population bees by 2.3 kg. Egg producing ability of the bee colonies of the Carnica Sklenar Crain breed is better than of the local bee colonies of the Carpathian breed. Thus, we recommend the Crain breed bees of the Sklenar line with other bees of zoned breeds in the East Kazakhstan Region.

Key words: Apiculture, Crain and Carpathian bee breeds, economic and valuable features.

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VIRULENCE PROPERTIES OF PASTERELLAS ISOLATED FROM SAIGAS IN THE WEST-KAZAKHSTAN REGION

Annotation

In addition to environmental and anthropogenic factors (poaching), wildlife populations may also be affected by infectious diseases.

Clinically healthy saiga are carriers of the pathogen, and in some cases the virulence properties of the pathogen can be exacerbated, causing massive sickness and death of animals.

The article presents the results of virulent properties of pasteurellas isolated from saiga in the West Kazakhstan region. The results show that pasteurella cultures isolated from dead saigas have high virulence and toxicity for white mice.

Key words: pasteurella, virulence properties, pathogenicity, toxigenicity.

Introduction

The number of saigas is influenced by many factors and anthropogenic factor is the main one. However, infectious diseases cause considerable damage to the population of these animals, although not constantly.

Analysis of the literature shows that the issues of the morbidity of saigas by infectious diseases have not been sufficiently studied. There are only isolated reports of diseases that occur in saigas. To date, it is not known what problems of infectious pathology are relevant for this type of cloven-hoofed animals [3, p. 3].

Pasteurella - carriage is a widely known fact among healthy animals. It is of a great importance in the spread pasteurellosis in animals, especially in the farms where repeated outbreaks of infection were recorded, and direct contact is considered the main pathway for the spread of the disease. According to A.A. Sidorchuk, pasturella carriage among cattle reaches 70%, sheep - 50, pigs - 45, rabbits more than 50 and among chickens - from 35 to 50% [4, p. 173].

It is recognized that saigas are also carriers of *Pasteurella multocida,* the bacteria that inhabits the bodies of most saigas, but does not affect healthy animals [5, p. 151].

There are many unresolved issues in studying pasteurellosis. There is no well-founded differentiation of the species belonging to *P. multocida* and *P. haemolitica*, the use of serological and cultural-biochemical tests does not give positive results. The issue of circulation of the pathogen among domestic and wild animals has not been sufficiently studied. Highly effective methods of research have not been developed, which makes it possible to establish a diagnosis in animals in a short time and to display an agent in animal products, which are often a source of human infection.

Morbidity and mortality during pasteurellosis depends on the virulence of the pathogen, the immunological state of the herd, the conditions of maintenance and feeding, presence of secondary infections and the timeliness of the healthcare measures. An important role in the

development of pathological processes is played by the toxic products of the pasteurellas - endotoxins and especially the aggressors produced by the pathogen, which inhibit the body's resistance [6, p. 46].

Many genes encoding proteins with different functions are associated with the virulence of *Pasteurella multocida*. Such as, adhesion and penetration into the host cell, the acquisition of iron, the porins of the outer membrane, neurominidase, superoxide dismutase, dermanecrotoxin and dermatidinase [7, p. 105].

The formation of a capsule is one of the factors of virulence. Analysis of a large number of strains made it possible to reveal a correlation between the thickness of the capsular layer and the serovariant attachment of the strain. It is noted that the serovariants A and D show strong encapsulation. In cells of this type, with additional stabilization, extracellular formation is observed with a thickness of 70-90 and 10-30 nm from the outer membrane, respectively, for the above-mentioned serovariants [8, p. 501].

