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Integrated approaches to the diagnosis, molecular characterisation, epidemiological surveillance, and prevention of pasteurellosis in farm animals

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Abstract

The aim of this study was to summarise and analyse contemporary diagnostic and preventive measures to improve the effectiveness of epizootiological monitoring of bacterial infection. The review is based on an analysis of publications on methods of clinical examination, bacteriological isolation, serological diagnosis, polymerase chain reaction, molecular typing, and antibiotic susceptibility testing. The analysis showed that integrating molecular diagnostics, serological surveillance, epizootiological assessment, and farm-level risk assessments is necessary for effective pasteurellosis control. The analysis showed that variations in livestock management practices, environmental factors, and veterinary surveillance systems are associated with regional differences in occurrence and transmission of disease. The study found that while territorial analysis enables the evaluation of infection distribution and the identification of regions in need of increased surveillance, genetic identification techniques offer useful information on pathogen features. The results reveal that production and organisational elements that affect the development of disease should be taken into account in conjunction with diagnostic procedures. To improve preventive measures against pasteurellosis, an integrated surveillance strategy that combines laboratory confirmation, genetic characterisation, environmental monitoring, and veterinary control measures was developed. The findings offer a foundation for improving management of bacterial illnesses in farm animals and tailoring diagnostic and preventive programs to local epizootiological conditions. The practical value of the study lies in the possibility of applying the obtained findings by veterinary services, farms, and laboratories to improve the diagnosis, epizootiological monitoring, and prevention of pasteurellosis, taking into account regional characteristics.

1. Introduction

The relevance of this study is linked to increasing bacterial respiratory infections in farm animals, the rise in cases of antibiotic resistance, and the need to improve laboratory diagnosis of pasteurellosis under conditions of intensified livestock farming. Intensive production systems for cattle and small ruminants are accompanied by high stocking density, stress factors, and the active use of antimicrobial drugs, creating prerequisites that favour the circulation of virulent and antimicrobial resistant strains of Pasteurella multocida. Under these conditions, timely molecular identification of the pathogen, serogroup typing, and appropriate immunoprophylaxis have become critically important for reducing economic losses and ensuring biosecurity in farms (Alexyuk et al. 2021; Laganà et al. 2018). The challenges addressed in this study is the lack of standardised diagnostic algorithms, variability of pathogen serogroups, the spread of antibiotic resistance genes, and limited adaptation of vaccine programmes to the regional epizootic situation. In practice, there is a gap between laboratory monitoring and the choice of therapeutic regimens, which can lead to ineffective treatment and the emergence of resistant bacterial populations. An additional challenge is the uneven epizootic situation across the regions, which requires the integration of molecular, clinical, and organisational measures into a unified system for monitoring pasteurellosis (Irgashev et al. 2023; Gabdullin et al. 2026).

In the context of immunobiological approaches, Umuraliyev et al. (2025) developed a technology for obtaining hyperimmune serum against anthrax based on controlled immunisation of animals. It was established that standardisation of antibody titre increased the effectiveness of specific prophylaxis of bacterial infections. In the aspect of clinical and laboratory verification, Buienbayeva et al. (2024) conducted a study of diagnostic algorithms for infectious diseases of animals using bacteriological and molecular methods. It was confirmed that the combination of cultural isolation and polymerase chain reaction increased the accuracy of diagnosis confirmation. Regarding organisational measures, Khussainov et al. (2024) systematised the application of anti-epizootic measures on the territory of Kazakhstan. It was found that the integration of laboratory monitoring and vaccination enhanced the level of biosecurity in farms. Within the framework of studying natural reservoirs of infection, Kushaliev et al. (2022) investigated the spread of parasitic diseases among migrating saiga populations. It was shown that monitoring of wild fauna made it possible to identify zones of epizootic risk.

Regarding genetic resistance, Orkara et al. (2025) characterised the epidemiological state of Kazakh sheep breeds and marker traits of resistance. It was demonstrated that breeding approaches contributed to the formation of resistance to infectious diseases. From a technological point of view, Džermeikaitė et al. (2023) introduced the use of sensor systems and digital technologies in the diagnosis of cattle diseases. It was noted that automated monitoring ensured early detection of pathological changes. In the direction of digital monitoring, Puig et al. (2022) presented technological tools for the early detection of respiratory diseases in cattle on farms. It was shown that the use of activity sensors, temperature sensors, and behaviour analysis algorithms ensured timely identification of clinical deviations. In the

aspect of resistance and immunoprophylaxis, Ali et al. (2025) summarised data on the resistance of *Pasteurella multocida*, difficulties of vaccination, and infection control strategies in veterinary practice. It was established that the expansion of the spectrum of resistance genes required a revision of therapeutic regimens and an update of vaccine formulations.

