Comparative evaluation of Yersinia dimethyl-sulfoxide antigens and antibodies obtained from it

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Abstract. A comparative assessment of the antigenic properties of dimethyl-sulfoxide fractions (DA) of Yersinia pseudotuberculosis and Yersinia enterocolitica, as well as antibodies to them. DA Yersinia pseudotuberculosis has been shown to have greater antigenic activity than DA Yersinia enterocolitica. Hyperimmune serum obtained for pseudotuberculous DA showed generic yersiniosis specificity and high antibody activity, which allows its use in diagnostic test systems.

1. Introduction
The causative agents of intestinal yersiniosis and pseudotuberculosis affect humans and animals. The number of infected pig farms in Europe, according to various researchers, ranges from 7% to 80% [1-4].

The bacteriological method used in the diagnosis of intestinal yersiniosis and pseudotuberculosis is complex, takes a long time to release pathogens, has low efficiency, and a large number of false secretions of yersiniosis cultures, which is associated with a significant contamination of pathological materials with intestinal microflora and imperfect methods of isolation [5, 6, 7, 8]. Serological diagnostic methods significantly increase the effectiveness of subsequent isolation of yersinia. At the same time, diagnostic preparations are required that allow simultaneous indication of Yersinia enterocolitica (Y. enterocolitica) and Yersinia pseudotuberculosis (Y. pseudotuberculosis), since in 10% of the intestines of pigs, the simultaneous circulation of both pathogens have been detected [5, 9]. However, the existing diagnostic drugs allow detection of only certain serovariants or species of Y. pseudotuberculosis and Y. enterocolitica. Therefore, the development of serological diagnostic methods for the simultaneous detection of pathogens of intestinal yersiniosis and pseudotuberculosis is an urgent task.

The most popular methods for the isolation and purification of antigens when creating diagnostic drugs are the low-cost methods. One of the widely used in medicine and a cheap solvent is dimethyl-sulfoxide (DMSO). DMSO is an inexpensive polar aprotic solvent with the formula (CH₃)₂SO. Widely used in medicine, as an anti-inflammatory and analgesic, facilitating transdermal drug transfer. Until recently, the use of DMSO for the production of antigenic and antibody preparations of microbial origin was carried out only on Mycobacterium tuberculosis and showed the promise of further studies in this area [10].
Previously, we isolated and studied dimethyl-sulfoxide antigen (DA) Y. enterocolitica, also obtained antibodies to it [11]. Antibodies obtained to Y. enterocolitica DA had generic specificity, which allowed to create two diagnostic test systems on their basis [12, 13].

In this article, we present the results of a study of the antigenic activity of the first DA Y. pseudotuberculosis obtained. A comparative assessment of the antigenic properties of DA pseudotuberculosis and intestinal yersinia microbes. The studied antigens are used to obtain hyperimmune rabbit blood serum, which are also subjected to a comparative assessment to identify the most promising diagnostic test system.

2. Materials and methods
Museum strains Y. pseudotuberculosis III O:3 serovarians and Y. enterocolitica № 66-82 O:3 serovarians taken from the state-owned collection of pathogenic microorganisms (SCPM) of the Federal state healthcare institution antiplaque research Institute "Microbe" were used to isolate DA.

The method for obtaining the DA of an intestinal yersinia microbe consisted in treating the dry acetone microbial mass of bacteria with DMSO, followed by fluid extraction, its release from DMSO, and lyophilization [11]. This is the first time, pseudotuberculosis DA has been isolated in this way.

The protein content in antigenic fractions was measured by Lowry’s method [14], carbohydrates were determined by the phenolic method [15].

Enzyme-linked immunosorbent assay was performed in an indirect version on titer plates (ELISA) [16].

The commercial diagnostic sera used in the study of DA are as follows:

- pseudotuberculosis and intestinal yersinia O:3, O:9 sera attached to diagnostic kits for the reaction of indirect hemagglutination (RIHA);
- pseudotuberculous O:1, O:3 and intestinal yersinia O:3, O:9 sera for an indicative agglutination reaction (IAR);
- brucellosis, salmonella ABCDE, escherichiosis OK sera.

Rabbits were immunized subcutaneously along the back at 3-4 points in a volume of 1 ml of the mixture. During immunization, the ratio of adjuvant to the DA solution was 1:1. 5 immunizations were carried out with an interval of 2 weeks. Blood for the study was taken from the ear vein in a volume of 5 ml a day before the introduction of the antigen, starting with the 1st immunization.

DA Y. pseudotuberculosis III and Y. enterocolitica № 66-82 at a concentration of 20 μg / ml and formalized Yersinia pseudotuberculosis I O:1 serovariant, Yersinia pseudotuberculosis III O:3 serovariant, Yersinia pseudotuberculosis IV O:4 serovariant, Yersinia pseudotuberculosis V O:5 serovariant (Y. pseudotuberculosis O:1, O:3, O:4, O:5), Yersinia enterocolitica 66-82 O:3 serovariant, Yersinia enterocolitica 383 O:9 serovariant (Y. enterocolitica O:3, O:9), Enterobacter aerogenes ATCC-13048 (E. aerogenes), Escherichia coli 4295 (E. coli), Proteus vulgaris 19 (P. vulgaris), Salmonella typhimurium 1626 (S. typhimurium) (obtained from SCPM Federal state healthcare institution antiplaque research Institute "Microbe"), as well as a single brutsellozny antigen produced by JSC "Pokrovsky plant of biological products" on the basis of Brucella abortus (B. abortus). Bacterial suspensions for ELISA were prepared with a concentration of 1 billion cells / ml.

