SEROLOGICAL DIAGNOSIS OF BRUCELLOSIS

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A b s t r a c t: *Aim*: To present a review and to describe the most widely used laboratory tests for serology diagnosis of brucellosis along with their pros and cons.

Methods: Review the recent literature on brucellosis serology diagnostic tests. The choice of the testing strategy depends on the prevailing brucellosis epidemiological situation and the goal of testing.

Results: The 'gold standard' for the diagnosis of brucellosis is isolation and identification of the causative bacterium, a member of *Brucella sp*. Isolation of *Brucella sp.* requires high security laboratory facilities (biological containment level 3), highly skilled personnel, an extended turnaround time for results and it is considered a hazardous procedure. Hence brucellosis is generally diagnosed by detection of an elevated level of antibody in serum or other body fluid. This is a presumptive diagnosis as other microorganisms and perhaps environmental factors can also cause increased antibody levels.

Conclusion: A large number of serological tests for brucellosis have been devised over the 100+ years since its initial isolation, starting with a simple agglutination test and progressing to sophisticated primary binding assays available today. However, no test devised to date is 100% accurate so generally serological diagnosis consists of testing sera by several tests, usually a screening test of high sensitivity, followed by a confirmatory test of high specificity.

Key words: Brucellosis, serology, diagnosis, conventional tests, primary binding assays.

Introduction

Intermittent fever in man has been recognized in the Mediterranean area since Hippocrates described it in 450 BC. Further evidence of the presence of the disease in the area was presented by Capasso [1], finding typical brucellosis

lesions in the bones of people killed in the eruption of Vesuvius in 79AD as well as the presence of coccoid cells consistent in morphology with *Brucella sp.* in carbonized cheese. These findings are in keeping with the nature of Roman diets of the day, containing both milk and cheese derived from small ruminants. Sir William Burnett, surgeon general to the British navy, differentiated the various fevers affecting British troops sent to Malta to recuperate in 1810. A British army surgeon, Jeffery Marston, contracted the disease and described his own symptoms in considerable detail in 1861. Sir David Bruce, a medical officer of the British army and after whom *Brucella* was later named, provided the first description of this pathogen. Considerable morbidity and at least one case of mortality of British soldiers stationed at garrison on Malta arose as it turned out because of consumption of fresh goat's milk. Dr. Bruce organized a team of scientists and clinicians who succeeded in isolating *Micrococcus melitensis* as the causative agent of the problem [2, 3]. The organism was later renamed *Brucella melitensis*. These findings helped to explain the epidemiology of the disease. For example, private soldiers were less likely to become ill because they drank less milk than the officers. Other species of *Brucella* include *B. abortus* isolated by Bang in 1897 [4], resulting in the term Bang's Disease and *B. suis* first described by Taum [5]. In terms of human public health and agricultural economics, these three species are the most important. There are several other species, including *B. ovis, B. canis, B. neotomae, B. microti* and of which only *B. canis* has been reported to infect man. Two other, *B. ceti* and *B. pinnipedialis* infect marine mammals and are potential human pathogens as well.

Brucella ovis and *B. canis* contain rough lipopolysaccharide (RLPS) in their outer cell wall whereas all the other species contain smooth lipopolysaccharide (SLPS). Smooth lipopolysaccharide contains a lipid A anchor to the cell wall, in the intermediate core region, and an immunodominant O-polysaccharide (OPS) which has been chemically defined as a homopolymer of 4,6-dideoxy-4-formamide-alpha-D-mannose linked via glycosidic linkages [6]. *B. ovis* and *B. canis* lack the OPS component [7]. Because all smooth species share common epitopes in the OPS, virtually all serological tests for an antibody to these bacteria use *B. abortus* antigen in the form of whole cells, SLPS or OPS [8] while RLPS is commonly used as the main antigen for detection of antibody to *B. ovis* and *B. canis* [7, 9]. Most recently developed tests use either SLPS or OPS antigens although some attempts at using protein antigen have been made.