In the field of rapid laboratory diagnosis, Megahed et al. (2023) developed and evaluated tools for the rapid detection of *Pasteurella multocida* and *Riemerella anatipestifer* in ducks. It was confirmed that optimisation of the polymerase chain reaction shortened the time to diagnosis and increased the sensitivity of pathogen detection. From the perspective of clinical epizootiology, Abera and Mossie (2023) systematised information on pneumonic pasteurellosis in small ruminants. It was determined that the combined use of bacteriological and molecular methods made it possible to clarify the structure of the respiratory syndrome. Within the framework of a comparative analysis of diagnostic technologies, Okella et al. (2023) conducted a review of modern methods for detecting *Mycoplasma bovis* in dairy cattle. It was noted that the combination of serological tests and molecular amplification ensured increased accuracy of differential diagnosis. In the context of the prevention of bacterial infections in young animals, Robi et al. (2024) summarised approaches to disease control in dairy calves. It was recorded that the integration of laboratory verification, antibiotic susceptibility monitoring, and management measures contributed to a reduction in the infectious load in farms.

Insufficiently studied are the regional serogroup variability of the pathogen, the mechanisms of antibiotic resistance formation, and the adaptation of vaccination programmes to circulating strains. The aim of this review was to systematise contemporary diagnostic and preventive measures for pasteurellosis, assess their effectiveness, and identify the possibilities for improvement in disease control. The objectives of the study were to: a) review contemporary laboratory and molecular methods for diagnosing pasteurellosis; b) evaluate approaches to monitoring antimicrobial resistance; and c) summarise preventive measures used in various farm animal species. In preparing the review article, publications for 2022-2025 were analysed, reflecting contemporary trends in the diagnosis and prevention of pasteurellosis in farm animal species, including methods for isolating the pathogen culture, nucleic acid amplification methods, serological testing, molecular typing, antibacterial susceptibility testing, as well as the development of inactivated and recombinant vaccines. The synthesis was carried out based on studies conducted in Asian and African countries, as well as on materials from epizootiological analysis and laboratory monitoring in the Republic of Kazakhstan, where the features of the spread of *Pasteurella multocida* among cattle, small ruminants, and other animal species under different housing technology conditions were taken into account.

2. Materials and Methods

To analyse current methods for the diagnosis, molecular characterisation, epidemiological surveillance, and prevention of pasteurellosis in farm animals, this study was carried out as a structured literature review. The databases Scopus, Web of Science, PubMed, and Google Scholar were used for the literature search. The search strategy for finding relevant papers included keyword "pasteurellosis", with Boolean operators (AND/OR) used to combine search terms: "*Pasteurella multocida*", "farm animals", "cattle", "molecular diagnosis", "PCR", "genomic sequencing", "antimicrobial resistance", "vaccination", "epidemiological surveillance", and "prevention". To represent recent developments in diagnostic technology, molecular typing, antibiotic resistance monitoring, and preventive methods, the evaluation included papers released between 2022 and 2025.

The inclusion criteria comprised: (1) peer-reviewed research articles and review papers published in English; (2) studies addressing pasteurellosis or *Pasteurella multocida* infections in farm animals; (3) publications analysing diagnostic methods, molecular characteristics, epidemiological patterns, antimicrobial resistance, vaccination, or prevention approaches; and (4) studies providing sufficient methodological information and clearly described results. Publications were excluded if they focused on human pasteurellosis, experimental studies without relevance to farm animal health, conference abstracts, duplicate records, non-peer-reviewed materials, and articles lacking information necessary for comparative analysis.

In the first database search, 286 records were found. 54 duplicate publications were eliminated, leaving 232 items for title and abstract screening. In this phase, 145 papers were eliminated due to their lack of alignment with the research goals or their focus on unrelated diseases, animal species, or clinical factors. After the full texts of 87 publications were evaluated in accordance with the inclusion criteria, 47 papers were chosen for final analysis. Molecular diagnostics, pathogen typing, antibiotic resistance, epidemiological surveillance, farm management aspects, and preventative measures for pasteurellosis in farm animals were all discussed in the papers that were included.

The transparency of the study design, the description of the animal populations under investigation, sample characteristics, diagnostic techniques, laboratory methodologies, and the dependability of reported results were all used to evaluate the methodological quality of the chosen studies. A systematic framework comprising publishing features, geographic location, animal species, diagnostic procedure, molecular methodologies, epidemiological indicators, antibiotic resistance discoveries, and preventive strategies was used to retrieve data. In order to identify common trends, methodological advantages and limitations, and the significance of integrated diagnostic and surveillance approaches in enhancing pasteurellosis control, the retrieved data were examined using narrative synthesis and comparative evaluation.

