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BIOLOGICAL PROPERTIES OF CIRCULATING STRAINS OF PASTEURELLA MULTOCIDA IN THE TERRITORY OF THE REPUBLIC OF KAZAKHSTAN

ANNOTATION

The article presents the results of studies on the study of cultural, tinctorial, biochemical and virulence properties of Pasteurella multocida strains isolated from cattle. In order to refresh the isolated strains and increase their virulent properties, successive passages were carried out through the body of white mice. According to the results of the bioassays, a reduction in the period of death of mice after infection with Pasteurella cultures was established, which proves an increase in their virulence. When studying the cultural and morphological properties, pure cultures of Pasteurella were grown on nutrient media at 37°C for 18–24 h. A biochemical test on Hiss medium was used to fully confirm the data obtained. The test cultures isolated from pathological material (heart, lung, spleen, liver, intestines) of cattle have cultural, biochemical and biological properties characteristic of the Pasteurella multocida species and form S-shaped colonies. Isolated cultures of Pasteurella multocida can be used to evaluate the immunogenicity of existing vaccines and use them as a basis for the development of anti-pasteurellosis polyvalent emulsion vaccines, anti-pasteurellosis hyperimmune serum and e.t.c.

Key words: Pasteurella multocida, passage, white mice, vaccine, strain, cattle.

Introduction. Pasteurellosis is a widespread highly contagious infectious disease of many species of domestic and wild animals, as well as all types of birds, and is accompanied in acute course by signs of septicemia, lobar inflammation and pulmonary edema, pleurisy, edema in various parts of the body, and in subacute and chronic course of purulent-necrotizing pneumonia, arthritis, mastitis, keratoconjunctivitis, endometritis and enteritis [1, 2, 3, 4, 7]. Rapid and accurate diagnosis of sources of infections is critical for both medical and veterinary activities, and it is important for improved understanding of disease mechanisms and measures to control the illness [17].

Scientific and practical interest in animal pasteurellosis is due to natural foci. Pasteurellosis of recovered animals, the appearance of not only isolated cases of the disease, but also large outbreaks both among domestic and wild animals [1-5]. Animal diseases associated with P. multocida such as fowl cholera in poultry and other birds, progressive atrophic rhinitis and pneumonic pasteurellosis in pigs, haemorrhagic septicemia and respiratory diseases in cattle and buffalos, leporeno atrophic rhinitis and pneumonic pasteurellosis, are of great economic significance in agriculture [6, 8, 11]. On the territory of Kazakhstan, according to veterinary reporting, there are enzootic outbreaks of pasteurellosis among farm
animals, mainly young animals. The disease is accompanied by high mortality, reduced productivity, long-term carriage of pathogenic forms of the microbe [9,11]. Pasteurellosis in cattle tends to spread rapidly and widely, seriously hindering the preservation and increase in the number of livestock, as well as increasing productivity and improving the quality of the products obtained. This disease leads to premature culling of animals, endangers the preservation of breeding herds, affecting the development of the economy, preventing the sale and exchange of animals [13]. Epizootic outbreaks of pasteurellosis among cattle are due to the virulence of Pasteurella, as well as the state of natural resistance of animals. The disease occurs as a result of suppression of immune mechanisms in animals due to adverse factors. Against the background of a sharp decrease in the resistance of the animal organism, pasteurellosis can manifest itself as a secondary infection and proceed as an epizootic [15,18]. The source of the causative agent of infection are sick animals and pasteurell-carrying animals. Under natural conditions, infection occurs when feeding feed, at a watering place contaminated with secretions of patients. A contact route of infection is possible, as well as through the respiratory tract in the form of an airborne infection [14,19]. The present work presents data on the study of cultural, morphological and biochemical properties of epizootic isolates of Pasteurella multocida isolated from cattle. The conducted studies make it possible to evaluate the possibility of using the obtained cultures of Pasteurella multocida both to assess the immunogenicity of existing vaccines and to use them as a basis for the development of modified vaccines.

