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Pasteurellosis in farm animals

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Abstract. The purpose of this study was to investigate the biological properties of *Pasteurella multocida* strains circulating among farm and wild animals in Kazakhstan, particularly saigas, to optimise the diagnostics and prevention of pasteurellosis. The study employed bacteriological, microscopic, and biochemical methods to analyse 20 samples of biomaterial collected from saigas and farm animals: 10 samples from specimens from Torgai village (Dzangeldy district, Kostanay region, Kazakhstan) and 10 samples from specimens from Kaztalov district (West Kazakhstan region, Kazakhstan). The study found that all isolates (100%, n = 60) belonged to *Pasteurella multocida* biovar *ovis*: greyish-white colonies 1-2 mm in diameter were formed on meat-peptone agar

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without haemolysis, gram-negative bipolar rods were detected in smears, and fermentation of glucose, sucrose, mannose, and trehalose was accompanied by the formation of acid without gas (in 100% of cases). Bioassays on 10 white mice (weight 16-20 g) showed 100% mortality within 48 hours after injection of 0.2 ml of culture, with haemorrhages in the heart, liver necrosis, and exudate in the abdominal cavity. Statistical analysis (Fisher's test) revealed no significant differences between the 2015 and 2023 isolates ($p > 0.05$), confirming the circulation of a stable clone. The findings reflect the key role of saigas as a reservoir of infection and the need for integrated measures: aerosol vaccination during migrations, introduction of polymerase chain reaction diagnostics, and antibiotic resistance monitoring. The data obtained are the basis for the development of regional antiepizootic strategies aimed at reducing risks to agriculture and conservation of wildlife populations in Kazakhstan

Keywords: saigas; epizootics; isolates; meat-peptone agar; bioassays

INTRODUCTION

Pasteurellosis continues to be one of the most dangerous infectious diseases of farm and wild animals, causing extensive economic damages due to massive livestock losses and treatment costs. This problem is particularly acute in regions with intensive livestock production, where outbreaks of the disease can lead to food security issues. In Kazakhstan, where the agricultural sector plays a key role in the economy, pasteurellosis poses an extra threat due to the migration of saigas, a species that is a natural reservoir of *Pasteurella multocida*. The increase in cases of the disease among wild populations, combined with the lack of comprehensive data on the biological properties of circulating strains, limits the development of effective anti-epizootic strategies.

Research in 2020-2025 highlighted the genetic diversity of *Pasteurella* and their ability to adapt to different ecological niches. Z.K. Buienbayeva *et al.* (2023) found that strains isolated from saigas have unique biochemical profiles compared to samples from domestic animals. This fact suggests intraspecific microevolution of the pathogen, which increases its adaptive potential. Such differences, according to S.J. Hasani *et al.* (2024), complicate the creation of universal vaccines. This is of key significance in regions with biodiversity of livestock and wildlife, where the pathogen circulates between different hosts. In Saudi Arabia, F.A. Alarawi and E.M. Saeed (2021) showed that even within the same animal species (e.g., sheep), pasteurellosis can cause distinct clinical forms, which is associated with the specificity of local strains. These findings reflect the need for a territorial approach to the analysis of the pathogen, which accounts for both genetic and epidemiological factors. In Central Asia, particularly in Uzbekistan, I.D. Sheraliyeva (2022) found a link between epizootics among sheep and changes in climatic conditions that intensify the transmission of the pathogen. This highlights another risk factor – global warming, which changes the seasonal dynamics of the spread of infections.

However, existing studies focus mainly on certain aspects of pasteurellosis, from molecular biology to clinical treatment. M. Umitzhanov *et al.* (2024) analysed the biochemical characteristics of *Pasteurella* in birds, while F. D'Amico *et al.* (2024) detailed the pathogenicity of strains in rabbits. However, there is a lack of studies that integrate epizootic, microbiological, and environmental data for concrete regions. For example, in Kazakhstan, where saigas are a key component of the ecosystem, the lack of a comprehensive analysis of their role in the spread of pasteurellosis limits the understanding of the mechanisms of infection transmission. It is particularly significant to investigate the virulence of strains. Experiments on laboratory animals conducted by T.D. Costa *et al.* (2023) revealed that even strains with low pathogenicity can cause systemic complications under certain conditions, especially in the presence of immunosuppression or stress factors. This indicates the potential of *Pasteurella* for latent persistence with subsequent reactivation. At the same time, M. Boulianne *et al.* (2020) noted that the effectiveness of existing diagnostic methods depends on the geographical origin of the pathogen, which confirms the need for localised studies. In Kazakhstan, according to Z.K. Buienbayeva (2024), even standard protocols for bacteriological analysis must be adapted due to the characteristics of local strains. This requires a review of existing methods, particularly in terms of the composition of culture media and test interpretation.

