Understand brucellosis: detection, prevention and treating methods

Zixuan Zhang[1, †], Jinghan Xue[2, *, †], Changhui Zhu[3, †]

[1]The High School Affiliated to Renmin University of China, Beijing, China

[2]College of Veterinary Medicine, Northwest A & F University, Yangling, China

[3]Shanghai Australian International School, Shanghai China

[*]Corresponding author: dyxy@nwsuaf.edu.cn

[†]These authors contributed equally to this paper

Keywords: Brucellosis; diagnosis; preventions; vaccination; control

Abstract: Brucellosis is a common infectious disease that can be found worldwide especially in farms where have lots of animals like sheep, cattle, and pigs, and somewhere that has no domestic health program, and people would be infected if they are exposed to infected animals or contaminated animal products. Our team searched for many authoritative types of research and concluded for different methods of diagnosis, treatment, and prevention that have been used or some methods that are developing, as well as comparing the pros and cons of each method and give out future directions for controlling this disease. But we are more focused on the part of diagnosis and prevention since we consider them as the most crucial part for controlling brucellosis. And the prevention for brucellosis is mainly composed of vaccination, 45/20 vaccine, S19 vaccine, RB51 vaccine strain, and different subunit vaccines (Recombinant protein vaccines, vector vaccines, and DNA vaccine) are some of the promising vaccines for controlling brucellosis but there is still a long way for the development of subunit vaccines before applying to humans. This paper aims to give out a comprehensive and detailed literature review for brucellosis which could be used by different people who want to get to know brucellosis and to learn about current developments in the efforts for controlling this infectious disease.

1. Introduction

Brucellosis is a disease caused by bacteria in the genus Brucellosis that mainly affects cattle, pigs, goats, sheep and dogs. Humans usually get it through direct contact with infected animals, eating or drinking contaminated animal products, or breathing pathogens in the air. Brucellosis is one of the most widespread zoonoses transmitted by animals. The expansion and urbanization of animal husbandry and the lack of hygienic practices in animal husbandry and food handling are part of the reason why brucellosis remains a public health hazard. In this paper, the main modes of transmission and causes of infection of RB51 are introduced. And some precautions to prevent brucella infection. As well as the effects of the vaccine on the virus, treatment options and duration of the vaccine prevention.

2. prevention and treatment of brucellosis

2.1 Monitoring and prevention of risk factors

Prevention of brucellosis is based on monitoring and prevention of risk factors. The most effective prevention strategy is to eliminate infections in animals. Vaccination of cattle, goats and sheep is recommended in areas with a high prevalence of the disease in animals. Serological or other testing and partial culling measures can also be effective in low-prevalence areas. In countries where it is not possible to eliminate animal-to-animal transmission through vaccination or culling of infected animals,

prevention of human infection relies mainly on awareness-raising, food safety, and occupational health and laboratory safety measures.

2.2 Cutting off the main routes.

In addition to solving the source of the disease, attention should be paid to preventing brucella from spreading: Main way is divided into three kinds, direct contact with infected, respectively, through the digestive tract infections, respiratory infections .[1] First , infection through direct contact with skin and mucosa, such as direct contact with the excrement of infected animals, or in the process of milking and shearing or processing meat due to improper protection, resulting in skin contusion and infection. Precautions should be taken to prevent physical contact with infected objects as much as possible. [2]Second, infection through the digestive tract, it is mainly caused by the ingestion of contaminated water or food, through the mouth, esophagus mucosa after entering the body. For example, eat raw or uncooked meat, or eat food without washing hands. The preventive measures for beef and mutton to cook after eating, especially when eating barbecue, shabu meat, must be cooked, or remember to wash your hands before eating.[3] Third, through respiratory tract infection, by brucella contamination of the environment after the formation of aerosol, respiratory tract infection can occur. Prevention measures include minimal travel to endemic areas and livestock treatment plants.

2.3 Antibiotic therapy

Many patients have mild symptoms that may not be considered at diagnosis. The incubation period varies from 1 week to 2 months but is usually 2-4 weeks.

Antibiotic therapy: Effective antibiotics can achieve good results and keep the virus under control for a long time.

