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Review Article

Diagnostic Approaches for Detection of Brucellosis in Dairy Animals

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A B S T R A C T

Brucellosis is a disease that causes severe economic losses by great reproductive losses in dairy animals. Abortion, placentitis, epididymitis, and orchitis are the most common clinical manifestations in animals. Clinical diagnosis of brucellosis is not easily achieved. Laboratory testing is therefore very important for a correct identification of the disease, detection and confirmation in animals. Definitive diagnosis is normally done by isolation and identification of the causative agent. While definitive, isolation is time-consuming, must be performed by highly skilled personnel, and it is hazardous. For these reasons, serological tests are normally preferred. Brucellosis serology has advanced considerably in the last decades with very sensitive and specific new tests available. Modern genetic characterizations of Brucellae using molecular DNA technology have been developed. Several PCR-based assays have been proposed, from the rapid recognition of genus to differential identification of species and strains. This review describes bacteriological, serological, and molecular methods used for the diagnosis of animal brucellosis.

Introduction

Brucellosis is a disease that causes severe economic losses for livestock farms in India. It is an infectious bacterial disease caused by member of genus Brucella. Brucella is obligate parasite requiring an animal host for maintenance. Brucella is small gram negative cocco bacilli measuring 0.6 to 1.5µm x0.5 to 0.7µm in size. They are typically arranged singly but also occur in pairs or clusters. No capsule, flagella or spores are produced. The A and M antigen are found in varying concentration among the different Brucella species. The classical Brucella species are B. abortus, B. melitensis, B. suis, B. ovis, B. neotomae, and B. canis. More recently, B. pinnipedialis and B. ceti were described (McDonald, *et al., 2006*), followed by B. inopinata and B.

microti (Scholz, *et al., 2010*). The different Brucella species exhibit host preferences and vary in severity of disease caused. Infection tends to localize to the reticuloendothelia system and genital tract with abortion in female and epididymis and orchitis in males the most common clinical manifestation. Appropriate samples for diagnosis of brucellosis depend upon the animal species affected, species of Brucella involve and clinical presentation. Abscess material, semen and vaginal fluid associated with recent abortions are useful for recovering organism anti mortem. Milk samples are used in anti- mortem isolation attempts and for immunodiagnostic evaluation. Serum is used for serological evaluation. Samples collected at necropsy should include spleen liver udder and multiple lymph node including supra mammary lumber, internal iliac and mesenteric lymph nodes. All specimens must be packed separately and transported immediately to the laboratory cooled or preferably frozen in leak proof containers.

Diagnostic procedures

Basic techniques

For the staining of Brucella organism smears prepared from fetal membranes, fetal stomach contents, vaginal swabs, semen, etc. The most common methods in use are the modified Ziehl-Neelsen and the modified Köster (Alton *et al.,* 1988) *Brucella* species are not truly acid-fast, but they are resistant to decolorization by weak acids, and stain red against a blue background **(**Fernando *et al.,* 2010**)**. Care must be taken as *Coxiella burnetii* and *Clamydophila abortus* may superficially resemble *Brucella* (Alton *et al.,* 1988**)**. For the culture of Brucella a wide range of commercial dehydrated basal media is available, e.g. *Brucella* medium base, tryptose (or trypticase)–soy agar (TSA). Serum–dextrose agar (SDA) or glycerol dextrose agar can be used (Alton, 1988). SDA is usually preferred for observation of colonial morphology. A nonselective, biphasic medium, known as Castaneda's medium, is recommended for the isolation of *Brucella* from blood and other body fluids or milk, where enrichment culture is usually advised.

Advance techniques

Serological tests

No single serological test is appropriate in all epidemiological situations; all have limitations especially when it comes to screening individual animals (Nielsen, *et al.,* 2006). Sometimes false positive reactions may be expected among the vaccinated animals because of antibodies cross-reacting with wild strain infection. Serological methods represent a standard of comparison with respect to expected diagnostic performance.