The current epidemiological situation in Central Asia demonstrates that the circulation of the same strain between domestic and wild animals is a real threat of interspecies transmission (Tursumbetov *et al.*, 2024). Particular attention should be paid to the areas of seasonal migration of saigas, which may be a mechanism for the transfer of the pathogen between natural and farmed ecosystems. The absence of systematic monitoring of this process limits the ability to identify epizootic nodes promptly and prevent their consequences. Thus, despite a considerable amount of accumulated data, the issues of interaction of *Pasteurella multocida* with different animal species in the specific environmental conditions of Kazakhstan are still unresolved. Comparison of the obtained findings with the data of reference strains allows determining the biovar affiliation of isolates and assess the level of virulence, which is key to the development of a national

strategy for the specific prevention of pasteurellosis in Kazakhstan. The purpose of the present study was to comprehensively investigate the properties of *Pasteurella multocida* isolated from farm animals in Kazakhstan to optimise diagnostics and prevention.

MATERIALS AND METHODS

The study was conducted in the bacteriology laboratory of the LLP "Kazakh Scientific-Research Veterinary Institute" (KazSRVI LLP, Kazakhstan) from 22 February to 2 March 2023. The object of the study included 20 samples of biomaterial collected from saigas and farm animals: 10 samples from specimens from the village of Torgai (Dzangeldy district, Kostanay region, Kazakhstan) and 10 samples from specimens from Kaztal district (West Kazakhstan region, Kazakhstan). The sample included animals with clinical signs of pasteurellosis (fever, haemorrhagic lesions, necrosis) and excluded samples from healthy individuals or animals with symptoms of other infections. Meat-peptone agar and meat-peptone broth manufactured by Sigma-Aldrich (USA), as well as Hottinger's agar and broth with 1% glucose (Merck, Germany) and 10% bovine serum (Thermo Fisher Scientific, USA) were used. For serological analysis, the study used an erythrocyte pasteurellosis antigenic diagnostic kit (series 010422 B/K No. 210, expiration date: 20.04.2024, manufactured by KazVetPharm, Kazakhstan). Biochemical studies were performed using Hiss kits for carbohydrate fermentation (HiMedia, India). Microscopic analysis was performed using an Olympus CX23 light microscope (Olympus Corporation, Japan) with an immersion system; Gram stain (Coico, 2006) (Bio-Rad, USA), and Romanowsky-Giemsa stain (Kuhlmann, 2020) (Sigma-Aldrich, USA) were used to stain the smears.

The pathological material was inoculated on meat-peptone agar, meat-peptone broth, and Hottinger's medium, and then incubated at 37°C for 24-48 hours. The cultural and morphological properties of Pasteurella (colony shape, presence of capsules) were examined after obtaining isolated cultures. After incubation, Gram-stained smears (Coico, 2006) (to detect Gram-negative bacilli) and Romanowsky-Giemsa microscopy (to visualise bipolar staining) were performed (Kuhlmann, 2020). Biochemical activity was studied by inoculating cultures on Hiss medium with glucose, sucrose, and other substrates; carbohydrate oxidation was assessed by changing the colour of the indicator (acid formation without gas). Catalase activity was determined by applying a 3% hydrogen peroxide solution to the surface of the cultures: a positive reaction was accompanied by the release of oxygen bubbles. To detect hydrogen sulphide, filter paper moistened with lead acetate (Sigma-Aldrich, USA) was used, and to detect indole – paper moistened with a saturated oxalic acid solution (Merck, Germany). Additionally, bacteriological analysis of pathological material from saigas that died during the 2015 pasteurellosis epizootic was conducted. Organ samples (heart, liver, spleen) were taken from each of the 20 animals for the study, a total of 20 samples.

The virulence of the isolated strains of *Pasteurella multocida* was studied in vivo in BALB/c white mice (Charles River Laboratories, USA), which were kept under standard laboratory conditions. Ten outbred mice aged 6-8 weeks and weighing 16-20 g were used. The animals were divided by randomisation into three experimental groups: two experimental and one control. In groups 1 and 2, animals were injected subcutaneously into the back area with 0.2 cm^3 of daily broth culture of *Pasteurella multocida* isolates 1 (from a farm animal) and 2 (from a saiga), respectively. The third group was left intact (without inoculation) and served as a negative control. The observation lasted for 10 days with daily clinical monitoring of general condition, activity, appetite, coat condition, mucous membranes, and registration of deaths. Statistical data processing was performed using Fisher's test (to compare the frequency of positive results between groups). Animal experiments were performed following the requirements of the Veterinary Legislation of the Republic of Kazakhstan (Law of the Republic of Kazakhstan No. 339-II, 2002) and the World Veterinary Association Position Statement on the Humane Care of Animals in Biomedical Research (2023). Animal experiments were conducted according to the Directive of the European Parliament and of the Council No. 2010/63/EU (2010).