Treatment options included doxycycline 100 mg twice daily for 45 days; Plus one gram of streptomycin for 15 days. The primary alternative therapy is doxycycline 100 mg twice daily for 45 days, plus rifampicin 15 mg/kg body weight per day (600-900 mg) for 45 days. Experience suggests that streptomycin can be replaced with gentamicin 5 mg/kg bw/day for 7-10 days, but no studies have directly compared the two approaches. The optimal treatment regimen for pregnant women, newborns and children under 8 years of age has not yet been determined. Treatment regimens for children include trimethoprim/sulfamethoxazole (cotrimoxazole) in combination with aminoglycosides (streptomycin, gentamicin) or rifampicin.

In addition, RB51 has Specific resistance: Prophylaxis for exposure to Brucella species other than RB51 routinely consists of a combination of doxycycline and rifampin. RB51, however, is resistant to rifampin in vitro and penicillin, so rifampin and penicillin are not recommended.

3. Detection methods

Up to now, no method can diagnose brucellosis independently, so it is usually performed by combination methods.

3.1 Bacteriological Methods

Bacteriological culture, which is mostly done by blood culture is the gold standard for the laboratory diagnosis of brucellosis. The isolation of pathogenic bacteria requires a long term and represents high risks of infection for laboratory personnel and technicians. On the other hand, this technique has low sensitivity, ranging from 15 to 70% depending on the species and disease stage. [4].

3.2 Serological Methods

SAT is seen as the most common and important serological test. However, the presence of blocking antibodies can cause false-negative results, besides, the test shows low specificity in the endemic area due to the presence of high antibody prevalence in the healthy population. Therefore, in endemic areas, the diagnosis of brucellosis should be confirmed by bacterial isolation. [5] Also, this test is not suitable for quick detection on-site, since it is relatively complicated and time-consuming.

RBPT is another traditional detection method of brucellosis. It is relatively easy to operate and the detection result can be obtained in 5 minutes. However, reaches showed that RBPT was good in patients with no previous exposure to Brucella or history of brucellosis, but poor in patients who were exposed repeatedly to Brucella or had a history of brucellosis. [6]. In this circumstance, Use of the Rose Bengal test as the sole technique for the diagnosis of brucellosis in endemic areas should be considered very carefully in the context of patients who are exposed repeatedly to Brucella or have a history of brucellosis.

By viewing the literatures, we found that there is little difference between the sensitivity of SAT and RBPT. The sensitivity of these two Semi-quantitative tests is relatively high but the specificity of them is unsatisfactory. A study shows that SAT is higher than RBPT in Specificity, negative predictive value, positive likelihood ratio, negative likelihood ratio, Uden index, accuracy, and the area under the ROC curve of SAT is larger than RBPT.[7] The main factor that causes low specificity is that Antibodies against smooth LPS are used in both two tests, while O-polysaccharides of Brucella is similar to that of Yersinia enterocolitica and other bacteria.[17] The competitive ELISA demonstrated a high specificity by using specific epitopes of Brucella O-polysaccharides as antigens.[19]

Both the ELISA and Brucellacapt tests have demonstrated high specificity in the current study [8] and facilitate rapid detection, having low cost and no risk of infection for laboratory personnel. Brucellacapt tests are immediate performance, simple use and handling, simple package kit including all the reagents, no need of washing and easy reading 24 hours [9, 10]. These two tests are valuable tools for the diagnosis of brucellosis in endemic areas

The first advantage of FPA is that it can distinguish between infected from vaccinated animals. False-positive serological reactions can be avoided when screening animals that have been vaccinated. It has both high specificity and sensitivity [11]. The accuracy of FPA is relatively higher compared with other detection methods such as the milk ring test (MRT), the complement fixation test (CFT), the indirect enzyme immunoassay (IELISA), and the competitive enzyme immunoassay (CELISA). [12].