Cattle infected with *B. abortus* generally produce an early IgM isotype antibody response, the amplitude of which is governed by a multiplicity of factors. It usually appears 5 to 15 days post exposure but may be delayed [10–12]. The IgM antibody response is followed very shortly by production of IgG1 isotype of antibody and subsequently by IgG2 and IgA [11–15]. Because of the IgM response commences early, theoretically it would be most suitable to measure this isotype as an indicator of exposure. There is, however, a number of other microorganisms containing antigens with epitopes similar to those of OPS

and the main antibody response to these cross-reacting antigens is IgM [16]. Therefore, measurement of IgM antibody may result in a false positive reaction in serological tests. False positive reactivity would lead to specificity problems which would be of considerable consequence in an early control programme resulting in unnecessary slaughter; in the last stages of an eradication programme and in free areas, resulting in expensive follow-ups. Production of IgG2 and IgA isotypes occurs later in infection and, as a result, measurement of these antibodies would generally lower assay sensitivity. Based on these observations, the most useful antibody for serological testing for brucellosis is IgG1 [12, 15, 17, 18].

An antibody produced in response to smooth vaccines may also result in positive serological reactions which may lead to misdiagnosis. Specifically, *B. abortus* S19, a vaccine used in many areas, may be retained over an extended period, causing problems [19]. Smooth vaccine SLPS is antigenically identical to that of pathogenic strains of *B. abortus;* however, administration of the vaccine to young animals, usually between 3 and 8 months of age or by the conjunctival route generally results in insufficient antibody levels to cause diagnostic problems by the time animals reach sexual maturity and are tested for brucellosis [20]. However, some animals do have residual antibody resulting in allowances for higher antibody levels in vaccinated animals. Most of these problems have been overcome by the development of improved serological tests, for example, the competitive enzyme immunoassay and fluorescence polarization assay [21] and the development of a live vaccine devoid of OPS (*B. abortus* RB51 developed by Schurig) [22].

Serological tests

Brucellosis was first diagnosed by a serological test by Wright and Smith in 1897 [23] using a simple tube agglutination test. Subsequently, various modifications to the tube agglutination test and numerous other tests have been developed to increase test accuracy. The procedures are divided into 2 categories, the Conventional Tests and Primary Binding Assays. All conventional tests rely on the antibody performing a secondary function, for instance fixation of complement while in primary binding assays the only function of the antibody is attachment to its antigen.

Conventional Tests

Agglutination tests:
Slow tests requiring incubation from 8 to 24 hours
- Standard tube (SAT)

- SAT with added reducing agents such as 2-mercaptoethanol or dithiothreitol
- SAT with addition of rivanol to precipitate glycoproteins
- SAT with addition of ethylene diamine tetraacetic acid to reduce IgM binding (EDTA)
- SAT with antiglobulin added to enhance agglutination
- Milk ring test

Rapid agglutination tests performed in minutes:

- Rose Bengal
- Modified Rose Bengal
- Buffered antigen plate agglutination
- Card
- Antigen with rivanol added
- Heat treatment of serum
- Addition of 10% sodium chloride

Precipitation tests:

- Agar gel immunodiffusion
- Radial immunodiffusion

Complement fixation tests:

- Warm
- Cold
- Haemolysis in gel
- Indirect haemolysis

Primary Binding Assays:

- Radioimmunoassay
- Fluorescence immunoassay
- Particle counting fluorescence immunoassay
- Indirect enzyme immunoassay
- Competitive enzyme immunoassay
- Fluorescence polarization assay

Each category of tests will be described and their performance will be discussed. There are variations on some of these tests and there are several other tests not commonly used which will be beyond the scope of this review.

Agglutination tests

In 1897, Smith and Wright [23] published the first description of a test for the serological diagnosis of brucellosis in man. This test used a mixture of bacterial cell antigens incubated with the patient's serum in a glass tube and if a 'mantle' pattern of cell sediment was observed, it was considered as an indication of infection while a 'button' pattern was considered as negative. This test is virtually the identical test still used in some countries, except that only *B. abortus* cells are used as the antigen. This test is performed at a near neutral pH and therefore detects IgM isotype of antibody efficiently and is therefore very sensitive. The SAT detects IgG less efficiently, especially IgG1, resulting in low assay specificity [13, 15, 24]. Therefore, the SAT is generally not used as a single test but rather in combination with other tests.

The production of IgM in response to cross-reacting antigens often induces significant levels of agglutinating antibody which causes specificity problems in the SAT. As a result, a number of modifications have been made to the SAT to lower the IgM levels thereby increasing the assay specificity. The most commonly used methods of IgM destruction is chemical treatment with 2-mercaptoethanol or dithiothreitol which reduce disulfide bridges in the molecule resulting in monomeric units of the pentameric molecule. The monomers are much less efficient agglutinins. Other methods include precipitation of glycoproteins using rivanol and addition of divalent cheating agents.

