DOI 10.7251/VETJEN1802392V **UDK 636.2.09:616.981.42**

Original scientific paper

THE IMPORTANCE OF BRUCELLIN ALGERIC SKIN TEST FOR DIAGNOSIS OF BOVINE BRUCELLOSIS[2*]

Lejla Velić [1]*, Toni Eterović[1], Silvio Špičić[2], Željko Cvetnić[2], Amina Hrković Porobija[1], Benjamin Čengić[1], Selma Filipović[1], Tarik Bajrović[1]

1 Veterinary faculty, Sarajevo, Bosna and Herzegovina
2 Croatian Veterinary Institute, Zagreb, Croatia
*Corresponding Author: Lejla Velić: lejla.velic@vfs.unsa.ba

Abstract: Infection with Brucella results in the induction of both humoral and cellular immune responses. Humoral immune resposne is based on monitoring the occurrence of specific antibodies against smooth lipopolysaccharide (S-LPS) of Brucella. However, in cattle, classical serological methods can detect antigenic determinants for other types of microorganisms (cross reactivity) such as *Escherichia coli* 0:157, *Yersinia enterocolitica* 0:9, *Salmonella urban*, *Pseudomonas malthopilia* and *Pasteurella*. The aim of our work was to determine the immunological response based on the use of standardized and purified allergen in which lypopolysaharid has been removed and doesn't induce humoral immune response. A total of 16 dairy cattle previously tested positive using RBT (Rose Bengal test) and CFT (complement fixation test) were tested for confirmation with BST (brucelline skin test) according to the instructions of the producer. *B. melitensis* B115 (Synbiotics Brucellergene OCB) was used in the test. 14 of 16 cattle reacted with skin thickening >1 mm after 72 hours from the application of brucellin. 2 animals with no skin thickening or thickening <1mm also reacted negative in CFT. This outcome can be attributed to cross reactions with other antigens than *Brucella* that commonly occurs in Rose Bengal test.

Brucellin allergic skin test is not recommended as a standalone diagnostic tool because all infected animals do not react therefore this test cannot be recommended as a self-sufficient diagnostic test or for the purpose of international trade. However, due to high specificity and adequate sensitivity at the herd level, it can be recommended for the control of herds in areas free of brucellosis.

Keywords:brucellosis, cattle, brucellin, humoral immunity, cellular immunity.

[2*] Presented at the 23rd Annual Consulling of Doctors of Veterinary Medicine of Republic of Srpska (B&H). Teslic, June 6-9, 2018

INTRODUCTION

Brucellosis is caused by facultative intracellular Gram-negative bacteria of the genus Brucella. In susceptible animals, brucella induces a humoral and cellular immune response. While the humoral immune response is based on monitoring the occurrence and growth of titres of specific antibodies against lipopolysaccharides - smooth strain of Brucella (S-LPS) (Benet et al., 1991), the cellular immune response is based on activation of macrophages by lymphokines secreted by T cells. Monitoring of the humoral immune response by serological methods is influenced by many factors such as the long and variable incubation period during which the serological tests are negative (Nelson et al., 1966), the immunological response generated by vaccination, variation in serological responses of individual cattle as well as stage of pregnancy at the time of infection (Fensterbank et al 1975). Numerous serological tests currently available clearly indicate that no test is "ideal" in terms of early infection detection during a long and variable incubation period, the presence of non-specific antibodies (cross-reactions), detection of latent or chronic carriers and the differentiation of infection from vaccination (Nielsen and Duncan, 1990) The aim of this paper is to determine the cellular immune response in serologically positive cattle using a purified and standardized Brucellin allergen, almost completely devoid of lipopolysaccharide and consequently does not lead to the development of humoral immune response . Also the study indicates the significance of this test in making the final diagnosis of brucellosis in cattle.

MATERIAL OF METHODS

Examined animals

A study was carried out on cattle (14 animals) The Rose Bengal test (fast serum agglutination test) and complement fixation reaction (CFR) determined the presence of Brucella-specific antibodies . The Rose bengal test was found positive in 2 cases while complement fixation reaction gave negative results. All examined bovine originated from farms where unvaccinated cattle and sheep were bred together. Bovine blood samples for serological testing were submitted for examination to the Laboratory for Virology and Serology, National Reference Laboratory for Brucellosis, within the annual order for the control of infectious and parasitic diseases in the Federation of Bosnia and Herzegovina for 2017.