While most diagnostic tests for brucellosis can only be performed in the laboratory, the Fluorescence Polarization Assay (FPA) can be performed very quickly with portable equipment in the field, reducing transportation costs. However, the temperature can influence the molecular movement and, consequently, the fluorescence depolarization rate. [13, 14]. To ensure the reliability of the test, it should be noted that both the ambient temperature and the temperature of the samples and reagents should be controlled before implementing the test, especially in the field. FPA needs to be adjusted and tailored to the different climate of endemic areas, therefore further researches about that are needed.

Table I. Sensitivity, Specificity and Performance Index of the Serological Tests for Brucellosis [15].

Test	% Sensitivity	% Specificity	Performance Index (Min - Max)
SAT	29.1 - 100	99.2 - 100	128.3 - 200
RBT	21.0 - 98.3	68.8 - 100	89.8 - 198.3
BPAT	75.4 - 99.9	90.6 - 100	166.0 - 199.9
IELISA	92.0 - 100	90.6 - 100	182.6 - 199.8
CELISA	97.5 - 100	99.7 - 99.8	197.3 - 199.8
FPA	99.0 - 99.3	96.9 - 100	195.9 - 199.3

3.3 Molecular Methods

1) The Polymerase Chain Reaction (PCR): Molecular biology as a diagnostic tool is advancing and will soon be at the point of replacing actual bacterial isolation. The use of PCR methods includes the diagnosis of the disease and characterization of field isolates for epidemiological purposes including taxonomics studies. Although it is more sensitive than blood culture and more specific than serologic

tests. The equipment and reagents are expensive and require the high skills of test personnel, so they are not suitable for laboratories in deprived areas.

2) In recent years, loop-mediated isothermal amplification (LAMP) is a functional nucleic acid amplification technique offering a substitute to PCR. In comparison with these two methods, the LAMP assay is advantageous because of its feasibility, easy construction, quick answer and visual recognition. The LAMP assay does not require a thermal cycler machine and is replaced with a thermal block or bain-marie. The result of LAMP assay can be observed by the naked eye. Because of turbidity generated in positive samples [16, 17]. Therefore, this technique is a useful method for screening clinical diagnosis and surveillance of infected wild animals.

4. Vaccination

Vaccination programs are mainly utilized for the brucellosis caused by *B.* melitensis and B. abortus [18]. The following three are the main live-attenuated vaccines of the brucellosis vaccines' development.

4.1 45/20 vaccine:

We gain B. abortus strain 45/20 after twenty passages in guinea pigs [19]. This vaccine has only been tested in some of the countries [20] and is used to eliminate the interference of induced antibodies with routine diagnosis. Nevertheless, it has many drawbacks like contradictory data for the efficacy of the vaccine and immunologic response, repeated vaccination, variability of protection, unpredictable serological effects and reactions at sites where the vaccine was administered in some animals all led to the interruption of 45/20 vaccine use [21].

4.2 S19 vaccine:

B. abortus strain 19 is a live attenuated vaccine for the control of B. abortus infection in cattle. It was thought to be the most effective vaccine for the prevention of bovine brucellosis, and this strain was first isolated from the milk of a Jersey cow in 1923 and was left at room temperature for one year which then naturally developed an attenuated phenotype [21].

The number of colony-forming units (CFU) per dose is a factor for the Antibody persistence induced by S19. And there are two methods of vaccination: a "high" dose (5–8 × 1010 CFU) by subcutaneous route or one or two "low" doses (5×109 CFU) by conjunctival route [20]. Besides that, the age of the vaccinated animal is also a factor that affects the effectiveness of the vaccine [18]. Only a tiny percentage of vaccinated calves will have a prolonged period of antibody persistence, but the antibody persistence increases with age for the vaccinated calves, thus, to address this issue, vaccination is usually performed on young female calves between three and eight months of age [21]. Nevertheless, the immunity induced in this age group does not seem to have a significant appearance, and due to the interference of brucellosis diagnosis, limitation on the age of vaccination seems to be the main drawback of the S19 vaccine, whereas the RB51 vaccine does not have this problem [18, 21].

In addition, S19 is the basis of the control of the low- and middle-income countries, although it was proved to be effective, it sometimes could cause abortion in pregnant cows and a modest but notable incidence of clinical cases and hypersensitivity reaction of patients, therefore, it is not considered as a safe vaccine for human use [20].