The study also included archival samples (n = 20) collected during the pasteurellosis epizootic among the saiga population in May 2015 in the Turgai Basin (Kostanay region, Kazakhstan). Biomaterials (liver, heart, spleen) were collected by specialists of KazSRVI LLP. The samples were delivered to the laboratory of KazSRVI LLP (Kazakhstan) and, after initial identification, preserved in liquid nitrogen cryostorage at -196°C. In 2023, these samples were revitalised by inoculation on Sigma-Aldrich meat-peptone agar (USA) and Hottinger agar (Merck, Germany) with bovine serum (Thermo Fisher Scientific, USA), followed by incubation at 37°C for 24-48 hours. The isolated cultures were re-checked for sterility, homogeneity of morphology, and absence of extraneous microflora. Further procedures for morphological analysis, biochemical testing, and bioassays on mice were performed using identical protocols as for the 2023 samples. This ensured comparability of results between the two-time samples, enabling a retrospective analysis of the stability of the biological properties of the isolates in time and geography.

RESULTS

The first stage of the study was to investigate the culture properties of *Pasteurella multocida* isolates obtained from saiga and domestic animals in 2015 and 2023. After 24-48 hours of incubation, a uniform

turbidity of the medium without the formation of sediment or film was observed in meat-peptone broth, which is typical for this type of bacteria and indicates the high adaptability of the microorganism to classical nutrient media (Fig. 1). Round, convex, translucent colonies of greyish-white colour with a shiny surface were formed on meat-peptone agar.

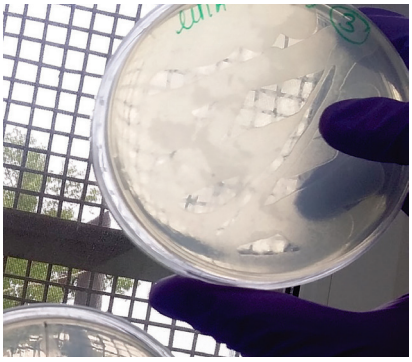

Figure 1. *Growth of Pasteurella multocida on meat-peptone agar*
Source: *created by the authors*

Microscopic examination of smears made from daily cultures and stained with Gram stain revealed small Gram-negative rods or coccobacilli with a clear bipolar tinctoriality. The bacteria were mostly isolated or formed small clusters in the form of chains or aggregates, which was also typical for this species. The presence of morphological homogeneity of cells in the field of view indicated the purity of the culture and a high degree of identification reliability. Figure 2 presents a typical microscopic image illustrating these diagnostic features. This tinctoriality, combined with morphological homogeneity, served as a key diagnostic feature for the initial recognition of the culture. Additionally, some Romanowsky-Giemsa-stained smears showed pronounced bipolar staining patterns, which further enhanced the differential diagnostic value of morphological analysis.

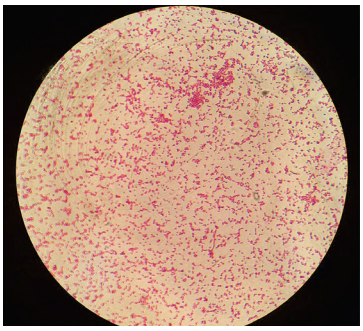

Figure 2. *Gram-stained daily culture*
Source: *created by the authors*

A particularly significant diagnostic feature was the absence of zones of haemolysis when the isolates were cultured on blood agar. In contrast to the haemolytic active species *Pasteurella haemolytica*, which forms pronounced zones of β-haemolysis, the isolates recovered from farm animals did not cause any visible changes in the erythrocyte layer. This allowed them to be reliably identified as *Pasteurella multocida* even at the initial stage, before more in-depth biochemical or molecular verification. Cultures isolated from these organs demonstrated stability of culture and morphological properties. Regardless of the type of biological material, all isolates showed a characteristic growth pattern, indicating the absence of microbial contamination by foreign cultures, as well as high conservation of the morphological and physiological properties of the pathogen. The isolation of pure cultures from different sources and animal species showed a wide range of *Pasteurella multocida* carriage, which is a serious threat to both the agricultural industry and biodiversity conservation. Thus, the first stage of the study – isolation and primary characterisation of *Pasteurella multocida* isolates – confirmed the presence of an epizootic strain circulating among animals of different taxonomic groups and demonstrating stable cultural and morphological characteristics inherent in the species.

