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ALLERGIC SKIN TEST AS A METHOD FOR DIAGNOSIS OF BRUCELLA INFECTIONS IN SMALL RUMINANTS

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Because the clinical signs of the brucellosis in small ruminants are not patognomonic and therefore diagnosis based on clinical symptoms is nontennable, additional laboratory tests are used for diagnostic of Brucella disease. Laboratory tests involve identification of the causative microorganism by classical laboratory techniques for isolation of Brucella spp. or PCR (Polymerase Chain Reaction) and measuring of the immune responses. Animals infected with *Brucella spp.* are usually diagnosed by serological tests, which measure the humoral immune response. Beside the humoral response, the infection with *Brucella spp* elicits cell-mediated immune response which can be detected by allergic skin test. Evaluation of the role of the cell-mediated immune response in infection by *Brucella spp.* and its detection, using delayed type of hypersensitivity reaction, is described in this paper.

Key words: *brucellosis, small ruminants, cellular immune response, allergic skin test.*

INTRODUCTION

Animals infected with Brucella spp. react by eliciting adoptive humoral and cellular immune responses to the microorganism, which has survived the innate immune defense mechanisms.

After being phagocytosed or engulfed in other ways by the professional antigen presenting cells (macrophages, dendtritic cells and B cells) bacterial proteins are processed into small peptides within the compartments of APCs and loaded on the surface of the cells in the conjunction with MHC Class II complex. This surface antigenic epitopes are recognized by the naive T cells which after encountering the antigen undergo the clonal expansion and became the armed effector T cells. This cells either leave the lymphoid organ to effect cell mediated immunity at the sites of infection or remain in the lymphoid tissues to participate in humoral immunity by activating antigen-binding B cells.

Besides the effector T cells, another subset of T cells - memory T cells is formed, which exist in the circulation long after their synthesis (many years) and they usually have an increased responsiveness to previously encountered pathogens. These memory T cells are the ones that are involved in allergic skin test used in this study.

Allergic skin test is based on type IV hypersensitivity, which involves primarily the

T cell responses to introduced antigens in previously sensitised animals with the same antigen. T lymphocytes involved in the reaction belong to the Th (helper) subpopulation of the CD4+ T cells. The allergic skin test is better known as a Delayed Type of Hypersensitivity (DTH) where the local visible reaction occurs 48-72 hours after the inoculation of the antigen. The test is used to detect the infections with the intracellular microorganisms.

When sensibilised animal is exposed again to the previously encountered antigen, the memory T cells elicit an immunologycal response of the delayed type hypersensitivity. The reaction is based on the activation of the inflammatory responses by inflammatory T (Th1) cells, which are mediated mainly by activated macrophages at the sites of inoculation, which are secreting inflammatory mediators. The clinical signs of the test, based on cell infiltration followed by redness and induration at the site of inoculation are visible after 48 - 72 hours of inoculation of the antigen.

MATERIALS AND METHODS

Two herds, each containing 20 sheep, were used for the study. One of the herd, herd A did not have a history of the disease while herd B had a Brucella infections were present for more than one year.

BRUCELLERGENE OCB is a protein antigen produced by Rhone Merieux used for the detection of the delayed type of hypersensitivity reactions in sheep, goat and cattle infected with Brucella spp. It contains LPS free cytoplasmatic proteins extracted from the rough strains of Brucella (strain B115). BRUCELLERGENE OCB antigen does not elicit antibody response, which would interfere in serological reactions, does not induce inflammatory reactions in non-sensibilysed animals and is not a sensibilising agent itself. O.1 ml of Brucellergene OCB were inoculated in each separate animal from both herds in the lower right palpebra, using a specially designed applicator.

Before inoculation of the antigen, each separate animal was bleed and tested for the presence of the specific antibodies in the serum using Rose Bengal and an Immunocomb test, developed in Israel.

Inoculated animals were tested for the presence of the allergic skin reaction 48 hours after the inoculation of the Brucellergen OCB antigen by palpation and comparison of the both, left and right lower palpebras for the presence of the inflammatory signs. Animals with thickened right lower palpebras were considered as a positive for the test.

RESULTS

All animals tested from the herd A were negative when tested with all three tests. Of 20 tested animals from herd B, 13 were negative with all tests.

Two animals numbered 54733 and 54735 were positive when tested with all three tests and one animal (54731) was positive only with Rose Bengal and Immunocomb test.

Two animals were positive when tested with immunocomb and allergic skin test, and two out of 20 tested animals were positive only with allergic skin test. Results from the tests are shown in table 1.

Table 1. Animals from herd B, which reacted positive with any of the three tests used.

N° of eartag	*Rose Bengal*	*ImmunoComb*	*Brucellergene OCB*
54723	-	+	+
54731	+	+	Not found
54733	+	+	+
54735	+	+	+
54736	-	-	+
54739	-	-	+-
54740	-	+	+

DISCUSSION

In the early stages of infection with Brucella spp infected animals elicit strong humoral immune response with high titter of IgM IgG and IgA antibodies. Agglutinating antibodies of the class IgM which are also the antibodies that react in the Rose Bengal test are present in high titter in the early stages of infection, but their titter decline sharply in the subacute and the chronic stages of infection, when antigenic microorganisms are present intracellular, hidden from the circulating lymphocytes. In those stages of the infection infected animals may not be detected as positive when screened with the routine Rose Bengal serological tests.

As shown from the results section in our study, 4 animals from herd B where Brucellosis persisted for more than one year were negative when tested with Rose Bengal test, while 3 of those 4 animals were positive when tested with allergic skin test. Allergic skin tests, which is based on the memory T cell reaction, in previously sensibilised animals may be good method for the detection of the chronically infected animals, where the titter of circulating antibodies has declined.

CONCLUSIONS

Allergic skin test is based on the detection of the cellular immune response, which can not be detected using serological methods. That could be important in the subacute and chronic stage of disease when the level of IgM is low and slide aglutination (Rose Bengal) test may gave negative results.

This test can be used as a simply applicable field method, as a routine screening test or as an additional method to Rose Benagal test for detection of herds infected with Brucella spp.

Obtained results showed that more than one test is needed for screening of the herds with history of Brucella infections.

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АЛЕРГОЛОШКИ ТЕСТ КАКО МЕТОДА ЗА ДИЈАГНОЗА НА БРУЦЕЛА ИНФЕКЦИИТЕ КАЈ МАЛИ ПРЕЖИВАРИ

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Бидејќи клиничките симптоми кај бруцелозата кај малите преживари не се патогномонични и затоа поставувањето на конечната дијагнозата на тој основ не е можна, се користат додатни лабораториски тестови во дијагностиката на оваа болест. Лабораториските тестови опфаќаат идентификација на предизвикувачот, со изолација на Brucella spp. или со примена на PCR-техниката, како и мерење на имунолошкиот одговор на животното. Животните инфицирани со Brucella spp. вообичаено се дијагностицираат со серолошките тестови кои го мерат хуморалниот имунолошки одговор. Покрај овој вид на имунолошки одговор, инфекцијата со Brucella spp предизвикува и клеточен имунолошки одговор кој може да биде детектиран со алергискиот коден тест. Проценката на улогата на клеточниот имунолошки одговор во инфекцијата со Brucella spp. и неговата детекција, користејќи го одлодениот тип на алергиска реакција, се опишани во овој труд.

Клучни зборови: *бруцелоза, мали преживари, клеточен имунолошки одговор, алергиски коден тест.*