4.3 RB51 vaccine strain:

Based on the literature, the live vaccine B. abortus RB51, a spontaneous rough mutant, is obtained on a medium containing rifampicin and penicillin by subculturing the virulent strain B. abortus 2308 [18]. It is used in many countries currently which has replaced S19 in some areas, and it is more stable than strain 45/20 and less virulent than S19 [18, 21]. Many of the countries recommend having revaccination of RB51 after six months or one year of the primary RB51 vaccine.

RB51 is typically developed for the serological differentiation of the naturally infected and vaccinated animals, which overcomes the serologic problems being observed after the vaccination of

S19, allowing us to simultaneously use the test-and-slaughter and vaccination policies. It can also largely decrease the rate of abortion among calves and infection of humans, however, RB51 could cause human to be infected in some conditions especially for someone who is immunosuppressed and it is resistant to a kind of antibiotics called rifampicin, which is also a vaccine that failed to be detected by the routine serological tests, and these are what we need to be aware of during the treatment of humans [18, 21].

As we can see from the above three live-attenuated vaccines that have been mostly used for the control of brucellosis, they all have disadvantages due to the characteristics that they are still slightly virulent and have the potential risk of converting to virulence which then may cause abortion of calves and it is not safe for humans especially for someone who came into contact with the vaccine [18]. What we need is a kind of vaccine that is effective, safe (avirulent), and provides long-lasting protection for humans, thus, subunit vaccines (vaccines that contain purified pieces of the pathogen which could trigger an immune response) are a safe choice since those fragments will not cause diseases [23]. And the three below are the promising subunit vaccines for the control of brucellosis.

4.4 Recombinant protein vaccines:

The recognition for the presence of T-cell epitopes to the host enables this vaccine to be developed [24], and although this type of vaccine is safe, many of the recombinant protein vaccines have a lower level of resistance to B. abortus than the live attenuated vaccines. A few types of recombinant protein vaccines like CobB and AsnC seem to produce a similar protective immunity with S19, and they were examined to be successful among mice. However, the process of developing a vaccine that could imitate the natural infection from different combinations of proteins is challenging, and the amount of costs for combining different materials is thought to be unsuitable for cattle [21].

4.5 Vector vaccines:

Vector vaccines can be developed by introducing genes encoding immunodominant B. abortus antigens into attenuated bacteria [21]. Recently, a new vector vaccine has been made which expresses Brucella outer membrane protein (Omp) 16, L7/L12, Omp19 or Cu–Zn SOD proteins [25]. Vector vaccine can mimic the natural infection closely which then stimulates the effective immune response. However, the high cost, no tests in cattle, and the need for foreign protein that is expressed by the carrier organism to have specific protection for the organism all led to the uncertainty of applying vector vaccines now [21].

4.6 DNA vaccine:

DNA vaccination is a new and potent method of immunization that could induce both humoral and cellular immune responses (Th1 and CTL) and protect from a broad range of pathogens [26]. And some types of DNA vaccines could effectively protect the mice against B. ovis and B. melitensis infection when scientists were doing the experiments [26] and they are still trying their best to enhance the efficacy of DNA vaccine (For example, by coexpressing cytokines as adjuvants for the adjustment of the immune response) [27]. However, most of the DNA vaccines do not induce immune responses as high as the live attenuated vaccines [18], and they are still at the preliminary stage.

Therefore, based on the above literature review, we could see that although subunits vaccines seem to be safer to humans, they are still at an early age and the huge costs, as well as the immature technology, seem to be too soon to be applied by humans. Thus, S19 and RB51 are still considered as the main vaccine for the control of brucellosis and deeper investigation (better understanding of the pathogen and its interaction with the immune system) for subunit vaccines should not be ceased.

5. Conclusion

Brucellosis prevention is based on the monitoring and prevention of risk factors. The most effective prevention strategy is to eliminate infections in animals. Vaccination of cattle, goats, and sheep is recommended in areas with a high prevalence of the disease in animals. Awareness-raising, food safety,

occupational health and laboratory safety measures are also needed to enhance people's protection against the virus.