The study found that all isolated Pasteurella isolates (n = 60) actively fermented a number of carbohydrates: glucose, galactose, sucrose, mannose, fructose, and trehalose. The results of the reaction were accompanied by a change in the colour of the indicator in the test tubes, indicating the formation of organic acids as fermentation products. All isolates demonstrated the same reaction, which was accompanied by a change in the colour of the indicator in the test tubes, reflecting the formation of organic acids as the primary metabolic product. Therewith, no gas was produced as a secondary metabolite, which is a typical property of *Pasteurella multocida* and allows differentiating it from some other enterobacteria capable of gas production. On the contrary, substrates such as lactose, rhamnose, raffinose, maltose, salicin, dulcit, and glycerol in the studied isolates were not fermented. This was confirmed by the preservation of the initial colour of the medium, suggesting the absence of acid production. Such a pattern of biochemical activity helped to clearly define the boundaries of the isolates belonging to the genus Pasteurella and to distinguish them from related species (*Pasteurella haemolytica*, *Pasteurella pneumotropica*), as well as from other Gram-negative facultative anaerobic bacteria that may be present in pathogenic material as concomitant or opportunistic pathogens. The high conservation of the biochemical properties of the isolates was confirmed by their comparative identification with the reference strain *Pasteurella multocida* B-0229, which is stored in microbiological collections. For comparison, two isolates were selected – No. 1 (from a farm animal) and No. 2 (from a saiga), which showed full compliance with the biochemical profile of the reference strain

(Fig. 3). This allowed identifying them as representatives of the species *Pasteurella multocida*, biovar *ovis*. This result is of major epizootological significance, as it confirms the circulation of a single stable strain with identical properties in animal populations in fundamentally different ecological niches.

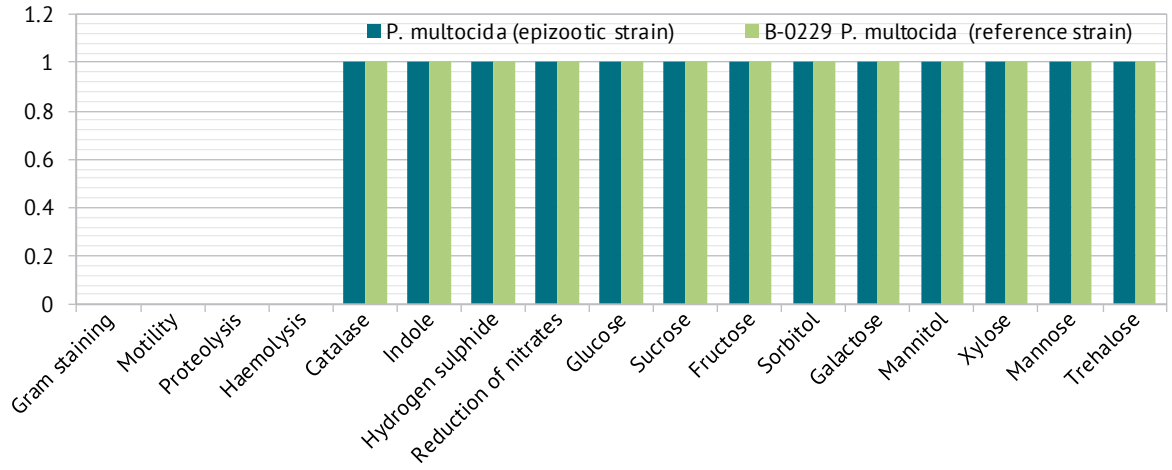

Figure 3. *Comparative biochemical characterisation of epizootic and reference strain isolates*
Source: *created by the authors*

The analysis of the biochemical characteristics of *Pasteurella multocida* biovar *ovis* isolates presented in Figure 3 indicates that their metabolic profile closely resembles the reference strain B-0229 stored in microbiological collections. The epizootic isolates were Gram-negative, non-motile, did not show proteolytic activity (did not liquefy gelatine) and did not form zones of haemolysis on blood agar, which enabled a clear differentiation from *Pasteurella haemolytica*, which exhibits β-haemolytic activity. Catalase activity was positive, indole was produced, and hydrogen sulphide was formed. The results of the Voges-Proskauer reaction were negative, which excluded the presence of acetone as a side metabolite. The isolates reduced nitrates to nitrites, which confirmed their belonging to the typical representatives of the genus Pasteurella. As for carbohydrate metabolism, the epizootic isolates fermented glucose, galactose, mannose, fructose, sucrose, xylose, trehalose, sorbitol, and mannitol to produce organic acids without gas, which was confirmed by the change in the colour of the indicator in the medium. This type of fermentation – acid production without gas formation – is a characteristic species feature of Pasteurella. Therewith, the isolates did not ferment lactose, dulcitol, raffinose, rhamnose, glycerol, salicin, maltose, and arabinose, which allowed clearly differentiating them as representatives of *Pasteurella multocida* biovar *ovis*. Specifically, the absence of lactose fermentation is considered a marker diagnostic feature for this biovar. Thus, the biochemical properties of the epizootic strains were typical for the genus *Pasteurella*, particularly *Pasteurella multocida*, and fully corresponded to the characteristics of the reference strain B-0229.