Skin test

The test was performed according to the OIE Manual (2016) and Seagerman et al., (1999) - brucelin, which is the

B. melitensis B115 extract (Synbiotics Brucellergene OCB). According to the manufacturer's instructions the hair was cut from the side and 0.1 ml of brucellin (2000 units / ml) was intradermally injected. Skinfold thickness was measured with a caliper prior to the application of brucellin. Brucellin was applied with an injector and a needle. A positive reaction is indicated by local swelling and induration. The test is read after 48- 72 hours, primarily by palpation, and then by measuring with a caliper. A positive reaction is evaluated qualitatively or by creating a local edema and induration. Any increase in the skin thickness greater than 1.5 mm was considered as a positive reaction. In order to reduce the possibility of variation in reading, the application and the reading were performed by the same person.

RESULTS

No disease, necrosis or swelling of regional lymph nodes on the side of the neck where the allergen was applied was observed in cattle used in the study. The reaction was read only once by measuring the thickness of the skin fold, seventy-two hours after theinjection of brucellin. The obtained measurement values are shown in Table 1.

Table 1. Skinfold measurements before and after brucellin application.

Cattle No.	Skinfold before administration of Brucellin(mm)	Skinfold 72 hours after administration of Brucellin (mm)	Difference in skinfold thickness (mm)
1.	10	21	11
2.	9	12	3
3.	12	20	8
4.	13	16	3
5.	10	17	7
6.	10,5	17	6,5
7.	9	10	1
8.	13	19	6
9.	20	22	2
10.	8	11	3
11.	9	13	4
12.	10	15	5
13.	6	8	2
14.	6	13	7
15.	7	7	0
16.	10	13	3

As seen in the table of 16 animals tested, the increase in skin fold was found in 14, while no changes were noted in two of them. An average value of thickness increase was 4.5 mm. Although the brucellin skin allergy test is based on a delayed-type hypersensitivity reaction, the phenomenon used in tuberculinization, the reaction after administration of Brucellin is two to three times less intense than the one in tuberculinization (Saegerman et al., 1999) (Fig. 1).

Measuring skinfold with a caliper

DISCUSSION

Making precise and definitive diagnoses in diseases such as brucellosis is of great importance in controlling the disease both in animals and humans. Since brucellosis in animals is manifested by abortions in the third trimester of pregnancy, clinical suspicion is based on anamnestic data related to reproductive disorders in the herd. The final diagnosis is based on the application of direct and indirect laboratory methods. The 'gold standard' in the diagnosis of brucellosis is the isolation of Brucella spp from the diagnostic material (Alton et al., 1988). However, this method requires an adequate biosecurity level - because it is a highly infectious agent. Molecular methods, on the other hand, are an important tool in brucellosis diagnosis - and in epizootiological studies, but they require expensive equipment as well as highly educated staff (Godfroid sar., 2010). The application of serological methods also has its advantages and disadvantages. Serological tests for do not require special conditions, they are less demanding, safer and more economical than bacteriological and molecular methods. Although it is generally accepted that serological tests are reliable in the diagnosis of brucellosis in cattle (FAO / WHO,