**Materials and research methods.** The following isolates and strain of Pasteurella multocida were used in the experiments: 1) an isolate isolated from pathological material from cattle from Bayserke-Agro LLP, Almaty region in 2021; 2) strains of Pasteurella multocida: equi (Collection number /B-0227/), ovis (Collection number /B-0228/), bovis (Collection number /B-0229/) were used as a control. To refresh and increase the virulent properties of the studied isolates, three successive passages were carried out through the body of white outbred mice weighing 16-18 g by subcutaneous infection in the back area with an 18-hour broth culture, in a volume of 0.5 cm3. Cultural-morphological and biochemical properties of epizootic strains of Pasteurella were studied according to generally accepted microbiological methods. When studying the cultural and morphological properties of isolated pure cultures of Pasteurella multocida strains, nutrient media MPA, Hottinger Agar and MPB were used. The isolate was incubated in biological test tubes and Petri dishes at 37°C for 18-24 hours [10]. Cell morphology was determined by electron microscopy, imprint smears were stained according to the method of Gram and Mikhin [12]. The biochemical properties of Pasteurella were studied by the method of their saccharolytic activity by seeding on Giss media using various carbohydrates and subsequent incubation at 37°C for 24 h. Carbohydrate fermentation was determined by changing the color of the medium [15,20].

The catalase test was carried out by mixing, applying a drop of catalase solution to the backmass in sterile glass slides. If bubbles of gas were formed, the result was considered positive.

**Results and its discussion.** When inoculating the Pasteurella multocida isolate isolated from cattle on MPA and Hottinger agar, after 24 hours of cultivation, round, convex, with a smooth wet surface, transparent and translucent colonies with smooth edges with a diameter of up to and more than 2.5 mm are formed. When cultivating the strains isolated from cattle, after 18-24 hours of incubation, the growth of translucent round colonies with smooth edges, a smooth surface of colonies of mucusy consistency, up to 2 mm in diameter, is observed. In transmitted light for 24 h, the culture has transparent amber margins with a slightly darkened center. The research results are shown in the figure. In test tubes with a liquid nutrient medium of the MPB, when growing the studied cultures of Pasteurella, in all cases, the formation of a mucous sediment was observed, which, when shaken, rose in the form of a characteristic pigtail, which also indicates that they belong to Pasteurella.

According to the results of the research, it was found that an essential morphological feature of Pasteurella is the temporal differences in the size and color of the colonies, as well as the growth rate on nutrient media.
Cell morphology of Pasteurella. According to the literature, a characteristic feature of this microorganism is bipolarity when staining smears (picture 2.) [6]. In the studied cultures of Pasteurella multocida, repeatedly reseeded on dense nutrient (MPA) and liquid (MPB) media, the microbe most often has the form of a coccus-shaped bacillus, diplococcus, most often located separately, but there were paired, group clusters and chains of short sticks of different lengths.

When studying the virulent properties of the test isolates, with an increase in the passage level, a decrease in the time of death of mice from 24 hours to 18-20 hours was noted, while 12-14 hours after infection of laboratory animals, depression, increased respiration, inactivity and ruffled hair were observed. As a result of the autopsy of mice, pathological changes were found in the internal organs: the lungs were hyperemic, hemorrhages on the epicardium of the heart, under the serous membrane of the liver, spleen, and hemorrhagic inflammation of the intestine. Microscopy of smears-imprints made from
the blood and organs (heart, liver, lungs, spleen) of laboratory animals experimentally infected with the studied Pasteurella multocida isolates revealed Gram-negative rods, more often of an ovoid form, in the field of view. The middle part of bacterial cells is colored paler than the ends. Pure cultures of Pasteurella were isolated from the organs of dead mice.

After the restoration of the virulent properties of Pasteurella, experiments were carried out to study the biochemical properties by inoculation in biological test tubes on Hiss media, catalase test. Data on the study of the glycolytic activity of pure cultures of Pasteurella multocida isolated from cattle were evaluated by color change in test tubes with media. Strains of Pasteurella multocida bovis, ovis, equi were used as a control, the results are presented in table 1 and in picture 3.