3. Results

The ratio of proteins and carbohydrates in DA Y. pseudotuberculosis was 30:1, in DA Y. enterocolitica – 10:1.

Table 1 presents a comparative analysis of the antigenic activity of DA intestinal yersiniosis and pseudotuberculosis microbes. Antigens at a concentration of 20 μg / ml were studied in ELISA with commercial yersiniosis sera.

The activity of both of the studied antigens was manifested with pseudotuberculosis and with intestinal yersiniosis sera, i.e. generic specificity was noted in these fractions. It should be noted that
intestinal yersinia serums for IAR had higher titers with Y. enterocolitica cells than pseudotuberculosis ones with Y. pseudotuberculosis cells, i.e. were more antibody-active.

Antigenic activity in pseudotuberculosis DA turned out to be higher than in intestinal yersinia DA, which makes it more promising for creating a diagnostic drug.

Table 1. Antigenic activity of DA enteric and pseudotuberculosis microbes with yersiniosis sera.

| Diagnostic serum                      | DA antibody titers |
|---------------------------------------|--------------------|
|                                       | DA Y. pseudotuberculosis | DA Y. enterocolitica |
| Pseudotuberculosis for RIHA           | 1:25600             | 1:12800             |
| Intestinal yersiniosis O:3 for RIHA   | 1:12800             | 1:12800             |
| Intestinal yersiniosis O:9 for RIHA   | 1:12800             | 1:12800             |
| Pseudotuberculosis for IAR O:1        | 1:50                | 1:50                |
| Pseudotuberculosis for IAR O:3        | 1:100               | 1:50                |
| Intestinal yersiniosis for IAR O:3    | 1:400               | 1:400               |
| Intestinal yersiniosis for IAR O:9    | 1:400               | 1:400               |

Table 2 presents the antigenic properties of two DA studied in ELISA with sera to bacteria of other genera: Brucella, Salmonella, Escherichia.

Table 2. Antigenic activity of DA intestinal yersiniosis and pseudotuberculosis microbes with sera to bacteria of other genera.

| Diagnostic serum   | DA antibody titers |
|--------------------|--------------------|
|                    | DA Y. pseudotuberculosis | DA Y. enterocolitica |
| Brucellosis        | 1:400               | 1:3200               |
| Salmonella ABCDE   | –                   | –                    |
| Escherichia        | OKA: 1:1600         | 1:800                |
|                    | OKB: 1:800          | 1:400                |
|                    | OKC: 1:100          | 1:50                 |
|                    | OKD: –              | –                    |
|                    | OKE: 1:800          | 1:400                |

The data in table 2 indicate that DA Y. pseudotuberculosis has a less nonspecific reaction with brucellosis serum than DA Y. enterocolitica, but large non-specific reactions with escherichia sera than other fractions. In both fractions we see a negative reaction with salmonella serum. However, in general, the set of nonspecific reactions is similar in the two fractions.

A comparative assessment of the sensitivity and specificity of experimental hyperimmune sera obtained from DA Y. pseudotuberculosis and Y. enterocolitica was carried out in ELISA and is presented in table 3.

Table 3. Antibody activity of DA experimental sera.

| Antigens used | DA serum                  |
|---------------|---------------------------|
|               | pseudotuberculosis | intestinal yersiniosis |
|               | Antibody titers       | Antibody titers       |
| DA Y. pseudotuberculosis | 1:819200               | 1:409600               |
| DA Y. enterocolitica       | 1:409600               | 1:409600               |
| Y pseudotuberculosis serovariant O:1 | 1:51200               | 1:6400               |
| cells                     | 1:51200               | 1:12800               |
As seen from the data presented, the serum obtained for pseudotuberculosis DA showed a higher sensitivity than serum to intestinal yersinia DA.

Both sera reacted with Y. pseudotuberculosis and Y. enterocolitica cells, indicating their generic specificity.

We additionally conducted tests for the interaction of experimental sera with cells: Enterobacter, Salmonella, Escherichia, Proteus, Serration, Brucella. With these bacteria, the titers in ELISA were not high and amounted to 1:200-1:400, which additionally indicates generic specificity of both serums.

4. Conclusion

DA Y. pseudotuberculosis was obtained, which has greater antigenic activity than DA Y. enterocolitica.

Experimental DA Y. pseudotuberculosis hyperimmune serum, showed a higher sensitivity than DA Y. enterocolitica serum.

DA of Y. pseudotuberculosis and Y. enterocolitica, as well as antibodies to them, have generic specificity.

DA of Y. pseudotuberculosis and its related hyperimmune serum can be recommended for the creation of diagnostic test systems.

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