A number of rapid agglutination tests have been devised. Some of these tests use a stained whole cell antigen stored in an acid buffer. This antigen is mixed with undiluted serum resulting in an acid test environment which in turn discourages agglutination by IgM and enhances agglutination by IgG1. Other tests use heat-treated serum or a high salt concentration to diminish reactivity by IgM.

Agglutination tests are generally not used for the diagnosis of infection with *B. ovis* and *B. canis*, rough species of *Brucella*, as the whole cell antigens tends to autoagglutinate. However, rapid slide agglutination tests have been developed for the serological diagnosis of *B. canis* infection [25–28] as well as a microagglutination test [29].

Acidified antigen modifications

Because of the cross-reaction of the LPS of *B. abortus, B. melitensis* and *B. suis*, only one antigen is required for serological diagnosis. Virtually all agglutination tests use the *B. abortus* antigen although in some cases different strains are used. The Buffered Antigen Plate Agglutination test (BPAT) has

been widely used [30] as has the Rose Bengal test (RBT) [31]. In these tests, *B. abortus* S99 or S1119.3 cell antigen, stained with Rose Bengal or Brilliant Green and Crystal Violet, respectively, and suspended in a buffer which when mixed with the appropriate volume of serum results in a final pH of 3.65. After thorough mixing of the serum and antigen, agglutination must be visible be within the specified time for each test (4 minutes for the RBT and 8 minutes for the BPAT). Incubation for extended periods of time may sometimes result in false reactions, often due to the formation of fibrin clots. The acid pH diminishes agglutination by IgM but encourages agglutination by IgG1, generally reducing cross-reactions [12, 13]. False negative reactions can occur in the acidified antigen tests, especially in the RBT, due to prozoning with sera containing very high levels of antibody. These tests are considered as suitable screening tests for brucellosis, followed by confirmatory testing. Antibody resulting from *B. abortus* S19 vaccination will react in these tests [8].

Reducing agents

Dithiotreitol [32] and 2-mercaptoethanol [33] have both been used for the serological diagnosis of brucellosis. Either chemical may be added to serum as a diluent, using dilutions starting at 1 : 25 and increasing. For the diagnosis of brucellosis, a reaction at a 1 : 25 serum dilution is considered significant. In general, reduction of IgM increases specificity. However, some false negative reactions may occur as some IgG molecules are also susceptible to reduction of disulfide bridges, rendering them unable to agglutinate.

Care must be taken when using 2-mercaptoethanol as it is quite toxic and should only be used in a well ventilated area or a chemical hood. Test employing reducing agents are usually used as confirmatory tests, however, antibody resulting from *B. abortus* S19 vaccination may interfere [34].

Precipitation of glycoproteins

Reduction of non-specific reactivity by precipitation of high molecular weight serum glycoproteins has been applied to serological diagnosis of brucellosis [34, 35]. This is commonly done by addition of rivanol (2-ethoxy-6,9-diaminoacridine lactate) to serum followed by removal of the precipitate by centrifugation and either a rapid plate type agglutination test with undiluted serum or a tube test using serum dilutions starting at 1: 25. Because the protocol is fairly labour-intensive, precipitation tests are generally used as confirmatory tests.

Use of EDTA

Addition of ethylene diamino tetraacetic acid disodium salt (EDTA) has proven to significantly increase SAT specificity [36–38]. The mechanism by which EDTA reduces non-specificity is not understood; however, it appears to eliminate attachment of immunoglobulins to the *Brucella* cell wall via the Fc piece. The modified SAT may be used in tubes or 96 well plates and incubation is usually overnight after which the cell sediment pattern is observed. The modified SAT has been used mainly as a screening test.

Milk ring test

The agglutination test has been adapted to test milk for antibody to *Brucella sp.* The format of the milk ring test (MRT) is a little different in that haematoxylin stained *Brucella* cells are mixed with whole milk or whole milk with cream added [35, 39, 40]. Immunoglobulins present in the milk will in part be attached to fat globules via the Fc portion of the molecule. If antibody to *Brucella sp.* is present, antigen will attach to it, resulting in a purple band in the cream layer. If no antibody is present, the fat layer will remain a buff colour and the purple antigen will be evenly distributed throughout the milk. This test may be applied to individual animals or to pooled milk samples using a larger volume of milk relative to the pool size. The milk ring test is prone to false reactions caused by abnormal milk such as mastitic milk, colostrums and late lactation cycle milk. Still, in spite of its problems, it may be used as an inexpensive screening test in conjunction with other tests.