The spectrum of pathological and anatomical changes in sick animals is very wide. The disease occurs in acute, subacute and chronic forms. The development and severity of the pathological process depends on the state of the animal and the virulence of the pathogen. The acute form is characterized by numerous hemorrhages on serous, mucous membranes and parenchymal organs. Inflamed swelling and gelatinous infiltration of subcutaneous tissue, and intermuscular tissue in the pharyngeal region, intermaxillary space, neck, and under neck region. The thoracic form is characterized by fibrinous pneumonia with various stages of hepatization, enlargement and swelling of the interlobular partitions of the lungs. In chronic course, there is catarrhal-hemorrhagic gastroenteritis [9, p. 823].

Due to the wide variability pasteurellosis, depending on various causes, it is necessary to confirm the etiology of virulence in each case of disease occurrence. Previously, we conducted microbiological studies to determine the pasteurellas in healthy saigas. In this regard, we conducted studies to determine the virulent properties of the pasterella isolated from saigas.

The aim of the work was to study the virulent properties of the pasteurellas isolated from the saigas' corpses on the territory of the West Kazakhstan region with the definition of LD_{50} and LD_{100} on a laboratory model.

The research included the study of pathomorphological changes in organs and tissues of dead saigas, and study of virulent properties of pasteurellas on white mice.

Materials and methods

The research was carried out on the basis of the biotechnology laboratory of the Research Institute of Biotechnology and Nature Management of the WKATU named after Zhangir Khan. As a material for the study, the corpses of 11 saigas that were shot were seized from poachers in January 2017 in the Bokeyorda region of the West Kazakhstan region and 6 dead bodies of saigas that were found in the same region.

For the study of animal corpses, a complex method was used, including examination of corpses, pathoanatomical dissection according to Shora's conventional method of organ evisceration, with the preparation of autopsy protocols and sampling for bacteriological examination [5, p. 151].

As a result of bacteriological research, 14 cultures were isolated, of which 8 were identified by biological properties as pasteurella. The task of our studies included a comparative study of the virulence of the pasterella isolated from the shot and dead saigas.

To reveal pathogenicity, white mice were infected with 24-hour broth cultures containing 1 billion microbial bodies (according to the optical turbidity standard of the Tarasevich GISC) in a dose of 0.5 cm^3.

LD_{50} was determined on white mice weighing 16-18 g with a subcutaneous injection of 0.5 cm^3 of 16-18 hour broth cultures in dilutions from 10^{-1} to 10^{-10}. The concentration of microbial cells was established by counting the colonies grown on petri dishes with blood agar after

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sowing of 0.1 cm^3 of bacteria at dilutions of 10^{-6} and 10^{-7}. LD_{50} and LD_{100} were calculated according to the Kerber method in the modification of I.P. Ashmarin [1, p. 14].

Detection of the toxicity of isolated pasteurella was carried out on white mice by intraperitoneal injection of filtrates of 72-hour broth cultures at a dose of 0.5 cm^3.

The ability to form exotoxins of isolated pasteurella strains was carried out as follows: Hottinger broth containing 10% horse serum was dispensed into the tubes, checked for sterility by incubating in a thermostat at 37 °C for 24 hours, after which it was sown and cultured for 24 hours at 37° C. The resulting broth culture was centrifuged at 5000 rpm for 30 minutes. The supernatant was filtered through sterilizing filters and checked for sterility by sowing 0.5 cm^3 of filtrate on blood agar. The filtrate was diluted from 1: 2 to 1:64 in sterile physiological solution, and injected intraperitoneally with 0.5 cm^3, 3 white mice per dilution. The mice were observed for 7 days. Dead mice were subjected to bacteriological examination.

Results of the study and discussion

In recent years, there have been frequent outbreaks of pasteurellosis among commercial animals due to virulent pasteurella strains, so the study of pasteurellosis in animals is highly relevant. In this regard, we continued research on the prevalence of pasteurellosis in agricultural and wild animals, through bacteriological and modern molecular genetic methods. However, confirmation of the disease causes and the mortality of animals from pasteurellosis requires a careful study of the pathogenic properties of isolated cultures of pasteurellas. Therefore, we conducted studies to determine the virulence of the isolated pasteurella culture by setting up a bioassay on laboratory animals. The criterion of pathogenicity of isolated pasteurella cultures was their ability to cause death of white mice. For this purpose, a biological sample was used, i.e. one-day broth culture was administered subcutaneously to mice at a dose of 0.2 cm^3 and monitored for 7-10 days.