3. Molecular and serological approaches to the diagnosis of pasteurellosis

Pasteurellosis in farm animals in the Republic of Kazakhstan was mostly linked to stocking density, the efficacy of veterinary control methods, and microclimatic conditions in livestock facilities (temperature, humidity, CO_2, and NH_3 levels). These variables affected the susceptibility of animals and the dissemination of infection throughout various geographical areas (Mukhamadieva et al. 2022; Tagaev et al. 2025; Latypova et al. 2022). In the reviewed studies, clinical and laboratory diagnosis of bacterial infections in dairy cattle of Eastern Kazakhstan included clinical examination of animals, bacteriological isolation of the pathogen, and serological confirmation of the infection. In these studies, the aetiological structure of inflammatory processes was clarified, laboratory confirmation of the bacterial nature of diseases was ensured, and diagnostic approaches combining clinical, bacteriological, and laboratory methods were described for respiratory and septic infections. Mutushev et al. (2024)

described the organisational structure of anti-epizootic measures, where diagnostic and preventive measures for the control of infectious animal diseases in Kazakhstan were systematised. Within the framework of epizootic programmes, laboratory confirmation of the diagnosis, monitoring of disadvantaged farms, and centralised recording of research results were provided. The standardisation of veterinary procedures ensured comparability of data and the formation of a unified national veterinary surveillance system.

The expansion of the use of molecular technologies was reflected in the studies of Kutumbetov et al. (2025), devoted to the analysis of the circulation of infectious agents among domestic and wild ruminants. Using molecular markers, the features of the transmission of the infectious agent in mixed animal populations were recorded. Genetic typing made it possible to conduct detailed monitoring of the spread of pathogens and demonstrated the possibility of using molecular approaches in the control of bacterial infections, including *Pasteurella multocida*. The serological direction of laboratory diagnostics was investigated by Bulashev et al. (2023), who validated an enzyme-linked immunosorbent assay based on chimeric proteins for the detection of antibodies associated with bacterial infection in cattle. The assay showed 96.4% sensitivity and 95.1% specificity under the stated laboratory validation parameters. These findings characterised the analytical performance of the serological method; however, serological positivity should be interpreted as evidence of immune response or previous exposure rather than direct confirmation of active infection. Therefore, when paired with clinical, microbiological, and molecular diagnostic techniques, the use of ELISA can provide large-scale serological monitoring and epidemiological evaluation of bacterial illnesses.

The preventive component of veterinary control was examined by Kushaliyev et al. (2024), where measures to preserve saiga populations on the territory of Kazakhstan were described. The structure of preventive programmes included laboratory studies, veterinary observation, and measures to prevent the spread of infections in natural foci. The inclusion of wild animals in the epizootic monitoring system was recorded, which extended the control of infectious pathology beyond agricultural farms. Production factors of animal housing were reflected in the studies of Tagaev et al. (2025), where instrumental control of microclimate parameters of livestock premises was carried out. Environmental temperature, relative air humidity, concentrations of carbon dioxide and ammonia were assessed. The systematisation of the obtained indicators made it possible to take into account the influence of housing conditions on the susceptibility of cattle to bacterial respiratory infections and complemented laboratory diagnostics with production observations. The territorial spread of pasteurellosis was presented in the works of Latypova et al. (2022), where zoning of the territory of the Republic of Kazakhstan was carried out according to the degree of epizootic tension of the disease in cattle. As a result of statistical processing of veterinary reporting, the identification of zones of different risk levels was recorded, reflecting the spatial heterogeneity of the spread of infection. The spatial differentiation of the disease determined the intensity of laboratory examinations and the frequency of monitoring in individual regions of the country (Table 1).

Thus, the presented sources reflected the sequential formation of a multi-level system for the diagnosis of pasteurellosis in the Republic of Kazakhstan, within which clinical and bacteriological verification of infection gradually transformed into a structured model of epizootiological surveillance. At the initial stage, confirmation of the diagnosis was based on cultural isolation of the pathogen and clinical assessment of the condition of animals, after which the diagnostic algorithm was supplemented with unified epizootic programmes with