Precipitin tests

Precipitin tests were shown to distinguish *B. abortus* S19 vaccinal antibody from the antibody resulting from infection with pathogenic strains [41, 42]. There are two basic formats: agar gel immunodiffusion in which soluble antigen(s) and test serum are inserted into adjacent wells, cut in an agar matrix 0.5 to 1.0 cm apart. The reagents diffuse into the agar for a period of time, resulting in the formation of a visible precipitin band where they intersect if the serum contains antibody. The second format, radial immunodiffusion, utilizes antigen placed directly in the agar matrix, pipetting test serum in a well cut in the agar and allowing the serum to diffuse radially to form a precipitin ring if antibody is present in the serum. Both tests use OPS antigens derived from *B. melitensis* [41] or native hapten [43]. Both formats proved to be relatively insensitive with OPS antigen [44] while the sensitivity was better with native

hapten antigen [43]. The tests are quite labour-intensive but provide results not available by any other test procedure at the time. Neither test is currently used extensively.

Precipitin tests are widely used for the diagnosis of *B. ovis* infection in sheep using RLPS or hot saline extracted antigens [8].

Complement fixation tests

The complement fixation test (CFT) requires a multitude of reagents and is technically challenging. However, in spite of this, it is a widely used confirmatory test for brucellosis. The basic test consists of *B. abortus* whole cell antigen incubated with dilutions of heat-inactivated serum (heated to destroy indigenous complement) and a titrated source of complement, usually guinea-pig serum. After a suitable time a pretitrated amount of sheep erythrocytes coated with rabbit antibody is added. If a primary immune complex (*B. abortus* cells and test serum) formed due to the presence of certain antibody isotypes mainly IgG1, in the serum, complement was activated and therefore not available to react with the secondary immune complex of sheep erythrocytes and rabbit antibody, resulting in no or only slight lysis of the erythrocytes. Alternately, if no primary immune complex was formed, complement would cause all the sensitized sheep erythrocytes to lyse. Thus the amount of haemoglobin in solution is a measure of anti-*Brucella* antibody activity. The complement fixation assay has been standardized [45, 46].

Because a number of reagents must be titrated daily and a number of controls for all the reagents and reactions are required, the test is time-consuming and technically challenging. It is also an expensive test because of the number of reagents used in the test and because it is labour-intensive, especially the daily titration routines. Since only IgG1 isotype of antibody fixes complement efficiently, the test specificity is high. The test does not allow for discrimination of *B. abortus* S19 derived antibody. Other problems include the subjectivity of the interpretation of results, occasional direct activation of complement by serum (anticomplementary activity), prozoning resulting in false negative results and the inability of the test for use with haemolysed serum samples. In spite of the shortcomings, the (CFT) has been and is a widely used as a confirmatory test in control/eradication programmes.

There are a number of variations of the test, including the indirect haemolysis test and the haemolysis in gel test [47–51]. These tests were not used extensively as diagnostic tests.

The (CFT) using a hot saline extracted antigen preparation has been used for the diagnosis of *B. ovis* infection in sheep [52–55].

Primary Binding Assays

Indirect formats

Indirect primary binding assays rely only on an antibody present in the test serum (or other body fluids) reacting with its antigen and then detection of the immune complex using a detection system with a 'marker' molecule. The tracer system usually comes in one of three formats: antiglobulins or bacterial cell receptors labelled with isotopes [48, 49, 50, 56–59]; fluorochromes [60–67] or enzymes (described initially by Carlsson et al, 1976) [68] and reviewed by Nielsen and Gall, 1994 [69–82].

The most commonly used system depends on enzyme conjugates for detection of antibody to SLPS preparations which are passively attached to a polystyrene matrix (usually in a 96 well format) to which diluted serum or milk is added. The detection system varies considerably but often a monoclonal antibody specific for an immunoglobulin heavy chain epitope of the test species and conjugated with peroxidase is used. Variation in the detection system includes the use of cellular receptors such as protein A, protein G, protein A/G or polyclonal anti-immunoglobulin reagents. Alkaline phosphatase or other enzymes can be used as well. Peroxide is the substrate used for peroxidase enzyme and a number of different chromogens (hydrogen acceptors) are available including ABTS and TMB.