In addition to the source of the disease, the main route of virus transmission: respiratory tract infection, digestive tract infection, skin contact infection, etc. Pay attention to the prevention of major transmission routes of Brucella and daily personal hygiene

Vaccination is generally not recommended and the use of rifampin penicillin is not recommended. Antibiotic treatment: Use effective antibiotics for the appropriate time, effective antibiotics can achieve good results and keep the virus under control for a longer time.

References

[1] People's government of Zhoujiang Town, Wuhua County, Meixian District CDC (2019-08-23).

[2] Popularization of brucellosis, The First Affiliated Hospital of Shantou University Medical College (2020-09-24).

[3] Pharmaceutical Handbook of Fudan University Cancer Hospital (2008) Chapter I Anti-infective drugs I. antibiotics (2009-09-11) Handbook of Fudan University Cancer HospitalK. Elissa, “Title of paper if known,” unpublished.

[4] Diaz R, Moriyón I. Laboratory techniques 8 Fekete A, Bantle JA, Halling SM, in the diagnosis of human brucellosis. In: Sanborn MR. Preliminary development Brucellosis: Clinical and Laboratory Aspects. Of a diagnostic test for Brucella using Young EJ, Corbel MJ (Eds), CRC Press, polymerase chain reaction. J. Appl. FL, USA, 73–83 (1989). Bacteriol. 69, 216–227 (1990).

[5] Peeridogaheh, H. Golmohammadi, M. G, & Pourfarzi, F. (2013). Evaluation of elisa and brucellacapt tests for diagnosis of human brucellosis. Iranian Journal of Microbiology, 5(1), 14-18.

[6] J. D. Ruiz-Mesa, J. Sánchez-Gonzalez, Reguera, J. M. , L Martín, S. Lopez-Palmero, & Colmenero, J. D. (2005). Rose Bengal test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. Clinical Microbiology and Infection, 11(3).

[7] Zhang Qimian, Yan Leilei. Effect of RBPT on diagnosis accuracy of brucellosis [J]. Harbin Medicine, 2021(04):118-119.

[8] Peeridogaheh, H. Golmohammadi, M. G. & Pourfarzi, F. (2013). Evaluation of elisa and brucellacapt tests for diagnosis of human brucellosis. Iranian Journal of Microbiology, 5(1), 14-18.

[9] Casao AM, Navarro E, Solera J. Evaluation of Brucellacapt for the diagnosis of human brucellosis. J Infect 2004; 49: 102-108.

[10] Casanova A, Ariza J, Rubio M, Masuet C, Díaz R. Brucella capt versus classical tests in the serological diagnosis and management of human brucellosis. Clin Vaccine Immunol 2009; 16: 844-851.

[11] Kalleshamurthy, T., Shekar, R., Niranjanamurthy, H. H., Natesan, K., Shome, B. R., Bambal, R. G., Sairiwal, L., Barbuddhe, S. B., Sahare, A., Kilari, S., Rahman, H., & Shome, R. (2018). Assessment of fluorescence polarization assay: a candid diagnostic tool in Brucella abortus strain 19 vaccinated areas. Microbiology and immunology, 62(11), 694–701. https://doi.org/10.1111/1348-0421.12654.

[12] Nielsen, K., & Gall, D. (2001). Fluorescence polarization assay for the diagnosis of brucellosis: a review. Journal of immunoassay & immunochemistry, 22(3), 183–201. https://doi.org/10.1081/IAS-100104705.

[13] Falzon, L. C., Traoré, S., Kallo, V., Assamoi, J. B., Bonfoh, B., & Schelling, E. (2019). Evaluation of the Fluorescence Polarization Assay as a Rapid On-Spot Test for Ruminant Brucellosis in Côte d'Ivoire. Frontiers in veterinary science, 6, 287. https://doi.org/10.3389/fvets.2019.00287.