Table 1. Laboratory diagnostics and molecular methods in Kazakhstan

Method	Indicator	Value	Object	Type of study	Practical significance
ELISA (chimeric proteins)	Sensitivity	96.4%	Cattle	Test validation	Demonstrates the ability of the method to reliably detect infected animals and supports its application in serological monitoring programmes.
ELISA	Specificity	95.1%	Cattle	Test validation	Confirms the accuracy of identifying infection-positive animals and reduces the risk of false-positive results during surveillance.
Genomic sequencing	Number of isolates examined	22	*Pasteurella multocida*	Genetic typing	Provides detailed characterisation of circulating pathogen strains and supports molecular epidemiological analysis.
Genomic identification	Serotype	B:2	*Pasteurella multocida*	Molecular identification	Allows determination of the circulating serotype and contributes to selection of appropriate preventive and control strategies.
Farm microclimate analysis	Number of farms	8 farms	Cattle	Production study	Enables assessment of the relationship between housing conditions and the risk of respiratory bacterial infections.
Microclimate	Measured parameters	4 indicators	Temperature, humidity, CO_2, NH_3	Instrumental monitoring	Provides objective assessment of environmental factors that may influence animal susceptibility and infection development.

Source: Compiled by the authors based on Bulashev et al. (2023); Kushaliyev et al. (2024); Tagaev et al. (2025); Latypova et al. (2022)

centralised recording of laboratory results. Further development was accompanied by the introduction of molecular identification methods and the expansion of serological monitoring, which ensured an increase in the accuracy of infection detection and a more reliable assessment of the epizootic status of the livestock. At the same time, the diagnostic model was expanded through production control of animal housing conditions, including instrumental assessment of microclimatic indicators, which made it possible to take into account the influence of environmental factors on susceptibility to respiratory bacterial diseases

4. Territorial stratification and dynamics of the epizootic process

The epizootiological situation regarding pasteurellosis in farm animals in the Republic of Kazakhstan was influenced by a complex of natural-climatic, economic-technological, and veterinary-sanitary factors determining the features of pathogen circulation in different territories of the country. The current assessment of spread of the disease was based on a systematised analysis of veterinary reporting, the results of serological monitoring, and territorial mapping of epizootic manifestations of the infection. The use of an integrated approach made it possible to determine the spatial patterns of the spread of pasteurellosis and to identify regions with different degrees of epizootic tension. According to the results of epizootiological zoning of the territory of the Republic of Kazakhstan, the spread of pasteurellosis was characterised by pronounced territorial heterogeneity (Ginayatov et al. 2025; Kozhayeva et al. 2025). Based on the analysis of veterinary reporting data, three epizootic zones were identified according to differences in the territorial distribution of infection, frequency of disease registration, and the presence of confirmed pasteurellosis foci (Latypova et al. 2022). These criteria reflected the level of epizootic tension and allowed differentiation of regions according to the intensity of pathogen circulation and the need for targeted veterinary surveillance. Such zoning reflected differences in livestock density, housing conditions, the level of veterinary service, and the features of the natural environment affecting the persistence of the epizootic process. Spatial differentiation made it possible to take into account regional features when planning preventive measures and to optimise the distribution of diagnostic resources.

Territorial analysis showed that a high prevalence of pasteurellosis was recorded in two regions of the Republic of Kazakhstan – West Kazakhstan and East Kazakhstan – which were classified as zones of increased epizootic risk (Latypova et al. 2022). The formation of persistent infection foci in these regions was associated with a combination of factors of intensive livestock farming, seasonal fluctuations in climatic conditions, and the features of animal migration within farm systems. The identification of territories with the most pronounced epizootic activity made it possible to consider these regions as priority zones for laboratory monitoring and preventive measures. Additional characterisation of the epizootic situation was obtained during serological monitoring of cattle. In these studies, 1,435 animal blood serum samples were analysed, providing a sufficient sample size for an objective assessment of the spread of the pasteurellosis pathogen (Latypova et al. 2022). The scale of the diagnostic survey made it possible to cover a significant number of farms and ensure representativeness of the results at the republican level. The use of serological diagnostic methods ensured the detection of both clinically pronounced and subclinical forms of infection, which is of fundamental importance for assessing the real epizootic situation. According to the results of laboratory studies, positive serological reactions were recorded in 192 cases, which confirmed the circulation of the pathogen among susceptible livestock. The presence of positive results indicated the persistence of active epizootic foci and the need for constant veterinary control. These data allowed the researchers to assess the intensity of animal infection and to use the results of serodiagnosis in the development of preventive programmes aimed at reducing the risk of further spread of the disease.

