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Evaluation of The Specificity of Diagnostic Hyperimmune Serum Produced Against Brucellosis in Heterologous Microorganisms

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Akmal Pulatovich Yusupov

Alfraganus University, Department of Medicine, PhD (Doctor of Philosophy), Associate Professor, Uzbekistan

Otabek Kuziev Jurakulovich

Alfraganus University, Head of the Department of Medicine, DSc, Professor, Uzbekistan

Abstract: The production of high-quality diagnostic reagents is essential for accurate serological diagnosis of brucellosis. This study aimed to evaluate the specificity of hyperimmune sera obtained by hyperimmunization of rabbits with the live Brucella abortus 19 (BA19) vaccine strain. The specificity was tested against a wide panel of clinical heterologous microorganisms from the Enterobacteriaceae family commonly associated with intestinal infections. Although weak positive reactions were observed in the slide agglutination test with a limited number of strains (Salmonella spp., Escherichia coli, and Citrobacter spp.), all reactions were negative in the confirmatory tube agglutination test. The results confirm the high specificity of the obtained diagnostic sera and support their reliable application in laboratory practice for the serological identification of Brucella pathogens.

Keywords: Brucellosis, Brucella abortus 19, hyperimmune serum, specificity, cross-reactivity, agglutination reaction, heterologous microorganisms, serological diagnostics.

1.Introduction:

Brucellosis remains one of the most widespread zoonotic diseases worldwide, causing significant economic losses and posing serious public health risks. Serological methods, particularly agglutination tests (Wright, Heddelson, and others), play a leading role in the laboratory diagnosis of brucellosis. The quality and specificity of diagnostic hyperimmune sera directly influence the accuracy of these tests.

However, hyperimmune sera can sometimes exhibit cross-reactivity with antigens of taxonomically related or unrelated microorganisms. Such non-specific interactions may lead to false-positive results and diagnostic errors, especially when identifying isolated bacterial cultures. In this regard, a comprehensive assessment of the specificity of newly developed diagnostic sera against heterologous microorganisms is a mandatory stage in their validation.

The purpose of this study was to determine the specificity of hyperimmune diagnostic sera produced against the Brucella abortus 19 strain using clinical strains of Enterobacteriaceae family bacteria isolated from patients with acute intestinal infections.

2. Methods

Experimental Animals The study used three clinically healthy male rabbits weighing 3.0–3.5 kg and aged 6–12 months. Prior to hyperimmunization, all animals were confirmed seronegative for brucellosis using the Heddelson and Wright agglutination tests. The rabbits were housed in a standard vivarium with controlled conditions: temperature 18–22°C, relative humidity 55–65%, 12-hour light/dark cycle, and free access to food and water.

Immunogen and Hyperimmunization Protocol The live vaccine strain Brucella abortus 19 (BA19), produced by Shchelkov Biokombinat (Russia, series No. 204, manufactured April 2022), was used as the immunogen. The vaccine was diluted in sterile 0.9% NaCl solution to a concentration of 4×10^9 CFU/ml.

Hyperimmunization was performed according to a four-stage scheme with 7-day intervals. The 1st and 4th immunizations were carried out using the antigen emulsified with incomplete Freund's adjuvant (6 ml vaseline oil + 2 ml BA19 suspension). The 2nd and 3rd immunizations used pure live vaccine without adjuvant. Each rabbit received a total volume of 1.6 ml of the preparation per immunization, administered at 8 sites (0.2 ml per site: 4 subcutaneous and 4 intramuscular). Blood samples were collected one day before each subsequent immunization. Final exsanguination was performed 13 days after the fourth immunization.

Serum Preparation and Storage Collected blood was incubated at 37°C for 30 minutes and centrifuged at 1500 g for 15 minutes. Sera were separated and preserved with sodium merthiolate (1:10,000 final concentration). Inactivation was carried out in a 56°C water bath for 30 minutes with continuous stirring. Sera were aliquoted and stored at 2–8°C for immediate serological testing or at –20°C for long-term storage.

Microorganisms Used for Specificity Testing Testing was conducted in the Bacteriological Laboratory of the Republican Specialized Scientific and Practical Medical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases. Clinical strains isolated from patients with acute intestinal infections and acute diarrhea syndrome were used, including:

- Enterobacteriaceae spp. (74 strains)
- Proteus vulgaris (86)
- Salmonella spp. (632, 660, 662, 663, 760, 766, 696)
- Klebsiella pneumoniae (97, 128, 139)
- Shigella spp. (5, 973, 1128)
- Escherichia coli (108, 109, 110, 111, 112, 114, 914)
- Citrobacter spp. (134, 140)

Serological Testing The specificity of sera obtained before immunization, during the hyperimmunization process, and after final blood collection was evaluated using the slide agglutination test (on glass slides) as the primary screening method. Sera showing any degree of agglutination with heterologous strains were subjected to confirmatory testing by the tube agglutination method using inactivated bacterial suspensions.

3. Results

In the slide agglutination test, weak positive reactions were recorded in isolated cases:

- Sera No. 5.10, 5.11, and 5.12 with Salmonella spp. strain 766;
- Sera No. 5.3, 5.4, 5.5, and 5.6 with Escherichia coli strain 108;

- Sera No. 5.5, 5.6, 5.7, and 5.8 with Escherichia coli strain 108;
- Sera No. 5.1, 5.2, 5.3, 5.9, 5.10, and 5.11 with Citrobacter spp. strain 140.

All other tested sera showed no reaction with the panel of heterologous microorganisms.

Importantly, when the sera that gave weak positive results in the slide test were re-examined by the tube agglutination method, all results were negative. No cross-reactivity was observed with Proteus, Klebsiella, Shigella, or the majority of Salmonella and E. coli strains.

4. Discussion

The obtained data indicate that the hyperimmune sera produced using the three-to-four-stage immunization scheme with the BA19 strain possess high specificity. The weak reactions observed only in the more sensitive slide agglutination test and their absence in the tube method suggest that these interactions are non-specific and of low avidity, which does not compromise the diagnostic value of the sera.

These findings are consistent with the literature, which notes that while certain cross-reactions between Brucella and Enterobacteriaceae can occur due to shared antigenic structures (e.g., lipopolysaccharide components), properly prepared and inactivated hyperimmune sera usually demonstrate acceptable specificity for practical use.

The high specificity of the studied sera reduces the likelihood of false-positive results in the serological diagnosis of brucellosis and during the identification of bacterial isolates, which is particularly important in regions where brucellosis is endemic and co-circulates with other intestinal pathogens.

5. Conclusion

The hyperimmune diagnostic sera obtained against Brucella abortus 19 demonstrated high specificity against a broad panel of heterologous clinical microorganisms of the Enterobacteriaceae family. Minor cross-reactions detected in the slide agglutination test were not confirmed by the tube method and are considered insignificant. The results justify the use of these sera in laboratory practice for the reliable serological diagnosis of brucellosis.

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