Diagnosis of Brucellosis at Satkania, Chattogram

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Abstract

Brucellosis is a zoonotic disease of great animal welfare and economic implications worldwide known since ancient times. The emergence of brucellosis in new areas as well as transmission of brucellosis from wild and domestic animals is of great significance in terms of new epidemiological dimensions. Brucellosis poses a major public health threat by the consumption of non-pasteurized milk and milk products produced by unhygienic dairy farms. Regular and meticulous surveillance is essentially required to determine the true picture of brucellosis especially in areas with continuous high prevalence. Additionally, international migration of humans, animals and trade of animal products has created a challenge for disease spread and diagnosis in non-endemic areas. Isolation and identification remains the gold standard test, which requires expertise. The advancement in diagnostic strategies coupled with screening of newly introduced animals is warranted to control the disease. One Health approach can aid in control of this disease, both in animals and man. Several serological tests, *viz*., RBPT, standard tube agglutination test (SAT), immune capture agglutination, CFT, milk ring, Coombs test, ELISA and lateral flow assay (LFA) are frequently employed to diagnose brucellosis.

Chapter 1

Introduction

Brucellosis is a bacterial disease associated with evolution of agricultural society, where animal husbandry is an integral part, with worldwide distribution. It is considered as one of the most prevalent zoonosis by Food and Agriculture Organization and World Health Organization (Schelling et al. 2003; WHO 2005, 2012; Corbel 2006). Office International des Epizooties (OIE) declares brucellosis as multiple species disease, infection and infestation (OIE 2018). The etiological agent of bovine brucellosis is a Gram-negative coccobacillus, *Brucella abortus* and occasionally by *Brucella melitensis* and *Brucella suis* (Moreno and Moriyon 2002; OIE 2016; CFSPH 2018a, 2018b). Human brucellosis is popularly known as undulant fever, Crimean fever, Mediterranean fever, remitting fever, Maltese fever, goat fever, Gibraltar fever and bovine brucellosis is called as contagious abortion or Bang's disease (Hayoun et al., 2020). *Brucella* species are among those pathogenic bacteria which have propensity to adapt to new host and they can either be naturally transmitted to their primary hosts by direct or indirect contact or sometimes inadvertently to other susceptible hosts (Moreno, 2014).

In this context, lack of enough awareness in public, safe husbandry practices, trading the infected animals and huge economic burden of diagnosis, vaccination and management have led to the persistence of brucellosis in India (Machavarapu et al., 2019). Effective control strategies of this disease include surveillance, prevention of transmission and controlling the reservoir of infection by different methods including culling (Rahman et al., 2011; Durrani et al., 2020).Some countries have controlled *Brucella* infection up to certain extent by implementing the strict immunization protocols such as use of suitable smooth live vaccines, reliable diagnostic tools, mass vaccination of large population, along with consistent culling of *Brucella*-positive animals. If proper vaccination and accurate diagnosis will not be performed, then in the absence of competent immune animals, disease may aggravate due to enhanced virulence, host jumping and wider transmission in different species (Moreno 2014).

In earlier times, when domestic animals were reared in close vicinity of animal owners and handlers, any loophole in the management of animals along with consumption of unsafe dairy or other animal products were major factors for spread of bovine brucellosis and its zoonotic form in humans. Not only domestication of animals, but anthropogenic adaptation

of wild animals also provoked this pathogen to widen its host range and jumping from one host to another with possible cross-species transmission. With the passage of time, brucellosis has become a disease causing serious economic losses, which is capable of affecting many species of animals as well as humans owing to the genetic adaptation of the pathogen against a variety of immune defense mechanisms of different hosts. However, humans act as dead-end host and brucellosis occurs with more severe clinical manifestation in man (Moreno 2014). Considering the anthropo-zoonotic potential of brucellosis, approximately 50,000 human cases were annually reported around the globe (Pappas, Papadimitriou et al., 2006). The main portal of transmission to human beings is through raw, improperly pasteurized or unpasteurized dairy products and contact with infected tissues or secretions (Moreno 2014).

The bacterium

They are Gram-negative, aerobic, facultative intracellular rods or coccobacilli, which lack capsules, endospores or native plasmids. The bacterium has a diameter of 0.5–0.7 μm and has 0.6–1.5 μm length, partial acid fast with oxidase, catalase, nitrate reductase and urease activity. The brucellae are able to survive freezing and thawing, but are susceptible to most of the common disinfectants. The bacterium remains viable in environment for months especially in cool and wet conditions. Pasteurization can effectively kill *Brucella* in milk. Though they are non-motile, yet they have all the genes except the genes required to form a flagellum (Fretin et al., 2005).