Already on the second day after inoculation of experimental mice with a daily broth culture of isolated Pasteurella, the death of most experimental animals was recorded. Out of ten mice, five died in the first 24 hours, and three died on the second day (Fig. 4). In two cases, the mice stayed alive on the first day, but showed signs of depression: apathy, decreased appetite, and limited motor activity. These data indicate the high virulence of *Pasteurella multocida ovis* isolates both for laboratory models and, probably, for natural hosts.

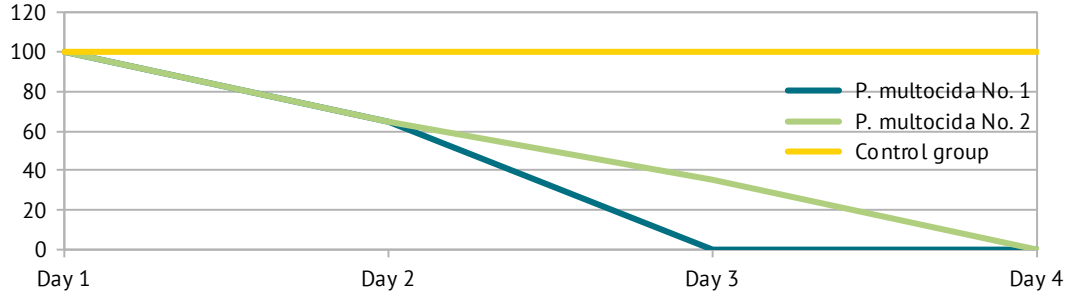

Figure 4. *Assessment of pathogenicity (survival)*
Source: *created by the authors*

Pathological examination revealed numerous punctate and striated haemorrhages on the pericardium, serous-exudative peritonitis with clear or turbid exudate in the abdominal cavity, and foci of yellow-grey necrosis in the liver. Cultures of pathological material on meat-peptone agar and broth gave rise to a characteristic culture of *Pasteurella multocida ovis*. Small, transparent, delicate S-type colonies were formed on meat-peptone agar, without contamination with foreign microflora. In the liquid medium, uniform turbidity without ring or sediment formation was observed, a typical sign of *Pasteurella multocida* growth. Repeated microscopy of smears from such cultures confirmed the presence of Gram-negative bipolar rods morphologically identical to the original pathogen.

Thus, the results of bioassays in laboratory mice provided convincing evidence of the high pathogenicity of the isolated strains. They simultaneously served as a diagnostic tool (pathogenicity bioassay) and experimental verification of the species by comparing clinical and anatomical changes and bacteriological results. As a result, the study of the biochemical properties and pathogenicity of *Pasteurella multocida* isolates helped to reliably determine their species characteristics, virulence, and potential for epizootic spread. The identity of biochemical profiles in samples from domestic animals and saigas indicates the circulation of a single clone or stable biovar of the pathogen, which is significant for the development of specific prevention strategies and epidemiological monitoring in mixed livestock and wildlife environments.

As a result of laboratory cultivation, isolates of *Pasteurella multocida* were obtained from these samples, which demonstrated a stable phenotype characteristic of this species. Microscopic examination of smears made from the culture material revealed Gram-negative rods with typical bipolar tinctoriality, which is one of the key differentiating features in the identification of *Pasteurella multocida*. The culture properties were also typical: the isolates did not cause haemolysis when grown on blood agar, which distinguishes them from some other respiratory pathogens such as *Mannheimia haemolytica*. Furthermore, they were characterised by stable growth in meat-peptone broth with the formation of turbidity without the formation of sediment or film, which confirms good adaptation to the culture medium (Fig. 5). Despite considerable environmental and geographical differences in the sampling sites – specifically, the steppe zones of the West Kazakhstan and Kostanay regions – the properties of the isolates stayed almost identical, indicating high stability and adaptability of the strain.

The biochemical properties of the isolates from saigas also confirmed their belonging to *Pasteurella multocida* biovar *ovis*. All strains were catalase-positive, produced indole, formed hydrogen sulphide, and reduced nitrate to nitrite. This set of traits, combined with negative reactions to lactose, rhamnose, and raffinose, allowed distinguishing the isolates not only from related species of the genus Pasteurella, but also from potentially comparable members of the family *Pasteurellaceae*, which may act as companions or contaminants in natural biocenoses. The circulation of the same epizootic strain among geographically distant animal populations, including saigas, cattle, and small ruminants, indicates an extremely high contagiousness and stability of the pathogen. This stability may be related to the genetic conservation of the strain, its ability to persist in the host for a prolonged period without clinical manifestations, and the possibility of transmission through contact and aerogenic routes.

