity and specificity, allowing it to be considered 'ideal', seropositive samples in screening tests should be evaluated using an established confirmatory and/or complementary strategy. It is recommended that all animals in an infected herd, including those that test negative during screening, should be successively evaluated using confirmatory tests.

Usually, specificity and sensitivity are inversely related. As sensitivity increases, specificity decreases, resulting in an increased number of false-positive results. Conversely, when sensitivity decreases, the proportion of false negatives increases. To diagnose and control brucellosis at the herd level, only a few serological tests with adequate sensitivity and specificity should be used, and these should be chosen based on the epidemiological features of brucellosis.

The different characteristics of tests and the types of herds or flocks to be monitored become crucial as the control programme progresses. When the prevalence of infection is high, a test with adequate sensitivity but high specificity is desirable, in order to allow efficient detection of the majority of diseased animals and herds and minimise the number of false-positive reactors. In contrast, in the final stages of eradication, when only a small number of false-positive reactions can be tolerated, a sufficiently specific but highly sensitive test is recommended. Since brucellosis is a herd or flock problem, the identification of one or more infected animals is sufficient evidence that the infection is present in the animal population, suggesting that other serologically negative animals may be incubating the disease.

At the final eradication stage, it is usually not cost effective to test all eligible animals and the surveillance programme should focus on problem herds, incidents of abortion, herds adjacent to known infected herds, and off-farm testing in markets and slaughterhouses (10).

The World Organisation for Animal Health (OIE) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (20) is the basis for identifying official tests for use in surveillance programmes. The *Manual* describes standardised and validated serological methods which have been designated as prescribed or alternative tests for international trade. It also indicates standards for comparison with respect to expected diagnostic performance.

However, any country may integrate its control and surveillance programmes with other diagnostic tests, for which sensitivity and specificity have been determined.

In 2006, the European Food Safety Authority (EFSA) was requested by the European Commission to give a scientific opinion on the suitability of current and new tests for

diagnosing animal brucellosis in intra-Community trade (9). Tests were designated as complementary or standard, with complementary tests not being allowed for use in intra-Community trade. In contrast, standard tests are tests conducted on individual animals and these have been approved for intra-Community trade.

Standard tests must have acceptable diagnostic sensitivity and specificity, and must perform equally as well as, or better than, the standard tests currently in use in the European Union.

Complementary tests do not qualify as standard tests, but have performance characteristics that support standard tests in brucellosis-free herds or herds that are officially free of brucellosis.

Scientific data on diagnostic tests for brucellosis in cattle, sheep and goats were reviewed and compared, using a meta-analysis approach, based on multiple sources of information. The scientific report examined the role of specificity for a given application. For cattle, the EFSA conclusions and recommendations were that the Rose Bengal test (RBT), complement fixation test (CFT) and indirect enzyme-linked immunosorbent assay (iELISA) demonstrated comparable performances, and were suitable as standard tests. The CFT also has a standardised system of measurement. The fluorescence polarisation assay (FPA) demonstrated sensitivity and specificity comparable to that of standard tests and was found to be suitable for intra-Community trade as a standard test.

As the iELISA and FPA are simpler to perform and have similar diagnostic performances to those of the other standard tests, they may be preferred.

In contrast, the serum agglutination test (SAT) was found to be unsatisfactory for international trade.

The review also found that the radial immunodiffusion test with native hapten (RIDNH) and the competitive ELISA (cELISA) could be suitable for intra-Community trade as complementary tests.

For sheep and goats, the RBT and CFT were found to be suitable to remain as standard tests. The RBT is useful for early detection of *B. melitensis*-infected flocks but it lacks specificity in low-prevalence areas and also lacks sensitivity, particularly in individual animals or when used in sheep.

However, the sensitivity of this test can be improved by increasing the amount of serum from 25 µl to 75 µl, while maintaining the volume of antigen at 25 µl – a modified RBT (MRBT).