A total of six classical and seven novel *Brucella* species have been recognized from a wide spectrum of susceptible hosts. Species affecting terrestrial animals are seven in number including *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae* and *B. microti* (Scholz et al., 2008): two other species, *B. ceti* and *B. pinnipedialis* affect marine mammals (Foster et al., 2007). *B. papionis* isolated from baboons and *B. vulpis* from red foxes were also added to the list of genus *Brucella* (Scholz et al., 2016). Seven biovars have been recognized for *B. abortus*, three for *B. melitensis* and five for *B. suis*. Rest of the species has not been characterized into biovars. The *Brucella* nomenclature is based on the principal host species (Verger et al., 1987). Reports also document the isolation of 36 atypical *Brucella* spp. from frogs (Scholz et al., 2016; Al Dahouk et al. 2011). As the list of species increases, it is essential to identify better prevention measures to control the spread of disease to man.

Pathogenesis

Brucella can be transmitted *via* horizontal or vertical route (Meltzer et al., 201o) B*rucella* organisms are found in higher concentration in the uterus of pregnant animals. The aborted fetuses, placental membranes and uterine discharges act as main source of infection. Organisms shed in the milk of infected animals may transmit the infection to the newborn. The organism may survive in the environment for months together especially in cold and moist atmosphere. The animals contract the infection by ingestion of contaminated feed and water or by contacting aborted fetuses, fetal membranes and discharges from uterus. Inhalation could also be a mode of transmission. Infected bulls may also spread infection by natural service or artificial insemination from one herd to another (Acha and Szyfers, 2001). Tukana and Gummow (2017) described that normal animals sharing common water sources with *Brucella*-positive animals is one of the most important reasons for the spread of brucellosis.

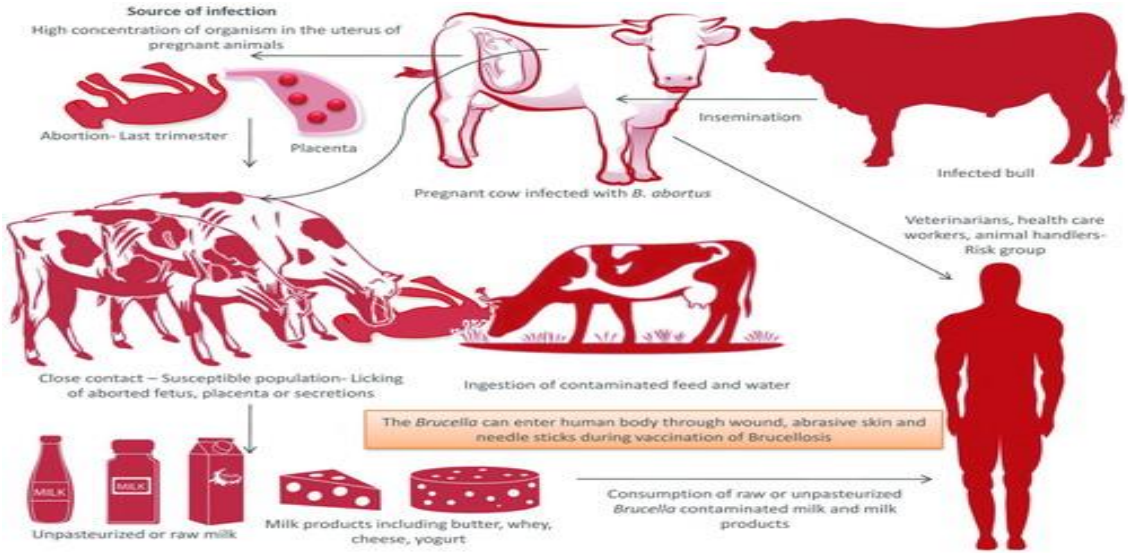

Figure1. Transmission of Brucellosis; Pregnant cows usually abort in the last trimester of pregnancy. Aborted fetus, placenta, and secretion from uterus act as the source of infection to other animals. Milk and milk products can act as source of infection to man, if consumed unpasteurized. Infected bulls serve as the lifelong source of infection.

Clinical signs

Various clinical signs have been described in infected animals, the main manifestation in *B. abortus* infection being reproduction failure in the form of abortion and birth of weak offsprings which remain as carrier in herd. The clinical signs, manifestations and multiple

complications in brucellosis in different animal species are firstly related to the reproductive tract. The incubation period could vary from two weeks to months together. Calves could be infected at early stage but no symptoms are seen till they mature. It is manifested by late abortions in pregnant animals, birth of weak calves, lowered fertility, retention of fetal membranes, endometritis and reduction in milk production (Kiros et al., 2016; Abdisa 2018). Abortion rate may vary from 30 to 80% in susceptible herds (Kiros et al., 2016). Calves borne at full-term may die very soon after birth. Fibrinouspleuritis coupled with interstitial pneumonia also appears in newborn calves and also in aborted fetuses (Carvalho Neta et al., 2010).

