Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Properties of *Pasteurella multocida* isolated from animals during the seasonal migration of saigas

Propriedades de *Pasteurella multocida* isolada de animais durante a migração sazonal de saigas

U. Taubaev[a], A. Ichshanova[a]*, Zh. Kirkimbaeva[b], B. Radojicic[c], K. Murzabayev[a], S. Bayantassova[a], F. Zakirova[a] and B. Yertleuova[a]

[a] Zhangir Khan West Kazakhstan Agrarian-Technical University, Uralsk, Republic of Kazakhstan
[b] Kazakh National Agrarian Research University, Almaty, Republic of Kazakhstan
[c] University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

Abstract

The paper describes data from the study of cultural, morphological, and biochemical properties and the pathogenicity and virulence of epizootic isolates of *Pasteurella multocida* obtained from cattle and saigas. The study aimed to investigate the properties of P. multocida isolated from saigas and cattle during their seasonal migration, with a focus on its role in the epizootic process and potential transmission to farm animals. The research was conducted in a laboratory setting at the West Kazakhstan Agrarian-Technical University. White mice, saigas, and cattle were examined, and pathological and bacteriological analyses were performed on tissues and secretions. Pathogenicity, virulence, and toxigenicity of the isolated *Pasteurella* cultures were determined through biological tests on white mice. The morphological, cultural, and biochemical properties of the isolates were studied using standard microbiological methods. The study found that *P. multocida* isolates from both saigas and cattle exhibited high pathogenicity and virulence when tested on white mice. The isolates from sick and dead animals displayed 65.3 and 83.3% pathogenicity, respectively. The isolates were toxic to white mice, with filtrate dilutions showing 100% toxigenicity. Comparative analysis showed morphological and cultural similarities between *Pasteurella* isolates from saigas and cattle, confirming their identity. This research demonstrates that *P. multocida*, isolated from both saigas and cattle, contributes to the epizootic process and poses a threat to farm animals. Saigas, in particular, play a role in disease transmission during seasonal migrations. Understanding the ecological interactions between wild and farm animals is crucial for implementing preventive measures to control the spread of infectious diseases, emphasizing the need for comprehensive monitoring and intervention strategies.

Keywords: seasonal migrations, epizootic process, saiga, cattle, pasteurellosis.

Resumo

O artigo descreve dados do estudo das propriedades culturais, morfológicas e bioquímicas e da patogenicidade e virulência de isolados epizoóticos de *Pasteurella multocida* obtidos de bovinos e saigas. A pesquisa foi conduzida em laboratório na Universidade Técnica Agrária do Cazaquistão Ocidental. Camundongos brancos, saigas e bovinos foram examinados, e análises patológicas e bacteriológicas foram realizadas em tecidos e secreções. A patogenicidade, virulência e toxigenicidade das culturas isoladas de *Pasteurella multocida* foram determinadas através de testes biológicos em camundongos brancos. As propriedades morfológicas, culturais e bioquímicas dos isolados foram estudadas utilizando métodos microbiológicos padrão. O estudo descobriu que isolados de *P. multocida* tanto de saigas quanto de gado exibiram alta patogenicidade e virulência quando testados em camundongos brancos. Os isolados de animais doentes e mortos apresentaram patogenicidade de 65,3 e 83,3%, respectivamente. Os isolados foram tóxicos para camundongos brancos, com diluições de filtrado apresentando 100% de toxigenicidade. A análise comparativa mostrou semelhanças morfológicas e culturais entre isolados de Pasteurella de saigas e bovinos, confirmando sua identidade. Esta pesquisa demonstra que *P. multocida*, isolado tanto de saigas quanto de gado, contribui para o processo epizoótico e representa uma ameaça para os animais de fazenda. As saigas, em particular, desempenham um papel na transmissão de doenças durante as migrações sazonais. Compreender as interações ecológicas entre animais selvagens e de criação é crucial para a implementação de medidas preventivas para controlar a propagação de doenças infecciosas, enfatizando a necessidade de estratégias abrangentes de monitorização e intervenção.

Palavras-chave: migrações sazonais, processo epizoótico, saiga, gado, pasteurelose.

*e-mail: aimanichshanova@gmail.com
Received: November 24, 2023 – Accepted: December 26, 2023

1. Introduction

Modern problems of the epizootic process of diseases of wild and farm animals depend on several factors that exacerbate the epizootic situation with the manifestation of the infectious process, including pasteurellosis (Mukhamadiyev et al., 2023; Myrzhieva et al., 2020; Sheralieva et al., 2023). The transformation of natural landscapes deprives wild animals of habitual living conditions (Dukenov et al., 2023). This leads to a decrease in their individual and group resistance and activates the source and factors of transmission of infection (Absatirov and Ichshanova, 2018; Zhanabayev et al., 2022; Barakhov et al., 2023).