A multistep washing procedure is used between each stage of the assay.

A number of other antigens have been used, including RLPS, used mostly for the diagnosis of *B. ovis* and *B. canis* infection [55, 81–86]. Numerous protein antigens have also been employed with various success in indirect assays [87–98].

The indirect enzyme immunoassays generally have very high sensitivity but because they are largely unable to distinguish *B. abortus* S19 vaccinal antibody and cross reacting antibody, the specificity can be slightly lower. These assays are available as commercial kits from numerous sources and while there is some variation in their accuracy, the kits as well as individually developed assays are excellent screening assays for the diagnosis of brucellosis, especially in individual animal tests of serum or milk.

Competitive immunoassays

There are two types of competitive assays used for brucellosis serology. In both cases, antigen is immobilized, a competing antibody, specific for OPS, with or without an incorporated detection system, is added at a predetermined dilution, followed by diluted test serum and in some cases by a separate detection system.

The particle concentration fluorescent immunoassay has been widely used only in the US [99,100]. Antigen coated polystyrene beads are added to test serum and polyclonal *Brucella* specific antibody labelled with a fluorochrome. Excess reagents are removed with washing through a filter in the bottom of the 96 well plate. The amount of fluorochrome labelled antibody attached to the beads is inversely related to the amount of antibody present in the serum. This assay can be automated for high throughput.

A second and more widely used competitive assay type uses SLPS passively immobilized in 96 well polystyrene plates. Competition between a monoclonal antibody specific for a common epitope of OPS and test serum, both appropriately diluted and added to the well, takes place. The monoclonal antibody may be labelled directly with enzyme or a secondary anti-mouse antibody labelled with enzyme may be added [75, 101–118].

Competitive enzyme immunoassays were developed in order to overcome some of the problems arising from residual *B. abortus* S19 vaccinal antibody and from cross reacting antibody. By selecting a monoclonal antibody with slightly higher affinity for the antigen than most of the vaccinal/cross-reacting antibody but with lower affinity than most antibody arising from infection, reactivity by vaccinal antibody could be eliminated in the majority of cases. The specificity of the competitive enzyme immunoassay is very high; however, it is slightly less sensitive than the indirect enzyme immunoassay. This assay is an excellent confirmatory assay for the diagnosis of brucellosis in most mammalian species. Competitive assay kits are available commercially from various sources.

Fluorescence polarization assay

The basis for the fluorescence polarization assay (FPA) is that the rate of rotation of a molecule in solution is inversely proportional to its size. A small molecule will rotate rapidly while larger molecules rotate more slowly. By attaching a fluorescing molecule to a small molecular weight antigen molecule, the time of rotation through a given angle can be measured using polarized light. For brucellosis serology, a small molecular weight subunit of OPS, labelled with fluoroescein isothiocyanate is used as the antigen. If antibody to the OPS is present in diluted serum, milk or whole blood to which the antigen has been added, the rate of rotation of the labelled antigen will be reduced. The rate of reduction is proportional to the amount of antibody present. The (FPA) was developed in 1996 [119] and has since been extensively validated [111, 119–127].

The (FPA) is a homogeneous assay, requiring no washing steps or removal of unreacted components. It can be performed in a 96 well format or in a tube format. The tube format can be used in the field for rapid diagnosis.

When testing the serum or milk, the incubation time is a minimum of 2 minutes while the whole blood assay requires a maximum of 15 seconds of incubation. Because only 2 reagents, antigen and diluent buffer are required, the test is technically simple and relatively inexpensive. It does require a fluorescence polarization analyzer of which several are available at various costs. Diagnostic kits are also commercially available from several sources at various prices and accuracy.

The (FPA) is very accurate and the sensitivity/specificity can be manipulated by altering the cut-off value between positive and negative reactions to provide a highly sensitive screening test as well as a highly specific confirmatory test. The FPA can distinguish vaccinal antibody in most vaccinated animals and it can eliminate reactivity by some cross-reacting antibodies as well.

Primary binding assays in general are highly sensitive and specific assays for detection of antibody in various species.

