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TAJRIBA HAYVONLARIDA BRUTSELLYOZ QO‘ZG‘ATUVCHILARIGA QARSHI IJOBIY STANDART POLIVALENT ZARDOBLARNI OLISH

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ANNOTATSİYA

Maqolada brutselloz infeksiyasini erta tashxislash va serologik usullarni takomillashtirish maqsadida brutselloz qo‘zg‘atuvchilariga qarshi standart polivalent zardob olish bo‘yicha olib borilgan tajribaviy tadqiqotlar natijalari bayon etilgan. Tadqiqotda 12 ta quyon 4 guruhga bo‘linib, ularga turli immunizatsiya sxemalari asosida B. abortus 19 tirik vaksinasi va faolsizlantirilgan brutsella kulturasi suspenziyalari turli konsentratsiyalarda yuborilgan. Olingan zardoblarda umumiy oqsil, albumin, globulin, IgA, IgM va IgG miqdorlari aniqlanib, ularning immun javob shakllanishidagi o‘zgarish dinamikasi baholangan. Tadqiqot natijalariga ko‘ra, tirik, virulentligi pasaytirilgan B. abortus 19 vaksinasi bilan immunizatsiya qilinganda barqaror va davomli immun javob shakllanganligi, to‘liq bo‘lmagan Freynd ad’yuvanti qo‘llanilganda esa immunoglobulinlar darajasi yanada yuqori bo‘lganligi aniqlangan. Olingan natijalar asosida milliy standart zardob tayyorlashning ilmiy-amaliy asoslari ishlab chiqildi. Ushbu zardob brutsellozning serologik diagnostikasini standartlashtirish, diagnostikumlar sifatini nazorat qilish hamda xalqaro miqyosda solishtiriladigan natijalarni ta’minlash imkonini beradi.

Kalit so‘zlar:brutselloz, Brucella abortus 19, standart zardob, immunizatsiya, immunoglobulin, Freund ad’yuvanti, serologik tashxis.

ABSTRACT

The article presents the results of experimental studies aimed at obtaining a standard polyvalent serum against brucellosis pathogens to improve early diagnosis and serological detection methods. The experiment was conducted on 12 rabbits divided into four groups, which were immunized with the live B. abortus 19 vaccine and inactivated Brucella culture suspensions at various concentrations following different immunization schemes. The obtained sera were analyzed for total protein, albumin, globulin, IgA, IgM, and IgG levels, and the dynamics of their changes were assessed during immune response formation. The results showed that immunization with the live attenuated B. abortus 19 vaccine produced a more stable and long-lasting immune response, while the use of Incomplete Freund’s Adjuvant (IFA) enhanced immunoglobulin production. Based on the obtained data, scientific and practical foundations were developed for preparing a national standard serum against brucellosis. The application of this serum will enable the standardization of serological diagnostics, quality control of diagnostic preparations, and ensure comparability of research results at the international level..

Keywords:brucellosis, Brucella abortus 19, standard serum, immunization, immunoglobulins, Freund's adjuvant, serological diagnosis.

АННОТАЦИЯ

В статье представлены результаты экспериментальных исследований, направленных на получение стандартной поливалентной сыворотки против возбудителей бруцеллёза с целью совершенствования ранней диагностики и серологических методов выявления заболевания. В эксперименте использовано 12 кроликов, разделённых на 4 группы. Животным вводили живую вакцину B. abortus 19 и инактивированные суспензии культур бруцелл в различных концентрациях по различным схемам иммунизации. В полученных сыворотках определяли содержание общего белка, альбумина, глобулина, IgA, IgM и IgG, а также оценивали динамику их изменений в процессе формирования иммунного ответа. Результаты исследований показали, что при иммунизации живой аттенуированной вакциной B. abortus 19 формируется более стабильный и продолжительный иммунный ответ, тогда как использование неполного адъюванта Фрейнда способствует усилению продукции иммуноглобулинов. На основе полученных данных разработаны научно-практические основы для приготовления национальной стандартной сыворотки против бруцеллёза. Применение данной сыворотки позволит стандартизировать серологическую диагностику бруцеллёза, контролировать качество диагностических препаратов и обеспечит сопоставимость результатов исследований на международном уровне.

Ключевые слова: бруцеллёз, Brucellaabortus 19, стандартная сыворотка, иммунизация, иммуноглобулины, адъювант Фрейнда, серологическая диагностика.

Introduction.

In the Republic of Uzbekistan, large-scale national programs have been implemented to improve the early diagnosis, treatment, and prevention of brucellosis infection and to reduce its complications. However, despite these measures, the incidence rate of brucellosis remains relatively high in certain regions of the country. This indicates the continued presence of active epizootic and epidemic factors and highlights the necessity of enhancing early diagnostic methods and improving preventive and anti-epidemic strategies [6].

In endemic areas for brucellosis, laboratory testing methods are used to effectively monitor the activity of epizootic and epidemic factors [1].

The clinical manifestations of brucellosis are highly diverse, and the course of the disease often resembles other infections. The absence of strictly specific symptoms frequently causes diagnostic difficulties or errors. Such cases are often associated with incomplete anamnesis, atypical clinical presentations, superinfection, reinfection, relapse, or latent forms of infection. In these situations, laboratory diagnostic methods play a crucial role [10].

Currently, serological tests are among the most reliable auxiliary methods for diagnosing brucellosis in both humans and animals. For humans, these include the Huddelson test, Wright's agglutination test, passive hemagglutination test, and the Coombs reaction; while for animals, the Rose Bengal test, agglutination reaction, and complement fixation test are commonly used [2, 4, 7].