The study of the environmental characteristics of pathogenic and conditionally pathogenic microorganisms makes it possible to determine the epizootic situation in the studied area and predict the probable outbreak of an infectious disease (Beishova et al., 2023). The study of the ecology of pathogens allows for determining their potential ability to cause an infectious process and finding methods and means of correcting the virulence of microbes in their ecological niche. In this process, the conditionally pathogenic microflora of wild animals, one of which is the saiga antelope, remains poorly studied (Gnezdilova et al., 2023).

Opinions among scientists on this important issue, which has not only theoretical but also great practical significance, are ambiguous. The success of controlling this disease depends to an extent on reliable knowledge of this issue. The stationarity of pasteurellosis is closely related to the natural foci. If we consider the fact that there are individual farms, regions, and even entire areas where pasteurellosis has been stationary for several years, it can be assumed that it is closely related to the significant spread of *Pasteurellae* in the natural biotope. The knowledge of the possibilities of cattle reinfection with pasteurellosis from saigas carrying this disease, or *Pasteurella* carrier state in animals, is of great practical interest.

Saiga is a paired mammal in the group of true antelopes. A unique animal of Western Kazakhstan, the saiga (*Saiga tatarica* L., 1766), a herd animal of the deserts and semi-deserts of Eurasia, has managed to survive for thousands of years in the conditions of the most severe natural disasters. The saiga of the Volga-Ural group lives in the West Kazakhstan region. The saiga spends the time from spring to autumn in the southern regions of the West Kazakhstan region and goes to the territory of the Atyrau region for the winter. In winter, saigas are located in the northwestern part of the interfluve area near the village of Karaoba, with subsequent migration to the Dzhangali and Bokeyordinsky districts (Absatirov and Ichshanova, 2018; Sidorchuk, 2007; Karmaliyev et al., 2019).

Saigas continuously move around their range throughout the year. These animals are characterized by regular annual seasonal migrations in Kazakhstan. Its duration and time depend on the period before spring.

The most dangerous infectious disease for an animal since ancient times is pasteurellosis. This epidemic is an infectious disease typical not only for saigas but also for humans, other mammals, and birds. The new results obtained in this direction will undoubtedly be applied in the development of measures to prevent the mass extinction of saigas from pasteurellosis and will contribute to the protection of not only farm animals but also people from the epidemic of zooanthroponosis (Ichshanova et al., 2018a; Kirkimbaeva and Oryntaev, 2011).

The intensity of the manifestation of the epizootological process depends on many factors, for example, biological (virulence of the pathogen, the number of microorganisms, i.e., the infecting dose, the degree of susceptibility of animals, the forms of manifestation of the disease, etc.); natural and geographical (the presence and density of carriers, the season of the year, the presence of natural reservoirs, etc.); economic (density of animal placement, intensity of their utilization, economic relations, hygienic condition, veterinary care, etc.) (Aubakirov et al., 2022). Depending on these factors, the degree of manifestation of diseases can vary from a single case to a mass epidemic (Ishchanova, 2012; Kirkimbaeva et al., 2018).

Farm animals are often present in natural disease foci, especially with pasture management (via common rodents, blood-sucking insects, contacts with wild animals and birds, etc.). These contacts are often broad. Changes in the number of wild animals and birds and their migration contribute to a change in the intensity of the epizootological process in the natural foci. They provide seasonal and periodic fluctuations in the incidence of not only wild but also farm animals (Ichshanova and Radojicic, 2018; Smith et al., 2021; Wilkie et al., 2012).

Wild animals pose a much greater danger to farm animals than vice versa, because they are poorly controlled by humans and latent (asymptomatic) infections are especially frequent among them (Ishchanova et al., 2018; Ichshanova et al., 2018b).

In some of the described cases, wild animals were the source of the pathogen causing pasteurellosis. Thus, saiga antelopes with pasteurellosis can serve as a source of the pasteurellosis pathogen for farm animals, including cattle.

The reason for the high mortality of saigas is the high activity of pasteurellosis against the background of the weakening of the organism in the breeder livestock during mass lambing after severe wintering (Nasambaev et al., 2022). The study of natural foci creates conditions for a better understanding of the patterns of epizootics of pasteurellosis and rational planning of prevention and control measures. Epizootics of pasteurellosis recorded among farm animals, wild animals, and birds cause not only great economic damage but also pose a serious threat to human health.

Thus, the main sources of the causative agent of pasteurellosis are sick animals. However, along with these sources of the causative agent of infection, many researchers give great importance to animals that carry *Pasteurella*. The study of the interaction between micro- and macroorganisms has shown that the bacterial carrier state happens without functional disruptions in the body. However, as a result of the weakening of the body under the influence of external factors, a healthy bacterial carrier may develop the disease.

The study aims to evaluate the influence of various factors on the manifestation of the epizootic process

with a decrease in the resistance of wild animals and the relationship of these animals with farm animals. Following the purpose of the study, we identified the following tasks:

- to determine the influence of factors and analyze *Pasteurella* carrier state;
- to study the ecology and properties of bacteria found in saiga and cattle in the West Kazakhstan region.