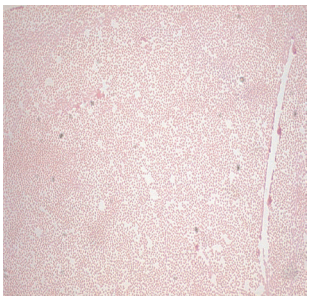

Figure 5. *Microscopy of the Pasteurella multocida isolate isolated in 2015 (Gram stain)*
Source: *created by the authors*

A separate threat is posed by the translocation of the pathogen through ecological corridors, particularly the migration routes of saigas that intersect with areas of seasonal grazing of unvaccinated livestock. It was found that during periods of intense migration load, animals can come into contact with pathogen sources at watering holes, pastures, and through mechanical vectors. Such situations contribute to the formation of a natural and anthropogenic epizootic chain, within which the pathogen spreads between wild and domestic populations (Davydovych *et al.*, 2025). Considering the cyclical nature of pasteurellosis outbreaks (with a frequency of 3-5 years) and the presence of numerous latent bacterial carriers, the development of a unified national approach to the prevention and control of the disease is crucial. Particular attention should be paid to the development of vaccine prophylaxis programmes for wild animals, which constitute a natural reservoir of infection (Abutalip *et al.*, 2017). It is advisable to introduce aerosol vaccination of saigas during calving, when the number of animals is the largest and contacts with other populations are minimal. This approach has already demonstrated effectiveness in the fight against other translocated zoonoses in Africa and Central Asia.

Studies confirm that the combination of culture, morphological, tinctorial, and biochemical methods with a bioassay on laboratory animals (white mice) enables a comprehensive assessment of the pathogenicity and taxonomic affiliation of the pathogen. The

bioassay showed high sensitivity: after inoculation, mice died within 24-48 hours, which corresponds to a prominent level of virulence of the isolates. This gave the study a high degree of reliability and opens up the prospect for further strain typing using molecular markers of virulence. Statistical analysis showed that the differences between the groups were not statistically significant ($p > 0.05$), suggesting that the biological properties of the isolates were stable regardless of the year of the epizootic and geographical origin. This confirms the circulation of a single conserved epizootic strain of *Pasteurella multocida* biovar *ovis*. Summarising the data obtained, it can be stated that regardless of the type of animal, region of distribution or conditions of keeping, the same stable epizootic strain of *Pasteurella multocida* biovar *ovis* circulates in the objects under study. It is characterised by a stable morphology, typical biochemical characteristics, and high virulence. This indicates the dominance of a pathogen clone that has adapted to many biological niches, and at the same time, a unique opportunity to create universal approaches to specific prevention, diagnostics, and control of this infectious agent. The data obtained are of both practical (for veterinary medicine and wildlife conservation) and theoretical (for evolutionary microbiology and epizootology) significance.

DISCUSSION

The results of the conducted study reflect that isolates of *Pasteurella multocida* biovar *ovis* isolated from farm animals and saigas in Kazakhstan have a prominent degree of stability of morphological, cultural, and biochemical characteristics. Specifically, all strains demonstrated typical growth on meat-peptone broth and agar, formed transparent S-colonies without haemolysis on blood agar, had Gram-negative bipolar tinctoria and a positive reaction to the fermentation of a series of carbohydrates (glucose, mannose, galactose, sucrose, etc.) with the formation of acid without gas. Such conservatism of the traits is consistent with the data of U. Taubaev *et al*. (2024) who described analogous properties of *Pasteurella multocida* strains isolated in the Western Kazakhstan region, particularly during saiga migrations. Analogous findings were reported by S. Boianovskiy *et al*. (2023), noting that the stability of the biochemical profile of *Pasteurella multocida ovis* is a diagnostically significant feature in outbreaks in mixed-type farms.

The identified virulence of the isolates, confirmed by bioassays in mice (70% mortality in the first 48 h), indicates a high pathogenic potential of these strains. The pathological changes, such as haemorrhages, serous-exudative peritonitis, and liver necrosis, correspond to the typical manifestations of septic pasteurellosis described by Y.A. Mirtneh *et al*. (2022) during experiments with inoculation of *Pasteurella multocida* in laboratory animals. Y.M. Hashem *et al*. (2022) observed an analogous pattern in cases of infection among wild ungulates in the Mediterranean region, which emphasised the universal pathogenesis of the infection regardless of the area. The similarities in pathogenesis found, regardless of host species or geographic location, emphasise the universal nature of infection mechanisms, suggesting the evolutionary stability of the virulence of the pathogen. These data are crucial not only for diagnosing and predicting the course of the disease, but also for developing effective measures to control and prevent pasteurellosis in livestock.