Their main advantages include:

- Electronic data assessment, precluding subjective observation errors;
- Easy data transmission;
- Adjustable sensitivity/specificity values depending on their use in disease control, eradication or surveillance;
- Commercial availability;
- Easily adapted to continuous quality control schemes;
- Some can distinguish vaccinal antibody;
- Most formats can be used to test multiple species of hosts;
- Some formats are rapid and may be used in the field;
- Some formats are simple to perform;
- Easily automated.

The main disadvantages include:

- Some commercial kits are very highly priced;
- Some commercial kits are more accurate than others;
- Expensive equipment but may be used for multiple tests;
- May be technically challenging due to high dilutions of reagents and multiple steps.

Other serological tests for brucellosis

The development of new test formats for serological diagnosis of infectious diseases is ongoing. The threat of bioterrorism has resulted in the infusion of funds for technology that detects minute quantities of biological agents. This

technology has also been applied to the serological diagnosis of brucellosis, resulting in tests that use modern technology for efficient antibody detection.

Fluorescence immunoassay using a capture and elution technique to measure antibody eluted from antigen with cyanine-5 was developed by Silva *et al*. 2004 [128]. This versatile, portable assay gave good specificity and sensitivity values at a low cost.

Chemiluminescence assays have also been developed both in a homogeneous format [118, 129] and in a format including washing procedures [129]. The homogeneous type of assay used a competitive based format in which 2 types of beads, a donor and an acceptor are pulled together by a reaction between their conjugates. Using laser excitation, singlet oxygen is formed in a positive reaction resulting in conversion to light emission by the acceptor. This assay was shown to have a performance index comparable to other primary binding assays. The assay format which included wash steps apparently did not improve assay performance.

Lateral flow assays have also been developed. These assays utilized coloured beads conjugated with a detection reagent for antibody bound to an immobilized antigen on a cellulose membrane matrix [130, 131]. This type of assay has a definite advantage because it requires no equipment for its performance*;* however, the interpretation is subjective, depending on the formation of a visible coloured line of reaction and the assay itself tends to be expensive because of the multiple ingredients/components included.

False Serological Reactors

Inaccurate serological results causing incorrect diagnoses are a continuous problem when testing for infectious disease agents in an outbred population of animals or in human beings. Because of the genetic diversity of populations, some animals will respond with low antibody levels to exposure to *Brucella sp*., resulting in false negative results. Other animals will respond with very high levels of antibody which may cause prozoning in some of the older assay types. High responders may also have elevated antibody levels to naturally occurring antibody caused by exposure to cross-reacting microorganisms. Exposure to cross-reacting microorganisms may also cause elevated antibody levels for various periods of time, some prolonged. Both scenarios will result in a false positive serological reaction, a major diagnostic problem in some areas where such microorganisms are endemic.

As described above, many modifications of various serological tests have been made to overcome the false positive reactor problem, some with limited success, some a little better. Virtually all serological tests for antibody to

smooth *Brucella sp*. use LPS, part of LPS or whole cells as the antigen. The immunodominant epitope on the surface of the smooth cell is O-polysaccharide (OPS) the outermost portion of LPS. O-polysaccharide is a homopolymer of 4-formamide-4,6-dideoxymannose. Most of the problems but not all arise from an immune response of the animal to another miccroorganism which shares epitopes with *Brucella sp.* OPS. The various cross-reactions have been reviewed in considerable detail by Corbel [132].

Many serological tests cannot distinguish these antibody responses, however, because often the cross-reacting antibody is of the IgM isotype, limiting the agglutinability of this antibody class somewhat diminishes the number of false positive reactors. Examples of IgM agglutination reduction include the use of dithiotreitol [133], 2-mercaptoethanol [33] and divalent cations [36].

A second line of reasoning has been to look for alternate antigens for serological tests. A number of protein antigens have been tried with limited success. For instance, *Brucella* Protein 26 (BP26) was cloned and the recombinant protein assessed for its value in the diagnosis of brucellosis. It was found to be of some potential using a western blotting method [134]. Further examination has demonstrated that while BP 26 may be useful, it requires combination with other tests for accuracy [135, 136]. Other candidate antigens include rough lipo-polysaccharide (RLPS) part of which is unique to *Brucella sp*. This antigen which is very hydrophobic and difficult to prepare was shown to be capable of some discrimination of antibody due to *Yersinia enterocolitica* O: 9 and other cross-reacting microorganisms [137–140]. Similarly, RLPS of *Yersinia sp*. was shown to eliminate *Brucella* cross-reacting antibody in some cases [138].