2. Materials and Methods

The study was carried out at the laboratory of the West Kazakhstan Agrarian and Technical University named after Zhangir Khan in 2022-2023. The object of the study was white mice, saiga antelopes, and cattle. Pathoanatomic and bacteriological studies were carried out in laboratories following current methods and veterinary and sanitary rules. To confirm the primary clinical and epizootological diagnosis for pasteurellosis, biomaterial obtained from the animals that had died or had been killed out of necessity (pieces of liver, spleen, lungs with regional lymph nodes, kidneys, heart) was examined by laboratory methods (Figure 1A, 1B).

To determine the influence of factors and conduct an analysis on *Pasteurella* carrier state in the West Kazakhstan region, the following works were carried out. To identify *Pasteurella* carrier state, discharge samples obtained from nasal cavities were examined by applying them on discs made of filter paper. The material was taken from 14 cows coming from three farms with registered pasteurellosis and seven saiga heads located in a nursery for the conservation and breeding of saigas.

At the farms, the discs were applied to the animal to be examined with tweezers on the mucous membrane of the nasal passages. The discs were very quickly soaked with discharge containing microorganisms. Then the discs were removed, placed in test tubes containing saline, and lightly shaken to be immersed in the liquid. In the laboratory, the contents of the test tube with the discs were poured into a sterile mortar and ground until a homogenate was obtained. Then, with a sterile pipette of 0.2 ml from the prepared suspension, the culture was seeded on nutrient media.

To study the ecology and properties of bacteria found in saigas and cattle, the following series of studies were carried out.

To clarify the involvement of saigas in maintaining the stationary status of the epizootic focus, material from saigas living in the West Kazakhstan region was studied. A total of six saigas were examined. Autopsy and pathoanatomic examination was carried out on the animals, and the material taken was carried out by laboratory method. Internal organs, blood from the heart, and nasal mucus were used for the study. In the beginning, imprint smears were made from the organs and stained according to Gram and Romanowsky-Giemsa; then, after appropriate preparation, they were sown on meat infusion agar (MIA) and meat infusion broth (MIB).

The study of morphological, cultural, and biochemical properties of the isolated microorganisms was carried out using methods of general microbiology (Ivanishchuk and Kovalev, 1993; Kovalev and Ivanishchuk, 1999; Terenteva et al., 2014).

Morphological properties of the isolated cultures of microorganisms were studied using light microscopy. Preparations were prepared from agar and broth cultures of microbes. An EX-30 biological microscope was used for light microscopy.

The cultural properties of the isolated bacteria were studied on liquid and solid conventional nutrient media (MIA, MIB).

The growth of microbial cultures on nutrient media was observed once a day for 7 days. The growth pattern of colonies and their sizes were noted, considering the shape of colonies, their edges, surface, gloss, color, profile, consistency, and structure. During the cultivation of microorganisms in liquid nutrient media, the presence of sediment, its quantity and nature, the degree of turbidity of the medium, the thickness and consistency of the film during surface growth of the culture, and a change in the color of the medium were noted.

To identify and differentiate the microbial isolates, the biochemical properties of the culture were studied. The biochemical properties of microorganisms were studied on a Hiss medium with an Andrade indicator. The cultures were sown according to the generally accepted method with a bacteriological loop. After incubation in the thermostat for 16 and 24 hours, the results of carbohydrate fermentation were considered by changing the color of the nutrient medium and the formation of gaseous substances.

The virulence, pathogenicity, and toxigenicity of the isolated *Pasteurella* cultures were determined by performing a biological assay on white mice.

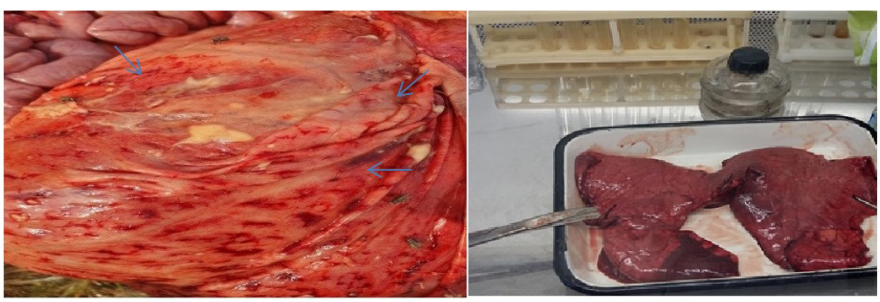

Figure 1. (A) Inflammation of the mesenteric lymph nodes of cattle; (B) Inflammation of the lungs of a dead saiga.

The pathogenicity criterion of the isolated *Pasteurella* cultures was their ability to cause the death of white mice. For this purpose, a biological sample was used, i.e., a one-day broth culture was administered subcutaneously to mice at a dose of 0.2 cm^3 and monitored for 7-10 days (Table 1).