As a result of the experiment, white mice, infected with a culture of pasteurellas isolated from dead animals, died 18-24 hours after inoculation with the daily culture of the pathogen, which confirms the high virulence of the isolated culture. At the same time, the cultures of the pasteurellas, isolated from healthy killed animals, did not cause the death of white mice after the first administration.

To determine the virulence, a suspension of pathogenic cultures of pasteurella with a known content of microbial cells per unit volume was prepared. Subsequent dilutions of the suspension were then performed in sterile saline and equal volumes of each dilution (0.5 cm^3) were administered intraperitoneally to susceptible laboratory animals (white mice). After that animals were monitored and the number of dead animals was taken into account to calculate LD_{50}.

Table - 1. LD_{50} values of pathogenic cultures of Pasteurella

Pasteurella suspension concentration, CFU	Infected mice	Dead mice	Survived mice	Lethality, %
10^2	6	0	6	0
10^3	6	1	5	16,6
10^4	6	3	3	50,0
10^5	6	5	1	88,3
10^6	6	6	0	100,0

At the same time, 12-14 hours after infection of laboratory animals, oppression, increased respiration, and lack of mobility were observed. An autopsy of dead experimental animals was performed and the the smears from pathological material (from the parenchymal organs) of dead

mice were prepared and stained, followed by their microscopy, and culturing on nutrient media. In all cases pure cultures of pasteurellas were isolated from dead mice.

During the autopsy inflammatory foci and puffiness of the subcutaneous tissue were observed at the injection site and pinpoint hemorrhages were clearly seen in the thoracic cavity and heart. Liver was swollen and filled with blood (Figure 1).

Figure 1 - Pathological changes in the parenchymal organs

Microscopy of smears from the parenchymal organs of white mice revealed Gram-negative rods, more often ovoid, dyed bipolar. Pure cultures of the pasteurellas were isolated from the organs of the dead mice (Fig. 2).

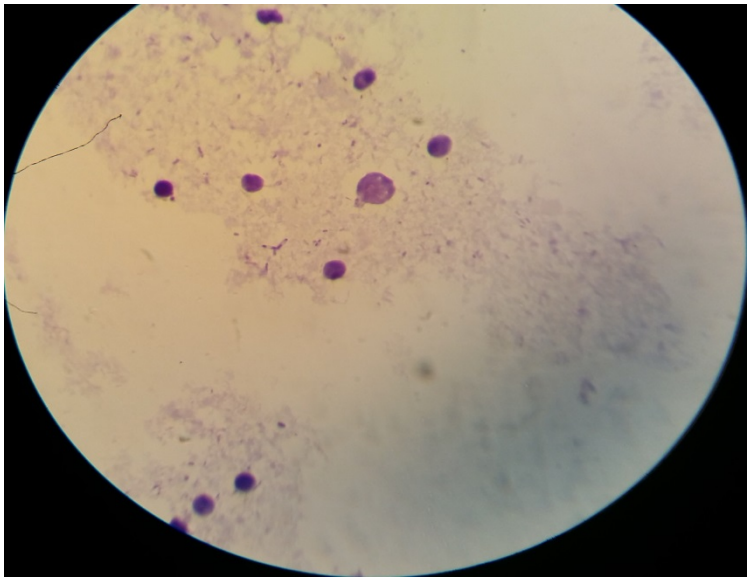

Figure 2 - Gram-negative sticks of ovoid form

Experimental studies on laboratory animals were carried out to determine the toxigenicity of the isolated strains. Preliminarily a filtrate of the pasteurella cultures was diluted in saline from 1: 2 to 1:64. Then, each dilution was infected intraperitoneally in a dose of 0.5 cm^3, 3 white mice per dose. The animals were monitored for 7 days. The dead mice were subjected to bacteriological examination.