Skin testing using a protein antigen derived from *Brucella* (Brucellergene, Brucellin or equivalent) is another approach to elimination of false reactions. While skin testing has certain logistical drawbacks, the test, in combination with serological tests, can provide part of a sensitive and specific protocol for detection of infected animals, especially latently infected animals devoid of measurable antibody. It was shown to be able to eliminate most false positive serological reactors [141, 142], however, in a recent review [143], both *B. abortus* vaccinated animals and animals infected with cross-reacting microorganisms gave skin tests reactions for a period of time.

Another method of detection cell mediated immunity involves the measurement of cell proliferation or gamma interferon produced in response to antigenic stimulation of sensitized peripheral lymphocytes. Thus *Brucella* or *Yersinia* experimentally infected cattle could be clearly differentiated by either blastogenesis or kin testing while both gave measurable serological responses [144]. These results were disputed [145] using a Brucellergene gamma interferon production assay, however, in more recent studies, the gamma interferon test also using Brucellergene as the lymphocyte stimulant have been shown to

discriminate *Y. enterocolitica* O: 9 infection in pigs with high specificity compared to serological tests [146, 147].

Conclusion

Accurate diagnosis of brucellosis in any species is generally fairly straightforward but may be very difficult in some cases. The only finite diagnosis, the 'gold standard', is the recovery of the causative agent from the host. Because of inherent problems with isolation of *Brucella sp.*: inefficiency, cost, danger and other factors, most laboratories prefer to use other, more cost-effective methods. Molecular biology as a diagnostic tool is advancing and will soon be at the point of replacing actual bacterial isolation. It is rapid, safe and cost-effective, the only real problems being some uncertainties regarding specificity.

Serological tests for the diagnosis of brucellosis have advanced considerably since their inception by Wright and Smith in 1897. The accuracy of modern assays has improved diagnosis resulting in more efficient control of the disease. However, the perfect test has still not been developed and probably never will be. In the meantime, the use of a vaccine that does not interfere with most serological tests and the validation and extensive use of primary binding assays has made diagnosis more manageable. Most likely the solution to the problems with accurate diagnosis will involve several tests for different functions of the immune response.

There are more than 5000 published manuscripts dealing with the diagnosis of brucellosis with approximately half describing serological diagnosis. Because of space limitations, only a sample of manuscripts have been cited. Omission of any reference is not a reflection of its quality.

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СЕРОЛОШКА ДИЈАГНОЗА НА БРУЦЕЛОЗА

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Цел: Да се даде преглед и да се опишат најкористените лабораториски тестови за серолошка дијагноза на бруцелозата заедно со нивните позитивни и негативни страни.

Методи: Преглед на поновата научна литература за серолошките дијагностички тестови за бруцелозата. Изборот на стратегијата за тестирање зависи од актуелната епидемиолошка ситуација на бруцелозата и од целта на тестирањето.

Резултати: Златен стандард во дијагнозата на бруцелозата е изолацијата и идентификацијата на бактеријата предизвикувач која припаѓа на

видот *Brucella*. За изолација на *Brucella* sp. потребни се лабараториски услови со висока сигурност (биолошка безбедност ниво 3), високо обучен персонал, доволно време за добивање резултати и истата се смета за ризична процедура. Заради тоа бруцелозата главно се дијагностицира со детекција на покачени нивоа на антитела во серумот или друга телесна течност. Ова претставува веројатна дијагноза затоа што и други микроорганизми и можеби фактори од околината можат исто така да ги покачат нивоата на антителата.

Заклучок: Пронајдени се голем број на серолошки тестови за бруцелоза во последните 100 години од нејзината првична изолација, почнувајќи од прост тест на аглутинација па сѐ до примарни врзувачки тестови што се достапни денес. Меѓутоа ниту еден тест откриен до денес не е 100% сигурен, па главно серолошката дијагноза се состои во тестирање на серуми со неколку тестови, обично скрининг тестови со висока осетливост, следени од тестови за потврда со висока специфичност.

Клучни зборови: бруцелоза, серологија, дијагноза, конвенционални тестови, примарни врзувачки тестови.

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