To determine virulence, a suspension of pathogenic *Pasteurella* cultures with a known content of microbial cells per unit volume was prepared. Then successive dilutions of the suspension were made on the sterile saline solution and equal volumes of each dilution (0.5 cm^3) were administered intraperitoneally to sensitive laboratory animals (white mice). Then the animals were monitored and the number of dead animals was considered to calculate the median lethal dose (LD_{50}) (Table 2).

At 12-14 hours after infection of laboratory animals, depression, increased breathing, and inactivity were observed. An autopsy was performed on the dead experimental animals, and smears were prepared from the pathological material (the parenchymal organs) of the dead mice. The smears were stained with subsequent microscopy, and seeding was done on nutrient media. In all cases, pure *Pasteurella* cultures were isolated from the dead mice.

During the autopsy of the animals at the injection site, inflammatory foci and swelling of the subcutaneous tissue were found, as well as spot hemorrhages in the chest cavity and heart. The liver was swollen and filled with blood (Figure 2A).

Experimental studies on laboratory animals were conducted to determine the toxigenicity of the strains. Previously, the dilution of the filtrate of *Pasteurella* cultures from 1:2 to 1:64 had been prepared in a sterile saline solution. Then, each dilution was injected intraperitoneally at a dose of 0.5 cm^3, three white mice per dose. The animals were clinically monitored for 7 days (Table 3). The dead mice were subjected to bacteriological examination.

The results of these studies were used to develop measures and recommendations to prevent the spread and reduce the intensity and manifestation of infectious diseases among agricultural and wild animals.

3. Results and Discussion

The intensity of the epizootic process in natural foci of diseases and the degree of danger of infection of farm and domestic animals depend on the number and migration activity of susceptible wild animals (Taubaev, 2007).

During the period of decreasing resistance or disease, saigas secrete pathogens of infectious diseases and

Table 1. Pathogenicity of *Pasteurella multocida* isolates for white mice.

No.	Isolates	Number of samples examined	Number of positive samples/% of the number of examined samples					
			Highly pathogenic		Weakly pathogenic		Non-pathogenic	
			number of PS	%	number of PS	%	number of PS	%
1	From nasal swabs obtained from healthy animals	28	-	-	8	28.6	20	71.4
2	From nasal swabs obtained from sick animals	98	64	65.3	30	30.6	4	4.1
3	From the organs of dead animals	24	20	83.3	4	16.7	-	-
	Total	150	84		42		24	

Note: (from nasal swabs of healthy animals: seven saigas, from nasal swabs of sick animals: 14 heads of cattle, from organs of dead animals: isolates obtained from six saigas). PS – Positive samples.

Table 2. LD_{50} indicators of pathogenic *Pasteurella* cultures.

No.	Concentration of Pasteurella suspension, colony-forming units (CFU)	Number of infected mice	Of them, died	Survived	Lethality, %
1	10^2	6	0	6	0
2	10^3	6	1	5	16.6
3	10^4	6	3	3	50.0
4	10^5	6	5	1	88.3
5	10^6	6	6	0	100.0

Figure 2. (A) Pathoanatomic changes; (B) Gram-negative rods (Gr-) in parenchymal organs of ovoid shape.

Table 3. Determination of the toxigenicity of *Pasteurella* in experiments on white mice.

No.	Dilution of Pasteurella culture filtrate	Number of infected mice	Of them, died	Survived	Toxigenicity, %	Note
1	1:2	3	3	-	100.0	Mice that died with signs of hemorrhagic septicemia
2	1:4	3	3	-	100.0	
3	1:8	3	3	-	100.0	
4	1:16	3	3	-	100.0	
5	1:32	3	1	2	33.3	
6	1:64	3	0	3	0	

conditionally pathogenic microflora into the external environment, where hay and haylage are harvested, and cattle are grazed. The dynamics of manifestations of the epizootic process among cattle herds are influenced by the intensity of migration of wild susceptible animals.

In the process of studying the epizootology of pasteurellosis in the selected farms, we were interested in the possibility of *Pasteurella* carrier state among saigas living in significant numbers in the vicinity of livestock farms and their epizootological role in maintaining the stationary nature of the disease. To solve this problem, we examined biological material from dead saigas of the West Kazakhstan region.

Pasteurella isolated from nasal mucus were pathogenic for white mice and therefore posed a danger as a source of the causative agent of infection.

The results of our study clearly show that in the farms with a constantly unfavorable pasteurellosis status, in combination with other unfavorable factors that negatively affect the animal organism, saigas carrying pasteurellosis are involved in the occurrence of periodic outbreaks in farms previously free from it.

