BRUCELLOSIS MELLITENSIS: DIAGNOSIS, SURVEILLANCE AND CONTROL

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Brucella spp. is the cause of the most common bacterial zoonosis disease with more than 500,000 new cases annually. Although it is endemic in many countries in the world, it is frequently under-diagnosed and under-reported. Although Brucella abortus has been successfully controlled or even eradicated in many countries, B. melitensis in small ruminants remains a common cause of disease. Due to the variable management practices practiced with small ruminants, food and hygiene practices are often suboptimal, increasing the incidence of human brucellosis as a result. In addition, diagnosis and surveillance of B. melitensis is often more challenging than with B. abortus. Control strategies are predominantly based on reducing prevalence and disease in susceptible animals, thus limiting the spread of organisms between and within flocks and herds using long term vaccination as the primary tool. Control of B. melitensis is based on determining the seroprevalance within a region using appropriate diagnostic testing under proper biosafety conditions and implementing risk-based vaccine programs. This presentation will address the proper methods for determining an accurate and representative survey of livestock, determining the appropriate diagnostic tests to be used in surveillance and the risk-assessment that is required for an efficacious vaccination strategy to be used.

Introduction and Literature Review. Brucellosis has been recognized as a cause of disease in animals and humans since ancient times. Brucellosis as a zoonotic disease causes both acute and chronic disease in humans often leading to severe debilitation and disability. The exact incidence of human brucellosis is unknown as infection and disease is frequently under-diagnosed and under-reported. However, it's been speculated that approximately half a million new human cases occur annually depending on the geographic location. Because human-to-human transmission is rare, most brucellosis in humans is attributed to either animal handling or consumption of infected milk or animal products. In animals, infection with *Brucella spp.* typically causes abortion and loss of production. Uncontrolled brucellosis can result in significant economic losses to producers. Most ruminants can be infected with more than one species of brucella. *B. melitensis* is the main cause of brucellosis in sheep and goats. It also is one of the most frequent *Brucell* organisms that cause infection and disease in humans. The primary source of infection for goats and sheep are fetal fluids and vaginal discharges following abortion or parturition. *B. melitensis* is also secreted in milk and semen.

A number of countries have expended significant resources and eradicated *Brucella abortus*. In contrast, *B. melitensis* control and eradication has been neglected due to the management styles associated with raising sheep and goats in small herds. In addition, small ruminant production is generally associated with low-income farming in marginal lands. The incidence of infection with *B. melitensis* is much less defined, and while some countries have never reported its presence, it also has been difficult to eradicate. However, diagnostics and intervention strategies are sufficiently validated to effectively fight *B. melitensis* in sheep and goats. What needs to be improved is the quality of the methodologies utilized by the veterinary services and administration. While sheep and goats are the primary reservoir of *B. melitensis*, other animals such as cattle, buffalo, camels, and yaks can also be infected. The knowledge of the incidence of infection in these animals is even more lacking.

Surveillance strategies. Animal disease surveillance is rapid evolving and undergoing considerable innovation due to the recognition of the frequency and impact of emerging diseases, increasing levels of international trade, increased consolidation of animal production, and threats of agroterrorism. As a result, there has been a demand for more powerful tools for identifying changes in disease patterns and detection. Disease surveillance is a management information tool undertaken to measure disease events in defined populations for decision making. The cornerstone of any epidemiologic endeavor is case definition. Accurate case definition is typically defined by the sensitivity and specificity of the diagnostic assay used to differentiate an infected population from a non-infected population. Determining the best assay to identify a "case" is critical to successful control and/or eradication of an organism. Understanding the methods used to determine the sensitivity and specificity of an assay is crucial in determining the potential long term success of your surveillance as well as control strategies. Risk-based surveillance is used to describe the use of limited surveillance resources that are applied where the risk is the greatest to increase the economic return. Veterinary services must select an approach compatible with the current socioeconomic conditions and infection status within a country or region. With this type of surveillance, sampling design and testing protocols are weighted to yield the most valuable mix of disease information. Effective implementation of risk-based or targeted surveillance strategies is reliant on valid epidemiological information that identifies the risk profile of the population involved. Aspects to be included are: knowledge of the local animal breeding practices, agreement regarding the strategy with the local administrators, and the availability of the people to carry out the work. Animal surveys should be combined with epidemiologic surveillance to detect human brucellosis in medical centers, which can provide important data in targeting the areas to be surveyed.

To begin a control program, a baseline seroprevalance survey using statistical methods to ensure adequate representation in the targeted areas is the first step. Following development of a control strategy, which usually includes vaccination (discussed later), but could consist of test and slaughter if resources are available, a surveillance system for early warning of change in disease status or spread to new regions is important.

Diagnostic Assays. The specificity and sensitivity of a diagnostic assay is usually determined by comparing it to a "gold standard" which determines accurately whether an animal is infected or not. For some diseases, there is no gold standard for diagnosis of infection. For brucellosis, culture of the bacterium is considered the gold standard to which all other assays are compared. Due to the inherent zoonotic risks of culturing *Brucella spp.*this is not a practical screening test. Biosafety training is of paramount importance when tissues or samples with the potential of being infected with *Brucella* due to the zoonotic potential and the high rate of laboratory infection.

Sensitivity is the probability that the test correctly identifies infected animals: true positives/(true positives + false negatives). For example, a test with a 80 % sensitivity would correctly identify an average of 80 % of infected animals as test-positive and would incorrectly identify 20 % as non-infected. The term sensitivity in diagnostic assays is also used to describe the minimal or lowest detection ability of the assay. This is tied to the overall sensitivity of the assay as accurate detection of low numbers of antibodies or organisms is critical for accurate testing.