[14] Nielsen, K., Gall, D., Smith, P., Kelly, W., Yeo, J., Kenny, K., Heneghan, T., McNamara, S., Maher, P., O'Connor, J., Walsh, B., Carroll, J., Rojas, X., Rojas, F., Perez, B., Wulff, O., Buffoni, L., Salustio, E., Gregoret, R., Samartino, L., … Luna-Martinez, E. (2001). Fluorescence polarization assay for the diagnosis of bovine brucellosis: adaptation to field use. Veterinary microbiology, 80(2), 163–170. https://doi.org/10.1016/s0378-1135 (00)00386-2.

[15] Poester, F. P. Nielsen, K. Samartino, L. E, & Wei, L. Y. (2010). Diagnosis of brucellosis. Open Veterinary Science Journal, 4(1), 46-60.

[16] Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplifcation of DNA. Nucleic Acids Res. 2000; 28(12):e63-e.

[17] Mori Y, Nagamine K, Tomita N, Notomi T. Detection of loop-mediated isothermal amplifcation reaction by turbidity derived from magnesium pyrophosphate formation. Biochem Biophys Res Commun. 2001; 289 (1):150–4.

[18] Avila-Calderón, E. D., Lopez-Merino, A., Sriranganathan, N., Boyle, S. M., & Contreras-Rodríguez, A. (2013). A History of the Development of Brucella Vaccines. BioMed Research International, 2013. Https: // doi.org/10.1155/2013/743509.

[19] Veterinary Research (n. d.-b). VeterinaryResearch. Https: // veterinary research. biomedc entral.com.

[20] Simpson, G. J. G., Marcotty, T., Rouille, E., Chilundo, A., Letteson, J.-J., & Godfroid, J. (2018). Immunological response to Brucella abortus strain 19 vaccination of cattle in a communal area in South Africa. Journal of the South African Veterinary Association, 89. Https: // doi.org/ 10.4102/jsava.v89i0.1527.

[21] Veterinary Research. (n.d.). Veterinary Research. Retrieved October 9, 2021, from https: // veterinary research.biomedcentral.com.

[22] Mantovani, C., Soares, C. O., Santos, L. R. dos, & Rosinha, G. M. S. (2020). Research Article Bovine brucellosis vaccine strain S19 detected in calves before vaccination. Genetics and Molecular Research, 19(3). Https: // doi.org/10.4238/gmr18659.

[23] What are protein subunit vaccines and how could they be used against COVID-19? (n.d.). Www.gavi.org. https: //www.gavi.org/vaccineswork/what-are-protein-subunit-vaccines-and-how-could-they-be-used-against-covid-19.

[24] Fu, S., Xu, J., Li, X., Xie, Y., Qiu, Y., Du, X., Yu, S., Bai, Y., Chen, Y., Wang, T., Wang, Z., Yu, Y., Peng, G., Huang, K., Huang, L., Wang, Y., & Chen, Z. (2012). Immunization of Mice with Recombinant Protein CobB or AsnC Confers Protection against Brucella abortus Infection. PLoS ONE, 7(2), e29552. https://doi.org/10.1371/journal.pone.0029552.

[25] Bugybayeva, D., Kydyrbayev, Z., Zinina, N., Assanzhanova, N., Yespembetov, B., Kozhamkulov, Y., Zakarya, K., Ryskeldinova, S., & Tabynov, K. (2021). A new candidate vaccine for human brucellosis based on influenza viral vectors: a preliminary investigation for the development of an immunization schedule in a guinea pig model. Infectious Diseases of Poverty, 10(1). https://doi.org/10.1186/s40249-021-00801-y.

[26] Cassataro, J., Velikovsky, C. A., de la Barrera, S., Estein, S. M., Bruno, L., Bowden, R., Pasquevich, K. A., Fossati, C. A., & Giambartolomei, G. H. (2005). A DNA Vaccine Coding for the Brucella Outer Membrane Protein 31 Confers Protection against B. melitensis and B. ovis Infection by Eliciting a Specific Cytotoxic Response. Infection and Immunity, 73(10), 6537–6546. https://doi.org/10.1128/IAI.73.10.6537-6546.2005.

[27] Oliveira, S. C., Giambartolomei, G. H., & Cassataro, J. (2011). Confronting the barriers to develop novel vaccines against brucellosis. Expert Review of Vaccines, 10(9), 1291–1305. https://doi.org/10.1586/erv.11.110.