The cell morphology was studied by light microscopy. According to the literature, a characteristic feature of this microorganism is bipolarity when staining smears. However, morphologically, the pathogens of pasteurellosis of different animal species do not differ (Taubaev, 2012; Eshmukhametov and Kamsaev, 2014; Kirkimbayeva et al., 2014). In our study, strain cells isolated from cattle (Figure 3A) are homogeneous and have an elongated shape (size 0.5×1.2 μm), while the ones obtained from saigas (Figure 3B) are also homogeneous, but more rounded (size 0.8×1.0 μm). There is a slight difference in size.

The cultural properties of nasal swabs isolated from sick animals showed weak turbidity on the MIB; sediment formed at the bottom of the test tube. On MIA, growth was observed 18-24 hours after seeding in the form of barely noticeable small, isolated transparent colonies with a bluish tinge (with smooth edges) resembling dew droplets. The cultural properties of swabs isolated from healthy animals on MIB: there was more intense turbidity of the medium and abundant precipitation formed at the bottom of the test tube, and small and medium-sized colonies were observed on MIA, more often with a matte shade. Cultures isolated from dead animals gave a very poor growth of transparent dewy colonies on agar, often with a bluish tinge.

Considering the biochemical properties, cultures isolated from healthy and sick animals fermented glucose, sucrose, mannose, mannitol, and sorbitol to acid without gas formation and did not ferment lactose, dulcite, and raffinose.

As a result of the experiment, white mice infected with a *Pasteurella* culture isolated from animals died 18-24 hours after inoculation with a daily culture of the pathogen, which confirms the high virulence of the isolated culture. At the same time, *Pasteurella* cultures isolated from healthy animals did not cause the death of white mice after the first administration (Table 1 and Figure 4).

As can be seen from Table 1 and Figure 4, isolates obtained from sick and dead animals showed high pathogenicity

Table 4. Comparative characteristics of morphological and cultural properties of *Pasteurella multocida* in cattle and saiga.

No.	Animal species	Cell morphology/μm	The shape of the bacterium	Bipolarity	Colony type	Growth conditions
1	Saiga	Gr -; 0.5×1.2 μm	more rounded	+	mild turbidity (MIB); small, with a bluish tint	optional anaerobe
2	Cattle	Gr -; 0.8×1.0 μm	elongated shape	+	mild turbidity (MIB); small, with a bluish tint	optional anaerobe

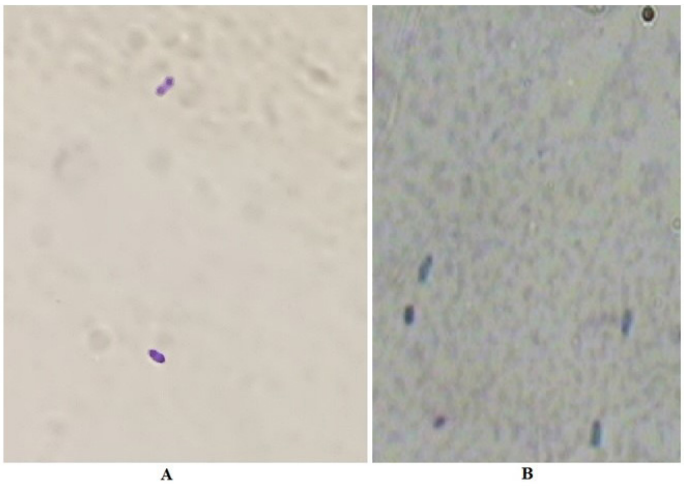

Figure 3. *Pasteurella*. A smear of the culture in MIB: (A) cattle; (B) saiga.

(65.3 and 83.3%, respectively), and weakly pathogenic isolates showed 28.6, 30.6, and 16.7%, respectively. Non-pathogenic isolates obtained from healthy animals accounted for 71.4% and the ones obtained from sick animals equaled 4.1%. Pathogens are transmitted from saigas to farm animals, and this contributes to an increase in morbidity.

When studying the virulent properties of the test isolates (Table 2), 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 breathing, a decrease in mobility, and disheveled hair were observed.

As is evident from Table 2, the results indicate that the cultures had virulent properties against white mice. The LD_{50} of pathogenic *Pasteurella* cultures was 10^4 CFU.

As a result of the autopsy of mice, the following pathological changes in internal organs were detected: hyperemic lungs, hemorrhages on the epicardium of the heart and under the serous membrane of the liver and spleen, and a hemorrhagic inflammation of the intestine. When microscopy of imprint smears made from organs (heart, liver, kidney, spleen) of laboratory animals experimentally infected with the studied isolates of *Pasteurella multocida*, gram-negative rods, more often ovoid and stained bipolar, were found in the field of vision. The middle part of the bacterial cells is colored paler than the ends.

The isolated *Pasteurella* cultures have high toxicity for white mice (Table 3).