The conservative biochemical profile of the identified strains (negative reaction to lactose, salicin, dulcite fermentation, etc.) helped to reliably differentiate them from other species of the genus Pasteurella, particularly *Pasteurella haemolytica* and *Pasteurella pneumotropica*. This approach is consistent with the recommendations of Y.A. Mirtneh *et al*. (2022), who emphasised the value of classical biochemical tests in the absence of molecular genetic capabilities. The results of the present study also confirmed the findings of D. Abera and T. Mossie (2023), who successfully identified *Pasteurella multocida* serotypes based on carbohydrate metabolism profiles. At the same time, the difference from the results of Z. Peng *et al*. (2022) should be noted, who found significant genetic heterogeneity of *Pasteurella multocida* isolates, even within the same host. In this case, the dominance of a single biochemical and morphological type suggests the presence of a circulating clone adapted to the conditions of the steppe ecosystem of Kazakhstan. A. Pascual-Garrigos *et al*. (2021) recorded an analogous trend in their study of clinical isolates in Europe, and H. Wang *et al*. (2023) – in their study of Chinese farms.

A significant finding was the detection of the same strain among isolates from saigas and domestic animals in different regions of Kazakhstan. Such geographical stability of the strain, combined with high virulence, indicates a strong epizootic potential and confirms the hypothesis of S. Boianovskiy *et al*. (2023) regarding the universality of pathogenic mechanisms of *Pasteurella multocida* biovar *ovis*. A vital role in the transmission of the pathogen is likely to be played by natural ecological corridors, primarily the migration routes of saigas that intersect with areas of seasonal cattle grazing. A. Abbas *et al*. (2023) presented analogous conclusions, studying the impact of joint grazing of wild and domestic animals on the risks of pasteurellosis transmission in Pakistan. S.A. Alemu *et al*. (2023) also demonstrated comparable findings in Ethiopia, where the key factors of epizootic spread included watering holes, mobile contacts between populations, and insufficient vaccination rates. In this case, these factors also play a critical role, which requires a review of anti-epizootic strategies at the state level.

One of the most reliable morphological markers for the primary identification of *Pasteurella multocida*

continues to be bipolar tinctoria in Gram or Romanowsky-Giemsa staining, as well as the absence of haemolysis on blood agar (Ospanov *et al.*, 2024). These features are the key differentiating criteria for distinguishing *Pasteurella multocida* from *Mannheimia haemolytica* and other Gram-negative facultative anaerobes, as noted by S. Sahay *et al.* (2020) and J. Xiao *et al.* (2021), which was also confirmed in the present study. In the current study, the absence of β-haemolysis zones was crucial for confirming the species status of the isolates before the use of biochemical analysis. However, the introduction of molecular methods continues to be relevant. Specifically, H. Wang *et al.* (2023) showed that multiplex PCR enables the simultaneous identification of several pathogens in mixed infections, which increases the sensitivity and specificity of laboratory diagnostics. This may be particularly relevant in cases of co-infections or in the early stages of an outbreak.

The obtained results confirm that *Pasteurella multocida* biovar *ovis* isolated from farm animals and saigas in Kazakhstan retains high conservation of cultural, morphological, and biochemical characteristics, which is consistent with the data of I. Cuevas *et al.* (2020) and F. Ahmad *et al.* (2024) on the stability of this pathogen in different biocenoses. The stability of the biochemical profile of the isolates, particularly the ability to ferment glucose, sucrose, and other carbohydrates to produce acid without gas, corresponds to the typical characteristics of the *Pasteurella multocida* biovar *ovis* species. Z. Peng *et al.* (2021) reported analogous results, noting that the biochemical stability of the strains ensures the reliability of classical diagnostic tests. However, the genotyping results obtained by these researchers indicate the presence of high genetic variability between strains isolated from different hosts. The spread of an identical strain among geographically dispersed groups of animals confirms its ability to adapt and transmit widely (Irgashev *et al.*, 2020). This is consistent with the studies of S. Ali *et al.* (2025), who found the circulation of unified strains of *Pasteurella multocida* among cattle and small cattle in Pakistan. A. Akane *et al.* (2022) made analogous conclusions, emphasising the role of mixed animal housing in the transmission of infection. Considering this, the need to implement integrated control measures was confirmed, especially in transboundary regions with intensive grazing.

Clinical and diagnostic features, such as the absence of haemolysis zones on blood agar and bipolar coloured bacilli, continue to be crucial markers for differentiating *Pasteurella multocida* from other pathogens such as *Mannheimia haemolytica* (Verzhykhovsky & Nedosekov, 2024). This is confirmed by the results reported by S. Sahay *et al.* (2020) and I. Cuevas *et al.* (2020), where the diagnostic significance of morphological and culture tests is noted. The researchers also emphasised that the combination of classical methods with modern approaches to identification can improve the accuracy of diagnostics and help prescribe the corresponding treatment promptly. The study found that the use of traditional microbiological methods in combination with bioassays on white mice provides high diagnostic accuracy. However, considering the requirements for pathogen detection, it is advisable to introduce genotyping and whole-genome sequencing to identify virulence genes and resistance markers. Thus, the data obtained indicate the existence of a stable epizootic strain of *Pasteurella multocida* biovar *ovis* circulating in both wild and domestic animal populations. This necessitates an interdisciplinary approach to prevention, which would include elements of environmental monitoring, vaccination and rapid laboratory diagnostics.