Serum agglutination test (SAT) is generally regarded as being unsatisfactory for the purposes of international trade. Diagnostically the complement fixation test (CFT) is more specific than the SAT, and also has a standardized system of unitage. The fluorochrome or peroxidase-labelled antibody conjugate based technique could also be used. (Alton G.G., 1988**)** In the comparison with CFT the diagnostic performance enzyme linked immune sorbent assays (ELISAs) and the fluorescence polarization assay (FPA) are better. ELISA and FPA are technically simpler to perform and more robust, their use may be preferred (Wright, *et al.,* 1997). The buffered *Brucella* antigen tests (BBATs), i.e. the Rose Bengal test (RBT) and the buffered plate agglutination test (BPAT), as well as the

ELISA and the FPA, are suitable screening tests For the control of brucellosis at the national or local level. Positive reactions should be retested using a suitable confirmatory and/or complementary strategy. The same serological procedures may be used for other species of animals like buffaloes (*Bubalus bubalus*), American and European bison (*Bison bison, Bison bonasus*), yak (*Bos grunniens*), elk/wapiti (*Cervus elaphus*), and camels (*Camelus bactrianus* and *C. dromedarius*), and South American camelids, *Brucella* sp. because infection follows a course similar to that in cattle (Nicoletti P. (1992), but each test should be validated in the animal species under study (Gall, *et al.,* 2001).

Method for recognition of Nucleic acid

Polymerase Chain Reaction (PCR) is very advance technique to differentiate different species of Brucella. Different type of PCR is used to differentiate high degree of DNA homology within Brucella species. To extent, differentiation between *Brucella* species and some of their biovars several molecular methods, including PCR, PCR restriction fragment length polymorphism (RFLP) and Southern blot, have been developed because there is high degree of DNA homology within the genus *Brucella* (Bricker (2002). Real time PCR provides an additional means of detection and identification of *Brucella* sp. (Bricker (2002))

IS*711* real-time PCR assay is a promising way to accurately detect *Brucella* spp. infections in wild boars so IS*711* real-time PCR as a complementary tool in brucellosis screening programs and for confirmation of diagnosis in doubtful cases (HINIĆ V., *et al.,* 2008). Multiple PCR assay for the identification and differentiation of all Brucella species and the vaccine strains Brucella abortus S19 and RB51 and Brucella melitensis Rev1. (García-Yoldi, *et al.,* 2006). Bricker B. J. and Halling (1994) described first species-specific multiplex PCR assay for the differentiation of *Brucella*. AMOS-PCR, an improved version of polymerase chain reaction was used for evaluation of the Brucella abortus species-specific polymerase chain reaction assay, for cattle (Bricker *et al.,* (2003)) based on the polymorphism arising from species-specific localization of the insertion sequence IS711 in the *Brucella* chromosome, and comprised five oligonucleotide primers that can identify without differentiating *B. abortus*, biovars 1, 2 and 4 but could not identify *B. abortus* biovars 3, 5, 6 and 9. To improve performance modifications to have been introduced over time, and additional strain-specific primers were incorporated for identification of the *B. abortus* vaccine strains, and other biovars and species (Bricker, *et al.,* 2003; Ocampo-Sosa, *et al.,* 2005).

Pulse-field gel electrophoresis has been developed that allows the differentiation of several *Brucella* species (Jensen, *et al.,* 1999). For rapid and simple one-step identification of *Brucella* a new multiplex PCR assay (Bruce-ladder) has been proposed (Garcia-Yoldi *et al.,* 2006).

It can identify and differentiate in a single step most *Brucella* species as well as the vaccine strains *B. abortus* S19, *B. abortus* RB51 and *B melitensis* Rev.1.

Vaccine strains S19, Rev.1 and RB51 may also be identified using specific PCRs. (Garcia-Yoldi, *et al.,* 2006). Basic techniques and serological techniques are easy to detect brucellosis in field condition but confirmatory identification can be done with the help of molecular techniques like PCR by identification of specific genes.

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