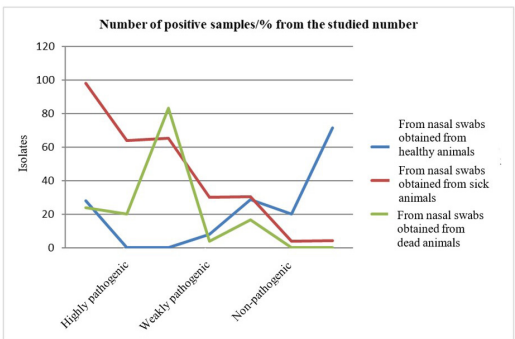

Figure 4. Pathogenicity diagram of *Pasteurella multocida* isolates.

As can be seen from Table 3, the study of the toxigenicity of *Pasteurella* isolated from the organs of dead saigas showed 100% toxigenicity for white mice in the dilution of filtrates equaling 1:2-1:16. When dissecting the corpses of dead mice, characteristic changes were noted for the acute course of pasteurellosis, namely, hemorrhage in all parenchymal organs.

The isolates obtained as a result of the study, isolated from saiga and cattle, were subjected to comparative analysis by morphological and cultural properties (Table 4).

From Table 4, it can be concluded that the comparative characteristics of *P. multocida* show the identical character of *Pasteurella* in the body of saigas and cattle.

4. Conclusions

When studying the tinctorial properties of isolates, bipolarity was established, and Gram staining was negative. The cells of the strains isolated from cattle were homogeneous and had an elongated shape (size 0.5×1.2 µm), and the ones from saigas were also homogeneous, but more rounded (size 0.8×1.0 µm). The cultural properties of nasal swabs isolated from sick animals showed weak turbidity on the MIB; sediment formed at the bottom of the test tube. On the MIA, growth was observed 18-24 hours after seeding in the form of barely noticeable small, isolated transparent colonies with a bluish tinge (with smooth edges) resembling dew droplets. With the growth of cultures from nasal swabs isolated from healthy animals, more intense turbidity of the medium was noted on the MIB, and abundant precipitation formed at the bottom of the test tube, while small and medium-sized colonies were observed on the MIA, more often with a matte shade. Cultures isolated from dead animals grew sparsely on the MIA with the formation of transparent, bluish-tinged dewy colonies.

Considering the biochemical properties, cultures isolated from healthy and sick animals fermented glucose, sucrose, mannose, mannitol, and sorbitol to acid without gas formation and did not ferment lactose, dulcite, and raffinose.

Pasteurella isolates were pathogenic and virulent in white mice.

Based on the conducted studies, it can be concluded that pure cultures of *P. multocida* isolated from the pathological material of cattle and saiga allow them to be attributed to the genus *Pasteurella*, the *P. multocida* species.

Acknowledgements

This study was funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. AP15473404 "Intensity of pasteurellosis manifestation during the seasonal migration of saigas and the relationship of disease occurrence in farm animals").

References

ABSATIROV, G.G. and ICHSHANOVA, A.S., 2018. Retrospektivnyi analiz massovoi gibeli i puti sokhraneniya saigakov v Kazakhstane [Retrospective analysis of mass deaths and ways of conservation of saigas in Kazakhstan]. In: *Stepi Severnoi Evrazii: Materialy VIII Mezhdunarodnogo Simpoziuma* [The Steppes of Northern Eurasia: Proceedings of the 8th International Symposium], 9-12 September 2018, Orenburg, Russian Federation. Orenburg: IS UrO RAN, pp. 132-337.

AUBAKIROV, M.Z., ABDYBEKOVA, A.M., KHASSANOVA, M.A., ISSABAYEV, A.Z., KAUMENOV, N.S., TEGZA, A.A., SAPA, V.A., DOMATSKY, V.N., ERENKO, E.N. and NAMAZBAI, K.N., 2022. Epizootology and epidemiology of opisthorchiasis in northern Kazakhstan. *Online Journal of Biological Sciences*, vol. 22, no. 3, pp. 340-346. http://dx.doi.org/10.3844/ojbsci.2022.340.346.

BARAKHOV, B., MYRZABEKOV, Z., SERIKBAI, N., NARBAYEVA, D., ALPYSBAYEVA, G. and BATYRBEKOV, A., 2023. Safe technologies of prophylactic disinfection in the presence of animals. *American Journal of Animal and Veterinary Sciences*, vol. 18, no. 2, pp. 107-116. http://dx.doi.org/10.3844/ajavsp.2023.107.116.

BEISHOVA, I., NURGALIYEV, B., BELAYA, A., CHUZHEBAYEVA, G., ULYANOV, V., ULYANOVA, T., KOVALCHUK, A., DUSHAYEVA, L., MURZABAYEV, K., TAIPOVA, A., ZHOLDASBEKOVA, A. and ISABAEV, A., 2023. Marking of genetic resistance to chlamydia, brucellosis and mastitis in holstein cows by using polymorphic variants of LTF, MBL1 and TLR9 genes. *American Journal of Animal and Veterinary Sciences*, vol. 18, no. 2, pp. 89-97. http://dx.doi.org/10.3844/ajavsp.2023.89.97.