To improve the standardization and efficiency of these diagnostic methods, the FAO/WHO Expert Committee on Biological Standardization has recommended that all countries develop their own national standard serum, expressed in international units (IU/ml),

to serve as a reference for comparison and calibration [3, 8].

At present, the production of brucellosis diagnostic reagents has been established in Uzbekistan for both medical and veterinary applications. However, the absence of a national standard serum poses significant challenges. Firstly, commercial analogs of standard sera are costly, making their use economically difficult. Secondly, the lack of a domestic standard serum creates problems in controlling the quality of both imported and locally produced antigens, hinders the development of national brucellosis diagnostic preparations, and reduces the overall efficiency of serological diagnostics in humans and animals [9].

Considering the aforementioned issues and in accordance with FAO/WHO recommendations, it is essential for the Republic of Uzbekistan to develop, validate, and produce its own standard serum that can serve as a national reference material for brucellosis diagnosis.

Purpose and Objectives.

To obtain positive standard polyvalent sera against brucellosis pathogens in experimental animals and to study their properties.

Methods and Organization.

The study was conducted on 12 healthy rabbits weighing 2.2–4.6 kg and aged 6–12 months. All animals were quarantined for 21 days prior to the experiment and divided into four groups of three rabbits each.

Group 1: Rabbits were immunized four times with a live attenuated Brucella abortus 19 vaccine (4×10^9 concentration).

Group 2: Immunization was performed with the same live vaccine mixed with Incomplete Freund's Adjuvant (IFA) during the 1st and 4th immunizations, and the live vaccine alone during the 2nd and 3rd.

Group 3: Rabbits received inactivated Brucella culture suspensions (1×10^9 and 4×10^9 concentrations) during the first three immunizations and the live vaccine during the fourth.

Group 4: Rabbits were immunized with inactivated Brucella culture mixed with IFA during the first immunization, followed by increasing doses (1×10^9 to 4×10^9) and the live vaccine at the final stage.

In all groups, the antigens (live or inactivated Brucella suspensions) were injected into eight sites (four subcutaneous and four intramuscular).

The bacteriological method used a low-virulent B. abortus 19 vaccine strain (produced by the ShchelkovBiocombinate, Russia, batch 204, April 2022). The serological tests followed the guidelines of the Ministry of Health of Uzbekistan (Order No. 177, May 1, 2015) for brucellosis laboratory diagnosis.

Immunological assays were performed using Vector-Best (Russia) ELISA kits to detect IgA, IgM, and IgG antibodies against Brucella antigens. Biochemical analyses of total protein, albumin, and globulin were conducted with the Mindray BA-88A biochemical analyzer according to the manufacturer's instructions.

Statistical analysis was performed using Excel Office 2013 software. The Student's t-test was applied, and differences were considered statistically significant at $p < 0.05$.

Results and Discussion.

Before hyperimmunization, blood samples were collected from rabbits, and sera were tested for brucellosis using the Heddelson and Wright agglutination tests. Immunological parameters, including total protein, albumin, globulin, and immunoglobulin classes (IgA, IgM, and IgG), were measured. Experimental animals were hyperimmunized four times at 7-day intervals. Blood was collected before each immunization and 13 days after the last one for serological and immunological evaluation.

The study involved 12 rabbits divided into four groups, each immunized with different antigen preparations: live attenuated Brucella

abortus 19 vaccine, the same vaccine with incomplete Freund's adjuvant, and inactivated Brucella culture suspensions with or without adjuvant. After hyperimmunization, sera were tested for cross-reactivity with heterologous gram-negative bacteria (Enterobacteriaceae spp., Citrobacter spp., Proteus vulgaris, etc.), which showed minimal (16.7%) weak cross-agglutination reactions.

Biochemical analysis revealed that total protein, albumin, and globulin levels increased significantly after the fourth immunization. In the first group, total protein rose from 59.57 g/L to 157.17 g/L, while IgM and IgG concentrations showed dynamic changes typical of primary and secondary immune responses. The second group, which received the vaccine with incomplete Freund's adjuvant, demonstrated a more stable and prolonged antibody response, indicating the adjuvant's enhancing effect on immunoglobulin production.

In the groups immunized with inactivated Brucella suspensions, immune responses were also observed, but antibody titers were lower than in the groups receiving live vaccine. The highest antibody titers were recorded in animals immunized with the live attenuated B. abortus 19 strain, confirming that it induces a strong and durable immune response.

The sera obtained from hyperimmunized rabbits are proposed as a basis for developing national standard polyvalent sera against brucellosis. Such sera can be used to standardize serological diagnostic tests, ensure quality control of diagnostic preparations, and harmonize brucellosis diagnostics in Uzbekistan with international standards.

Conclusion

The study demonstrated that multiple immunizations in experimental animals led to a significant increase in total protein, albumin, globulin, IgM, and IgG levels, indicating a typical immune response to Brucella antigens. The primary immune response was characterized by elevated IgM during the first week, followed by an increase in IgG in the second week, representing the secondary immune phase.

Immunization with the live attenuated Brucella abortus 19 vaccine produced a more stable and long-lasting immune response compared to inactivated Brucella culture suspensions. The use of incomplete Freund's adjuvant further enhanced antibody production and contributed to a more consistent immune reaction.

Although inactivated Brucella antigens elicited detectable antibody titers, these were considerably lower than those induced by the live vaccine. Therefore, to obtain fully active hyperimmune sera suitable for serological standardization, the use of live attenuated Brucella strains is essential..

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