CONCLUSIONS

As a result of the studies, it was found that mass animal deaths in Kazakhstan in 2015 and 2023 were caused by the circulation of a stable strain of *Pasteurella multocida* biovar *ovis*. Cultural and morphological analysis revealed that all isolates had typical characteristics: uniform turbidity was observed on meat-peptone broth within 24-48 hours without precipitation and rounded greyish-white colonies 1-2 mm in diameter were formed on meat-peptone agar. Microscopic examination revealed typical Gram-negative rods with a pronounced bipolar tinctoriality. Biochemical analysis of the isolates showed a high degree of homogeneity. All 20 isolates (100%) actively fermented glucose, galactose, sucrose, mannose, fructose, and trehalose to produce acid, but without gas. Therewith, all of them gave negative results for the fermentation of lactose, rhamnose, raffinose, maltose, salicin, dulcit, and glycerol. Additional tests confirmed the presence of catalase activity, indole production, hydrogen sulphide production, and the ability to reduce nitrate to nitrite in all samples tested. Evaluation of the pathogenic properties of the strain in BALB/c laboratory mice showed high virulence. After infection, 50% of the animals (5 out of 10) died within the first 24 hours and 80% (8 out of 10) – within 48 hours. Pathological examination revealed haemorrhages in 100% of cases and necrotic changes in the liver in 75% of cases, which correlated with the results of repeated isolation of the pathogen from pathological material.

Notably, the complete convergence of all the studied characteristics of the isolates obtained in 2015 and 2023, which indicates the extraordinary stability of the circulating strain. The findings substantiate the need to develop a comprehensive prevention programme, which should include regular vaccination of farm animals covering at least 80% of the population and aerosol immunisation of saigas during the period of their encirclement. Particular attention should be paid to monitoring of common watering places, which are recommended to be inspected 3-4 times a year. Such measures will effectively control the spread of this highly pathogenic strain among both wild and

domestic animals. A limitation of this study was the restrictive genetic analysis of isolates, which did not allow definitively determining the intraspecific structure of circulating strains. Furthermore, the study was focused mainly on phenotypic and biochemical characterisation, without broad coverage of other possible carriers in the wild fauna. Prospects for further research include the use of whole-genome sequencing to determine the phylogenetic relationship of strains, immunogenicity assessment for the development of specific vaccines, and the study of antibiotic resistance, which is crucial considering the growing threat of antibiotic resistance in wild and agricultural fauna.

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Анотація. Метою даного дослідження було вивчення біологічних властивостей штамів Pasteurella multocida, що циркулюють серед сільськогосподарських та диких тварин в Казахстані, зокрема сайгаків, з метою оптимізації діагностики та профілактики пастерельозу. У дослідженні використовувалися бактеріологічні, мікроскопічні та біохімічні методи для аналізу 20 зразків біоматеріалу, зібраного від сайгаків і сільськогосподарських тварин: 10 зразків від особин із села Торгай (Джангельдинський район, Костанайська область, Казахстан) і 10 зразків від особин із Казталовського району (Західно-Казахстанська область, Казахстан). Дослідження показало, що всі ізоляти (100 %, n = 60) належали до Pasteurella multocida biovar ovis: на м'ясо-пептоновому агарі утворилися сірувато-білі колонії діаметром 1-2 мм без гемолізу, у мазках виявлено грамнегативні біполярні палички, а ферментація глюкози, сахарози, манози та трегалози супроводжувалася утворенням кислоти без газу (у 100 % випадків). Біоаналізи на 10 білих мишах (вага 16-20 г) показали 100 % смертність протягом 48 годин після ін'єкції 0,2 мл культури, з крововиливами в серці, некрозом печінки та ексудатом в черевній порожнині. Статистичний аналіз (тест Фішера) не виявив істотних відмінностей між ізолятами 2015 і 2023 років ($p > 0,05$), що підтверджує циркуляцію стабільного клону. Отримані результати свідчать про ключову роль сайгаків як резервуару інфекції та необхідність комплексних заходів: аерозольної вакцинації під час міграцій, впровадження діагностики методом полімеразної ланцюгової реакції та моніторингу антибіотикорезистентності. Отримані дані є основою для розробки регіональних антиепізоотичних стратегій, спрямованих на зменшення ризиків для сільського господарства та збереження популяцій диких тварин у Казахстані

Ключові слова: сайгаки; епізоотії; ізоляти; м'ясо-пептоновий агар; біоаналізи