DUKENOV, Z., RAKHIMZHANOV, A., AKHMETOV, R., DOSMANBETOV, D., ABAYEVA, K., BORISSOVA, Y., RAKYMBEKOV, Z., BEKTURGANOV, A., MALENKO, A., SHASHKIN, A. and TRUSHIN, M., 2023. Reforestation potential of tugai forests in the floodplains of Syr Darya and Ili Rivers in the territory of Kazakhstan. *SABRAO Journal of Breeding and Genetics*, vol. 55, no. 5, pp. 1768-1777. http://dx.doi.org/10.54910/sabrao2023.55.5.28.

ESHMUKHAMETOV, A.E. and KAMSAEV, E.M., 2014. *Laboratornaya diagnostika pasterelleza bakteriologicheskom metodom: standartnaya operatsionnaya protsedura* [Laboratory diagnosis of pasteurellosis using the bacteriological method: standard operating procedure]. Astana, 7 p.

GNEZDILOVA, L., FEDOTOV, S., MURADYAN, Z., ROZINSKY, S. and REDDY GADE, R., 2023. Effect of mycotoxins on reproduction and milk quality of dairy cattle and its amelioration by natural adaptogen "Betulin". *Qubahan Academic Journal*, vol. 3, no. 4, pp. 400-408. http://dx.doi.org/10.58429/qaj.v3n4a231.

ICHSHANOVA, A.S. and RADOJICIC, B., 2018. Analysis of results of DNA isolation from biological samples. *Science and Education*, vol. 53, no. 4, pp. 184-188.

ICHSHANOVA, A., TAUBAEV, U., ABSATIROV, G. and KIRKIMBAEVA, Z., 2018a. Carrier state of pasteurellosis in saiga antelopes in the western Kazakhstan region. *The Indian Veterinary Journal*, vol. 95, no. 8, pp. 17-18.

ICHSHANOVA, A.S., TAUBAEV, U.B., KIRKIMBAEVA, Zh.S. and SHUZHEBAYEVA, G.D., 2018b. Extraction of *Pasteurella multocida* gDNA from organs and tissues of animals using various methods. *Science and Education*, vol. 51, no. 2, pp. 61-65.

ISHCHANOVA, A.S., 2012. Vydelenie shtammov pasterelly iz patologicheskogo materiala i izuchenie ikh svoistv [Isolation of Pasteurella strains from pathological material and study of their properties]. In: *Evraziiskaya Integratsiya: Rol Obrazovaniya i Nauki v Realizatsii Innovatsionnykh Programm, Materialy Mezhdunarodnoi Nauchno-Prakticheskoi Konferentsii* [Eurasian Integration: the Role of Education and Science in the Implementation of Innovative Programs, Proceedings of the International Research and Practice Conference], 2012, Uralsk, Kazakhstan. Uralsk, vol. I, pp. 210-213.

ISHCHANOVA, A.S., BILJANA, R. and NURZHANOVA, F., 2018. Vydelenie epizooticheskogo shtamma *Pasteurella multocida* i issledovaniya ikh svoistv [Isolation of an epizootic strain of *Pasteurella multocida* and a study of its properties]. *Mnogoprofilnyi Nauchnyi Zhurnal 3i: Intellekt, Ideya, Innovatsiya*, vol. 1, no. 1, pp. 35-40.

IVANISHCHUK, P.P. and KOVALEV, A.V., 1993. The effect of the fluid medium on the completeness of skin restoration in rats. *Morfologiia*, vol. 105, no. 11-12, pp. 78-81. PMid:7874295.

KARMALIYEV, R.S., USSENOV, Z.T., SIDIKHOV, B.M., AITUGANOV, B.E., YERTLEUOVA, B.O. and GABDULLIN, D.E., 2019. Helminthofauna of the digestive tract of cattle and saiga in West Kazakhstan. *International Journal of Engineering and Advanced Technology*, vol. 9, no. 1, pp. 2600-2604. http://dx.doi.org/10.35940/ijeat.A9377.109119.

KIRKIMBAEVA, Zh.S., ICHSHANOVA, A.S., TAUBAEV, U.B. and AIDARBEKOVA, S., 2018. Virulence properties of pasterellas

isolated from saigas in the West-Kazakhstan region. Issledovaniya. *Rezultaty*, vol. 77, no. 1, pp. 46-51.

KIRKIMBAEVA, Zh.S. and ORYNTAEV, K.B., 2011. *Bakteriologicheskaya diagnostika infektsionnykh boleznei zhivotnykh* [Bacteriological diagnosis of infectious animal diseases]. Almaty, pp. 87-98.

KIRKIMBAYEVA, Zh.S., MAULANOV, A.Z., ERMAGAMBETOVA, S.E., BIYASHEV, B., MAKBUZ, A.Z.H., KUZEMBEKOVA, G.B. and KUSHALIEVA, A.A., 2014. The feature of course and pathological changes of Pigs' Pasteurellosis. *Global Veterinaria*, vol. 12, no. 6, pp. 823-828.