1986), none of the available serological tests can detect specific antibodies at all stages of the infection (Nielsen, 2002) In the researches carried out in the 60's and 80's of the last century, it was found that in the serum of bovine infected with Brucella present in small numbers, the immune response was weak or absent (Rose and Sar., 1964; Nicoletti and Muraschi, 1966; Ray et al., 1988). Also, in cases of brucellosis in the late stage of pregnancy, normal delivery may occur, but such animals are infected even though in serum of such animals a significant antibody titer can rarely be detected (Cunningham, 1968). The drawback of serological tests is the presence of cross-reactions with antigenically similar microorganisms such as Escherichia coli 0: 157, Yersinia enterocolitica 0: 9, Salmonella urban, Pseudomonas malthopilia and Pasteurellae (Corbel, 1985, Kittelberger et al., 1995). Consequently there is no serological test that could accurately detect all stages of brucellosis (Mylrea and Fraser, 1976, Nielsen, 2002). In our research, we used a purified and standardized Brucellin allergen completely devoid of lipopolysaccharide which can not lead to the production of specific antibodies. Cellular immune response is the most important defense mechanism in Brucella infection. The mechanism involves lymphocytic stimulation resulting in inhibition of macrophage migration, lymphocyte blastogenesis and delayed-type hypersensitivity development (Soper et al., 1978). An increase in skinfold in 14 cattle in which both serological methods (RB and CFR) gave positive results shows that the skin allergy test is the most specific indirect test for the diagnosis of brucellosis in unvaccinated animals. Although the sensitivity and specificity of the skin allergy test depends on the chosen criteria for interpreting the results (De Massis et al, 2005), - the results obtained by these studies coincide with the results of other authors' research (MacDiarmida and Hellstrom, 1987, Pouillot et al., 1997, Seagerman et al., 1999). Many authors (Bercovich et al., 1992, Plommet, 1984, Seagerman et al., 1999) consider that skin-fold thickness values- equal to or greater than 1mm - is not taken as a limit value, but that each visible and / or tangible reaction is considered positive.

The negative result of the skin allergy obtained in two cases where Rose Bengal test was positive, while CFR gave a negative result. According to Corbel, (1985) and Kittelberger et al. (1995) this happens due to cross-reacting with other antigenically related microorganisms. It is important to note that animals vaccinated with B. melitensis Rev.1, B. abortus S19 or RB51 can react in a skin allergy test for years after vaccination (Pouillot et al., 1997; De Massis et al., 2005). Therefore, this test can not be recommended as the only diagnostic test, nor for the purpose of international trade in the areas where Brucella vaccine is used. It is also important to note that not all infected animals react, therefore this test can not be recommended as

an individual diagnostic test or for the purpose of international trade. However, due to high specificity and adequate sensitivity at the herd, it can be recommended for the control of herds in areas free of brucellosis (REI 2016).

CONCLUSION

Based on the above, it can be concluded that the use of a skin allergy test in bovine brucellosis diagnosis has a particularly high value in case of suspicious (unclear) result of serological testing as a confirmatory method in unvaccinated cattle.

The introduction of an additional test in brucellosis diagnosis such as a delayed-type brucellosis hypersensitivity is a useful diagnostic tool if brucellosis is of enzootic character and where vaccination of small ruminants is applied as well as in conditions where sheep and goats are held together with cattle on the same pastures and habitats. The lack of a skin test is reflected in the fact that it is not applicable in cases of animal vaccination and that before the repetition of a skin allergy test it is necessary to wait 6 weeks to desensitize the organism (OIE 2016).

LITERATURA

1. Alton G.G., Jones L.M., Angus R.D. and Verger J.M. (1988): Techniques for the Brucellosis Laboratory, Institut National de la Recherche Agronomique (INRA), Paris.
2. Benet J.J., Massard C., Garin-Bastuji B., Moutou F., Dufour B., Schaeffer C. and Cotton T. (1991): Reactions serologiques atypiques dans le depistage de la brucellose bovine: Enqune Bpidemiologique dans les departements condemes. Epidtmiol. Sante Anim., 19: 97-130.
3. Bercovich Z., Ter Laak E.A., Vanlipzig J.H.H. (1992): Detection of brucellosis in dairy herds after an outbreak of the disease using a delayed-type hypersensitivity test. *Preventive Veterinary Medicine*, **13**, 277–285.
4. Corbel M.J. (1985): Recent advances in the study of Brucella antigens and Their serological cross-reactions. Vet Bull 55, 927-942.
5. Cunningham B.(1968): The control and eradication of brucellosis. I. Serological response in cattle following vaccination with S,9 and killed *Brucella 45120* adjuvant vaccine, *Vet. Rec.,* 82, 7.
6. De Massis F, Giovannini A., Di Emidio B., Ronchi G.F., Tittarelli M., Di Ventura M., Nannini D and Caporale V. (2005): Use of the complement fixation and brucellin skin tests to identify cattle vaccinated with Brucella abortus strain RB51. Veterinariy italiana, 41 (4), 291-299.