Male animals show clinical manifestations in the form of orchitis and epididymitis, whereas, hygroma is witnessed in chronic infections (Corbel, 1997). Cervical bursitis in cattle has also been reported due to brucellosis (de Macedo et al. 2019). In seminal vesicles, the acute inflammatory phase is followed by a chronic stage with considerable fibrinoid induration. Areas of dry necrosis develop and become encapsulated by fibrinous tissue, which eventually contracts, often leaving the testicles smaller than normal. In some cases, it may soften with the production of a soft fluctuating lesion containing thin purulent exudate.

Chapter 2

Diagnosis of Brucellosis

Principle

Bovine Brucella Antigen rapid test kit uses rapid immune chromatographic test to detection brucellosis antigen on bovine, ovine, swine and dogs. After the sample to be added to the loading hole, it moves along the chromatographic membrane together with colloidal gold labelled anti brucellosis monoclonal antibody. When a brucellosis antigen is present in the sample, it shows a red wine colour by binding to the antibody on the assay line. No colour reaction occurs if no brucellosis antigen is present in the sample.

Chapter 3

Materials and Methodology

Materials

1. Test device
2. Disposable dropper
3. Swab
4. Dilution buffer

Procedure

1. Specimen

A. Pure culture bacteria

Picking up a single colony of suspected brucella to sample buffer, covering & shaking the tube vigorously for more than 30 seconds, then keeping it stand for 5 minutes. After the large particle settle, absorb the supernatant.

B. Clinical Specimens

Using swab dipped in suspected brucella in inguinal lymph node acupuncture aspirates, vaginal discharge, diseased tissue homogenates. Immediately inserting the swab into the test tube containing the sample buffer, dissolving the sample then standing for 5 minutes until the large particles precipitated supernatant as a test solution.

C. Milk

Collection of 20 ml raw milk, centrifugation for 15 minutes at 2000rpm, discarding the supernatant, dipping a centrifuge tube sediment with swab, then immediately inserting the cotton swab into the sample buffer, placing the swab in the test tube repeatedly force the wall rotation at least 10 times, and mixing the solution. Dissolving the sample then standing for 5 minutes until the large particles precipitated supernatant as a test solution.

D. Serum, plasma, Blood

Addition of 2 drops serum, plasma or 1 drop blood into sample buffer, mixing it evenly.

2. Removal of the test device from the foil pouch and placing it on a flat and dry surface.
3. Taking 5 drops of sample test solution to the test device sample well (S mark).
4. At this time, will see the wine red liquid flow through the observation window. Reading test result in 15-20 minutes. Result will invalid after 20 minutes.

Chapter 4

Results

1. **Positive:**

Both T line and C line being seen wine red color reaction, and the more antibody exist, the thicker the color appear.

2. **Negative:**

No color reaction on test line (T line), only on control line (C line) being seen wine red color reaction.

3. **Invalid:**

No color reaction on C line.

Limitations

This kit is a qualitative Screening reagent, can detect brucellosis antigen accurately, but if sample is not enough or concentration of antigen is *lower than sensitivity, it may appear negative result.*

Figure: Detection of brucellosis

Chapter 5

Discussion

The test had performed the rapid antigen test for brucellosis upon 24 animals from 6 farms at Satkania, Chattogram. Four animals have selected from each farm. From them, 17 are cow and remaining are Bull. All rapid antigen test is found negative in that area.

Figure: Negative result of Bovine Brucellosis

Negative result of brucellosis is confirmed by ULO, Satkania and Poultry Research and Training Center (PRTC). PRTC provided the diagnostic kit, which was imported from Uruguay, for the diagnosis of Brucellosis in field level.

LFA (Lateral Flow Assay) or rapid antigen test for detection of brucellosis is performed by Theresia Abdoel et al., 2008.

Conclusion

Brucellosis is among the most prevalent animal and zoonotic diseases with worldwide occurrence. Brucellosis reveals mostly an endemic pattern of disease in developing regions. The prevalence of this disease is on the rise owing to numerous hygienic, social, economic, cultural and political factors. Brucellosis is diagnosed by history, symptoms of disease, bacteriological isolation and identification, serological tests, and various molecular tests including PCR-based assays. However, all the tests have some strengths and limitations. The major ailments caused by brucellosis include abortions, retained fetal membranes, endometritis, orchitis, epididymitis, *etc*., in animals and undulant fever in human beings. The disease causes colossal economic losses globally in terms of reduced animal health and production and effect on public health, yet robust surveillance, prevention and control measures are lacking.

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