Specificity is the probability that the test correctly identifies non-infected animals: true negatives/(false positive + true negatives). A test with a 90 % specificity would correctly classify 90 % of non-infected animals on average as negative and falsely classify 10 % of the animals as infected (false positives). Specificity is also used to describe the likelihood of cross-reactivity with other pathogens. For example, assays that detect *Brucella*-induced antibodies do not differentiate between the species and may detect antibodies toother irrelevant bacteria.

As expected, high diagnostic sensitivity and specificity is desired for assays. But often the need for higher sensitivity or specificity is needed. For example, a *Brucella*-negative breeder would desire a 100% sensitivity to prevent infection from entering his herd. High specificity is needed in eradication programs where economic losses from false positives can be substantial.

For surveillance and determination of incidence of infection in a country or region, serology is the most convenient tool. Serological diagnostic tests have not been developed specifically for *B. melitensis* so assays for *B. abortus* that have been adapted to detect *B. melitensis* are typically used. Research documenting the sensitivity and specificity of these assays for *B. melitensis* is minimal. The Rose-Bengal (RB) and complement fixation (CF) tests that were developed for *B. abortus* are the most common assays used in the diagnosis of *B. melitensis* exposure. The current assays used most commonly in brucellosis surveillance do not differentiate between antibodies generated from infection and those generated from vaccination (this will be discussed later). There are also indirect enzyme-linked immunosorbent (iELISA) assays available. It is critical that the tests used in sero-surveillance are standardized according to OIE requirements. Specificity of these assays, the likelihood of false positives, is considered low due to their inability to differentiate vaccinated animals from infected animals. In addition, antibodies to a number of other bacteria may cross-react in the assay resulting in false positive results. The RBT assay is good for screening flocks or herds for antibodies, but lacks specificity in low prevalence areas and sensitivity on an individual level, especially for sheep. The CF assay is most commonly used as a confirmatory test, but overall appears to have the same sensitivity as the RBT and iELISA. Using the RBT and the CF assay in parallel increases the sensitivity of the diagnosis as any animal positive with either test is considered positive. If both tests are used in a series, only animals positive with both tests are considered positive. Newer assays being used are the Fluorescent Polarization Assays (FPA) and Time-Resolved Fluorescent Energy Transfer Assays have also been developed. The use of polymerase chain-reaction (PCR) is useful to identify the species of *Brucella* present and can be done directly from samples which can preclude the necessity and concerns associated with culture.

Vaccines and Strategies. Control or eradication of *Brucella spp.* is typically dependent on the use vaccines to reduce the transmission between animals and humans. While test and slaughter is the only method to eradicate brucellosis, for most countries, there are insufficient resources to use this with *B. melitensis*. Currently, the only vaccine available for use against *B. melitensis* is Rev-1. Effective vaccines to protect against brucellosis are all currently attenuated, live vaccines, whether it is for *B. abortus* in cattle or *B. melitensis* in small ruminants. As a result, care must be taken so that the person administering the vaccines does not inadvertently become infected from the vaccines. Suggested biosafety measures would include safety glasses and gloves, which are often impractical under field situations. Rev-1 is administered by either the conjunctival or subcutaneous routes. Administration of Rev-1 by the conjunctival route results in effective immunity; however, the level of antibodies induced is lower making it potentially possible to differentiate vaccinated from infected animals. Vaccination using the subcutaneous route results in long-lasting antibody levels making differentiation between vaccinated and infected animals impossible. Vaccination of pregnant animals can result in abortion, so vaccination is not recommended mid-gestation. Conjunctival vaccination prior to the mating season, late in lambing or during lactation is the safest course for mass herd vaccination.

There are several strategies for vaccination. One involves complete herd vaccination every two years, which may result in mass abortions depending on the timing of vaccination in gestation. Another mass vaccination strategy would be one time mass vaccination and then careful identification and vaccination of replacement animals in subsequent years. This option often fails in countries with large nomadic populations. Other strategies include vaccinating young animals, but again that is dependent on the management style of the producer. Control in countries in which *B. melitensis* is endemic is important for the health of their populations. New vaccine technologies are needed to control *Brucella spp.*and unfortunately; most developed countries have either controlled or eradicated brucellosis so the low-income countries suffer. Hopefully, as new vaccine technologies become more cost efficient, new *Brucella* vaccines will be developed that will be able to differentiate infected animals from vaccinated animals and will help control this disease throughout the world.

Conclusions. *B. melitensis* remains a serious health risk in much of the world. The incidence of brucellosis in humans is typically under-diagnosed and under-reported. Because the infected population of animals is sheep and goats, which are often the primary livelihood of less affluent cultures, control strategies become more problematic. Because *B. melitensis* is not a major disease problem in much of the developed world, little research is ongoing to produce improved diagnostics or vaccines. It is critical that new technologies are used to control this important disease as they become available and cost-effective.

References

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БРУЦЕЛЬОЗ *MELITENSIS*: ДІАГНОСТИКА, МОНІТОРИНГ ТА КОНТРОЛЬ

Текер Е.

Сільськогосподарське представництво Сполучених штатів Америки, Белтсвіл, США

Види Brucella спричиняють найбільш відоме бактеріальне зоонозне захворювання. Більш ніж 500 000 нових випадків захворювання реєструється кожний рік.

У той час, як Brucella abortus успішно контролюється та навіть викорінена у багатьох країнах, то B. melitensis залишається розповсюдженим збудником захворювання у дрібної рогатої худоби.

У роботі розглядаються належні методи щодо достовірного обстеження худоби, визначення відповідних діагностичних тестів для спостереження та оцінки ризиків, що має важливе значення для використання ефективної стратегії вакцинації.