Table - 2.Determination of toxicity of pasteurella in experiments on white mice

Dilution of pasteurella culture filtrate	Infected mice	Dead mice	Survived mice	Toxigenicitty, %	Note
1:2	3	3	-	100,0	Dead mice had the signs og hemorrhagic septicemia
1:4	3	3	-	100,0	
1:8	3	3	-	100,0	
1:16	3	3	-	100,0	
1:32	3	1	2	33,3	
1:64	3	0	3	0	

As can be seen from the Table 2, a study of the toxicity of the pasteurellas isolated from the organs of dead saigas showed 100% toxicity for white mice in the dilutions of filtrates from 1: 2 to 1:16. When autopsies of dead mice were performed, changes typical for the acute course of pasteurellosis-hemorrhage was observed in all parenchymal organs.

Conclusion

The obtained results indicate that the cultures isolated from dead saiga had virulent properties on white mice.

Also the conducted studies have established that saiga of all ages are susceptible to pasteurellosis and can be pasteurella - carriers. The disease occurs on the background of a sharp decrease in the resistance of the organism due to unfavorable factors.

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БҚО-НДАҒЫ АҚБӨКЕНДЕРДЕН БӨЛІНІП АЛЫНҒАН ПАСТЕРЕЛЛАЛАРДЫҢ ВИРУЛЕНТТІЛІК ҚАСИЕТТЕРІ

Аңдатпа

Жабайы жануарлар популяциясында экологиялық және антропогенді факторлардан (браконьерлік) басқа, жұқпалы сипаттағы аурулардың да мәні болуы мүмкін.

Клиникалық сау ақбөкендер ауру қоздырушысын тасымалдаушылар болып табылады және кейбір жағдайда оның вируленттік қасиеттері күшейіп, жаппай ауру тудыруы және жануарларды өлімге ұшыратуы мүмкін.

Мақалада Батыс Қазақстан облысы аумағындағы ақбөкендерден анықталған пастереллалардың вируленттік қасиеттерін зерттеу нәтижелері берілген. Алынған нәтижелер өлген ақбөкендерден бөлініп алынған пастерелла өсінділері ақ тышқандар үшін жоғары вируленттік қасиетке және уыттылыққа ие екенін куәландырады.

Кілт сөздер: пастереллалар, вируленттік қасиеттер, зардаптылық, уыттылық.

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ВИРУЛЕНТНЫЕ СВОЙСТВА ПАСТЕРЕЛЛ, ВЫДЕЛЕННЫХ ОТ САЙГАКОВ В ЗАПАДНО-КАЗАХСТАНСКОЙ ОБЛАСТИ

Аннотация

Кроме экологических и антропогенных факторов (браконьерство), лимитирующих популяцию диких животных, значение могут иметь и болезни инфекционной этиологии.

Клинически здоровые сайгаки являются носителями возбудителя болезни и, видимо, при определенных условиях его вирулентные свойства усиливаются, что может вызвать массовое заболевание и гибель животных.

В статье приведены результаты изучения вирулентных свойств пастерелл, выделенных от сайгаков на территории Западно-Казахстанской области. Полученные результаты свидетельствуют о том, что выделенные от павших сайгаков культуры обладали вирулентными и токсигенными свойствами в отношении белых мышей.

Ключевые слова: пастереллы, вирулентные свойства, патогенность, токсигенность.

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ВОЗРОЖДЕНИЕ СЕЛЕКЦИИ КРАИНСКИХ ПЧЕЛ В ВОСТОЧНОМ КАЗАХСТАНЕ

Аннотация

Приведено описание краинской породы пчел, показано преимущество перед другими