The CFT is the most widely used confirmatory test for small ruminants, despite its complexity and the heterogeneity of techniques used. The MRBT, the iELISA, the cELISA, the FPA and the brucellin skin test (BST) were also found to be suitable for intra-Community trade in bovines as their sensitivity was found to be equal to that of standard tests. With the exception of the BST, these tests have specificity values that are lower than those of the standard tests or else their specificity has not been sufficiently documented.

With the exception of the BST, the MRBT, iELISA, cELISA and FPA were found to have unsuitable sensitivity and specificity for sheep and goats in intra-Community trade – at least until new data demonstrate that these tests are at least as specific as the standard tests. The sensitivity of RIDNH was lower than that of standard tests, so this test is also unsuitable for intra-Community trade (9).

In conclusion, serological surveillance of humans and animals is a cornerstone for brucellosis control. Surveillance can provide essential scientific information for informing decision-makers, stakeholders and consumers, and drive the most suitable strategy to limit brucellosis in susceptible hosts. Serological surveillance can be exploited for three different purposes:

- for epidemiological studies
- to determine suitable control programmes
- to monitor the efficacy of control programmes based on vaccination.

In this context it is important to understand the reliability of the acquired information. The quality of the information acquired by serological surveillance is influenced by, and depends on, the capability of the Veterinary Services within the targeted area. Information is reliable if these Veterinary Services are able to effectively control animal movement and trade and gather detailed information on the presence and numbers of animals within the targeted area.

An effective animal identification system alongside the ability to control animal movement is highly encouraged if the surveillance programme is to depend upon a test-and-slaughter strategy. This may be less important if the scope of the surveillance is just for epidemiological assessment.

Épidémiosurveillance de la brucellose

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Résumé

La brucellose est l'une des maladies infectieuses les plus importantes dans le monde. Or, bien qu'étant endémique dans de nombreuses régions, elle ne fait pas partie des priorités des systèmes de santé nationaux et internationaux, ce qui fait d'elle une zoonose négligée. Les mesures destinées à prévenir et à contrôler la brucellose sont basées sur des méthodes directes qui visent à minimiser le risque de propagation de l'infection dans les populations animales. Prises ensemble, ces mesures tendent à réduire l'exposition des animaux à *Brucella* spp. et à renforcer la résistance des animaux sensibles à l'infection. Une condition essentielle à la mise en œuvre d'une stratégie efficace de lutte contre la brucellose est la collecte d'informations détaillées concernant la présence de la bactérie dans un territoire donné. La priorité doit donc être accordée à la surveillance active

au moyen de méthodes sérologiques. La surveillance sérologique fournit des informations utiles pour déterminer les structures épidémiologiques à l'œuvre et pour évaluer l'impact de la brucellose dans la zone étudiée, ce qui permet ensuite de rechercher les méthodes les plus adaptées pour maintenir la maladie à un niveau acceptable.

Mots-clés

Brucella – Brucellose – Diagnostic – Santé animale – Sérologie – Surveillance – Zoonose.



Vigilancia epidemiológica de la brucelosis

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Resumen

La brucelosis es una de las enfermedades infecciosas más importantes en todo el mundo, pese a lo cual, y dado que en muchas regiones donde es endémica los sistemas nacionales e internacionales de salud no le otorgan prioridad, se considera que es una zoonosis desatendida. Las medidas destinadas a prevenirla y combatirla reposan en métodos directos, que apuntan a reducir al mínimo el riesgo de propagación de la infección entre los animales. Tomadas en conjunto, esas medidas tienden a reducir el grado de exposición de los animales a las distintas especies de *Brucella* y a conferir a los animales susceptibles mayor resistencia a la infección. Para aplicar una estrategia eficaz de lucha contra la enfermedad es esencial disponer de información detallada sobre la presencia del patógeno en un determinado territorio. Por este motivo hay que hacer especial hincapié en la vigilancia activa con métodos serológicos. La vigilancia serológica proporciona información útil para ayudar a entender los patrones epidemiológicos y evaluar el impacto de la brucelosis en la zona estudiada, allanando así el camino para definir los planteamientos idóneos para mantener la enfermedad dentro de límites aceptables.

Palabras clave

Brucella – Brucelosis – Diagnóstico – Sanidad animal – Serología – Vigilancia – Zoonosis.



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