7. FAO/WHO (1986): Joint FAO/WHO expert committee on brucellosis, 740:1-132.
8. Fensterbank R., Plummet M., and Pardon, P. (1975): Traitement de Ia brucellose bovine par I' oxytetracycline, Ann. Rech. Vet., 6, 43.
9. Godfroid J., Nielsen K., Saegerman C. (2010.): Diagnosis of brucellosis in livestock and wildlife. Croat. Med. J., 15;51, 4:296-305.
10. Kittelberger R., Hilbink F., Hansen M.F., Ross G.P., Joyce M.A., Fenwick S., Heesemann J., Wolf-Waltz H., Nielsen K. (1995): Serological crossreactivity between *Brucella abortus* and *Yersinia enterocolitica* 0:9 II the use of Yersinia outer proteins for the specific detection of *Yersinia enterocolitica* infections in ruminants.Vet Microbiol. 47(3-4):271-80.
11. MacDiarmid S.C., Hellstrom J.S. (1987): An intradermal test for the diagnosis of brucelloisi in extensively managed cattle herds. Preventive Veterinary Medicine, 4, 361-369.
12. Mylrea P.J., Fraser G.C. (1976): The use of supplementary tests in the serological diagnosis of bovine brucellosis. Aust. Vet.J. 52: 261-266.
13. Nelson C. J., Anderson R. K., Kimberling C. V., and Pietz D. E. (1966): Enzoologic factors of bovine brucellosis: comparative bacteriologic studies of infected herds, Am. J. Vet. Res., 25, 1515.
14. Nicoletti P. and Muraschi T. F. (1966): Bacteriologic evaluation of serologic test procedures for the diagnosis of brucellosis in problem cattle herds, *Am. 1. Vet. Res.,* 27, 689.
15. Nielsen K. (2002): Diagnosis of brucellosis by serology. Vet Microbiol; 90: 447–59.
16. Nielsen K., Dunkan J.R. (1990): Animal Brucellosis, Animal Diseases Research Institute Agriculture Canada, Nepean, Ontario.
17. OIE (2016): "Terrestrial manual, *Bovine brucellosis*," in *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Brucellosis (*Brucella abortus, B. melitensis* and *B. suis*)(NB: Version adopted in May 2016.
18. Plommet M. (1984): Les dernieres etapes de la prophylaxie de la brucellosis bovine. Bull Mens Soc Vet Prat Fr, 68, 507-520.
19. Pouillot R., Garin- Bastuji B., Gerbier G., Coche Y., Cau C., Dufour B et al (1997): The brucellin skin test as a tool to discriminate false positive serological reactions in bovine brucellosis Vet.Res.28 (4), 365-374.

20. Ray W.C., Brown R.R., Stringfellow D.A., Schnurrenberger P.R., Scanlan C.M., Swann A.I. (1988): **Bovine brucellosis: an investigation of latency in progeny of culture-positive cows.** J. Am. Vet. Med. Assoc., 192, 182-186

21. Rose J, E., Lambert G., and Roepke M. H. (1964): Ultra-centrifugation and heat. Inactivation studies on serogglutinins of pregnant heifers artificially infected with virulent *Brucella abortus, Am. J. Vet. Res.,* 25, 329.

22. Seagerman C., Vo T.K., De Waele L., Glison, D., Bastin A., Dubray G., Flanagan P., Limet J.N., letesson J.J and Godfroid, J. (1999): Diagnosis of Bovine Brucellosis by Skin Test: Conditions for the test and evaluation of its performance. The Veterinary Record, 145: pp 214 – 218.

23. Soper F. E., Muscoplat C. C., and Johnson D. W. (1978): *In vitro* stimulation of bovine peripheral blood lymphocytes: analysis of variation of lymphocyte blastogenic response in normal dairy cattle, *Am. J. Vet. Res.,* 39, 1039.

Paper received: 05.06.2018.

Paper accepted: 31.10.2018.