Table 1 – Study of the glycolytic activity of Pasteurella on Hiss media

<table>
<thead>
<tr>
<th>Carbohydrates/alcohols</th>
<th>Tested cultures of Pasteurella</th>
<th>The control P. multocida ovis</th>
<th>The control P. multocida equi</th>
<th>The control P. multocida bovis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dulcete</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes: 1 "+" - positive reaction, 2 "-" - negative reaction

The data in the table 1 and picture 3 indicate the fermentation of carbohydrates and alcohols by *Pasteurella*: mannitol, glucose, sucrose, inositol, xylose, except for test tubes containing dulcitol, lactose and raffinose.

Simultaneously held catalase test showed a positive result. The results obtained allow us to attribute the studied Pasteurella to the species *Pasteurella multocida*, which are consistent with the materials of V.I. Pokrovsky [3].

![Picture 3 – Determination of glycolytical properties of strains *Pasteurella multocida* on Hiss media](image)

Table 2 – Results of a biochemical test for the identification of Pasteurella multocida

<table>
<thead>
<tr>
<th>Used tests</th>
<th>Tested cultures of Pasteurella</th>
<th>From cattle</th>
<th>The control P. multocida ovis</th>
<th>The control P. multocida equi</th>
<th>The control P. multocida bovis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lysine</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Othine</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Urase</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Escaulin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Salicin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Notes: 1 "+" - 80-100% positive reaction, 2 "(+)" - 70-79% positive reaction, 3 "±" - variable reaction, 4 "(-)" - 16-30% negative reaction, 5 "-" - 0-15% negative reaction

From the data presented in Table 2, we can conclude that during the biochemical test, all tested samples and control gave a positive result for indole, sucrose, trehalose, glucose, mannose. At the same time, the samples were stained: with indole - pink, sucrose - yellow-green, trehalose, glucose, mannose - yellow. A color change during a biochemical test indicates a positive reaction, which is a marker for Pasteurella multocida [9].

**Conclusion.** Pasteurella isolated from pathological material from cattle, after six consecutive passages on laboratory animals, cause death in 18-20 hours, which proves their high virulence. Based on the studies carried out, it can be concluded that pure cultures of Pasteurella multocida isolated from the pathological material of cattle form S-shaped colonies and allow them to be attributed to the genus Pasteurella, the species Pasteurella multocida.

Isolated cultures of Pasteurella multocida can be used to assess the immunogenicity of existing vaccines and their application as the most promising for the development of anti-pasteurellosis vaccines.

**REFERENCES**


ТУЙІН

Бұл мақалада ірі қара малдан бөлініп алынған Pasteurella multocida штамдарының культуралық, тинкториалдық, биохимиялық және вируленттілік қасиеттерін зерттеу бойынша ұнтықкерлер көрсетілген. Бөлініп алынған штамдардың жанарту және олардың вируленттілігіне қарсы поливалентті эмульсиялық вакциналарды құруға болады.

РЕЗЮМЕ

В данной статье представлены результаты исследований по изучению культуральных, тинкториальных, биохимических и вирулентных свойств полевого штамма Pasteurella multocida, выделенных от крупного рогатого скота. С целью повышения вирулентных свойств выделенных...
штаммов проводили пассажи через организм белых мышей. По результатам проведенных биопроб установлено, что сокращение срока гибели испытуемых лабораторных мышей после заражения культурами Pasteurella multocida обусловлено повышением их вирулентности. Изучение культурально-морфологических свойств пастерелл проводили путем выращивания их на питательных средах мясо-пептонного агара и бульона при температуре 37 °C в течение 18-24 часов. Также для полного подтверждения полученных результатов использовали биохимический тест на средах Гисса. Испытуемые культуры, выделенные из патологического материала (сердце, легкие, селезенка, печень, кишечник) крупного рогатого скота показали, что обладают культуральными, биохимическими и биологическими свойствами, которые характерны для вида Pasteurella multocida и образуют колонии S-формы. Выделенные культуры Pasteurella multocida могут быть использованы для оценки иммуногенности существующих вакцин и применения их в качестве основы для разработки противопастереллезных поливалентных эмульсионных вакцин, гипериммунных противопастереллезных сывороток и т.д.