KOVALEV, A.V. and IVANISHCHUK, P.P., 1999. Partial regeneration of the tail tip of newborn rats in a liquid medium. *Ontogenez*, vol. 30, no. 1, pp. 47-50. PMid:10205789.

MUKHAMADIYEV, N.S., CHADINOVA, A.M., SULTANOVA, N., MENGDIBAYEVA, G.Z. and ANUARBEKOV, K.K., 2023. Development of environmentally friendly protection measures against pests and diseases. *Online Journal of Biological Sciences*, vol. 23, no. 2, pp. 243-250. http://dx.doi.org/10.3844/ojbsci.2023.243.250.

MYRZHIEVA, A.B., SHABDARBAEVA, G.S., TURGANBAEVA, G.E., BALGIMBAEVA, A.I. and IBAZHANOVA, A.S., 2020. Ixodid ticks: epizootic status and methods for tick population size reduction. *Online Journal of Biological Sciences*, vol. 20, no. 4, pp. 166-175. http://dx.doi.org/10.3844/ojbsci.2020.166.175.

NASAMBAEV, E., AKHMETALIEVA, A.B., NUGMANOVA, A.E. and DOSZHANOVA, A.O., 2022. Breeding young Kazakh white-headed cattle in different calving seasons. *Annals of Agri Bio Research*, vol. 27, no. 1, pp. 131-137.

SHERALIEVA, Z., NURALIYEVA, U., KARYMSAKOV, T., TADZHIYEV, K., MOLDAKHMETOVA, G. and TADZHIYEVA, A., 2023. Climate zones and morphometric parameters of *Apis mellifera carnica* bees. *American Journal of Animal and Veterinary Sciences*, vol. 18, no. 4, pp. 243-248. http://dx.doi.org/10.3844/ajavsp.2023.243.248.

SIDORCHUK, A.A., 2007. *Infektsionnye bolezni zhivotnykh* [Infectious diseases in animals]. Moscow: KolosS, 671 p.

SMITH, E., MILLER, E., AGUAYO, J.M., FIGUEROA, C.F., NEZWORSKI, J., STUDNISKI, M., WILEMAN, B. and JOHNSON, T., 2021. Genomic diversity and molecular epidemiology of *Pasteurella multocida*. *PLoS One*, vol. 16, no. 4, e0249138. http://dx.doi.org/10.1371/journal.pone.0249138. PMid:33822782.

TAUBAEV, U.B., 2007. Patogennost i toksigennost epizooticheskikh izolyatov *Pasterella multotsida* [Pathogenicity and toxigenicity of epizootic isolates of *Pasteurella multocida*]. *Vestnik selskokhozyaistvennoi nauki Kazakhstana*, no. 580, pp. 46.

TAUBAEV, U.B., 2012. Morfologicheskie, kulturalnye i biokhimicheskie svoistva shtammov *Pasteurella multocida* [Morphological, cultural and biochemical properties of *Pasteurella multocida* strains]. In: *Evraziiskaya Integratsiya: Rol Obrazovaniya i Nauki v Realizatsii Innovatsionnykh Programm, Materialy Mezhdunarodnoi Nauchno-Prakticheskoi Konferentsii* [Eurasian Integration: the Role of Education and Science in the Implementation of Innovative Programs, Proceedings of the International Research and Practice Conference], 2012, Uralsk, Kazakhstan. Uralsk, vol. I, pp. 304-307.

TERENTEVA, T.E., GLOTOVA, T.I., GLOTOV, A.G. and DONCHENKO, N.A., 2014. Sravnitelnaya effektivnost razlichnykh metodov diagnostiki boleznei krupnogo rogatogo skota, vyzyvaemykh bakteriei *Pasteurella multocida* [Comparative effectiveness of various methods for diagnosing cattle diseases caused by the bacterium *Pasteurella multocida*]. *Sibirskii Vestnik Sel'skokhoziaistvennoi Nauki*, vol. 3, pp. 90-95.

WILKIE, W., HARPER, M., BOYCE, J.D. and ADLER, B., 2012. *Pasteurella multocida*: diseases and pathogenesis. *Current Topics in Microbiology and Immunology*, no. 361, pp. 1-22. http://dx.doi.org/10.1007/82_2012_216. PMid:22643916.

ZHANABAYEV, A.A., NURGALIEV, B.Y., KEREYEV, A.K., PARITOVA, A.Y., USSENBAYEV, A.Y., BAYANTASSOVA, S.M., SEITKAMZINA, D., ZHUMAGALIYEVA, G.K. and YELEMESSOVA, B., 2022. Parasite fauna and infection of sheep with parasitosis. *Online Journal of Biological Sciences*, vol. 22, no. 4, pp. 404-414. http://dx.doi.org/10.3844/ojbsci.2022.404.414.