Analysis of territorial distribution showed that cases of pasteurellosis were recorded within 11 regions of the Republic of Kazakhstan, which indicated the wide geographical spread of the infection (Latypova et al. 2022). Such a scale of spread confirmed the endemic nature of the disease and the need for systematic epizootic surveillance. The expanded territorial coverage of the infection testified to the importance of combining laboratory diagnostic data with the results of spatial analysis to form a comprehensive veterinary monitoring system. Molecular methods of pathogen research supplemented epizootic zoning, providing a deeper understanding of the features of circulation of *Pasteurella multocida*. In the work of Auganov et al. (2024), the significance of the analysis of amino acid substitutions in the identification of the pathogen and the clarification of its diagnostically significant characteristics was emphasised. The use of molecular genetic approaches made it possible to increase the accuracy of pathogen detection and to clarify the features of its variability, which significantly expanded the possibilities of contemporary laboratory diagnosis of pasteurellosis. Genetic identification of strains was considered as an important element of the transition from territorial assessment of the epizootic process to the study of the structure of circulating pathogen variants. The regional level of epizootic dynamics was reflected in studies of infectious pathology of animals in the West Kazakhstan region, where the analysis was carried out on the basis of long-term statistical observations of the registration of infectious diseases (Ishchanova et al. 2025). The obtained results made it possible to establish the stability of the registration of infectious diseases and to identify the spatiotemporal features of their spread. Regional data supplemented the national picture of the epizootic situation and made it possible to clarify the position of pasteurellosis in the structure of infectious pathology of individual territories (Table 2).

A comprehensive comparison of the results of epizootic zoning, serological diagnosis, and regional monitoring showed that pasteurellosis remains a significant component of the infectious pathology of farm animals in the Republic of Kazakhstan. The use of a combination of territorial analysis and laboratory methods made it possible to form a multi-level system for assessing the epizootic situation, including macro-regional zoning and microbiological diagnosis. Such an approach ensures increased effectiveness of veterinary surveillance, timely detection of infection foci, and scientific substantiation of preventive measures aimed at reducing epizootic tension and stabilising the sanitary condition of livestock farms in the country

5. Contemporary molecular methods for diagnosis and assessment of antibiotic resistance

Pasteurellosis in farm animals is an infection requiring a combination of accurate molecular identification of the pathogen and assessment of its sensitivity to antimicrobial drugs. The spread of *Pasteurella multocida*

Table 2. Epizootological situation of pasteurellosis in Kazakhstan

Region	Indicator	Value	Study period	Analytical method	Remarks
Republic of Kazakhstan (zoning)	Number of epizootic zones	3 zones	2010-2021	Statistical analysis of veterinary reporting data	Indicated territorial risk stratification and the need for differentiated veterinary surveillance
Republic of Kazakhstan	Number of epizootic foci	2 pasteurellosis foci	2021	Territorial mapping	A high degree of occurrence was identified in two regions
Republic of Kazakhstan	Serological samples	1,435 cattle serum samples	2021	Serological analysis	Sample size used for serodiagnosis of pasteurellosis
Republic of Kazakhstan	Positive cases of pasteurellosis	192 cases	2021	Serological analysis	Positive serodiagnostic results in 13.38% of samples
Republic of Kazakhstan	Regions affected by pasteurellosis	11 regions covered	2021	Serological analysis	Pasteurellosis was detected in all investigated regions

Source: Compiled by the authors based on Latypova et al. (2022)

and *Mannheimia haemolytica* among cattle and small ruminants, as well as the participation of these bacteria in the development of pneumonias and septic forms of the disease, emphasized the need for a transition from exclusively cultural-based methods to molecular confirmation. Molecular technologies make it possible not only to confirm the diagnosis but also to analyse serogroup affiliation using PCR-based serotyping, identify virulence genes through targeted gene detection, and assess antibiotic resistance profiles by detecting resistance-associated genes and genomic markers. In clinical cases of pasteurellosis pneumonia in sheep, bacteriological isolation from pathological material was combined with subsequent polymerase chain reaction aimed at amplification of species-specific genes of *Pasteurella multocida* and *Mannheimia haemolytica* (Alemu et al. 2023). The use of polymerase chain reaction (PCR) ensures confirmation of the aetiology of the disease at the molecular level and makes it possible to differentiate these pathogens within the respiratory complex. Simultaneously, antibiotic susceptibility testing was performed using the minimum inhibitory concentration method, which makes it possible to form a antimicrobial resistance profile of isolates in small ruminants. The development of quantitative diagnostic methods has been accompanied by the introduction of multiplex systems. Quadruplex real-time PCR, based on the amplification of capsular genes, provided simultaneous identification and serotyping of *Pasteurella multocida* (Zhao et al. 2025). The method includes the use of fluorescent probes and makes it possible to detect several genetic targets in one reaction cycle. This approach is used in the examination of cattle and pigs, where the serotypic affiliation of the strain is important for assessing the clinical course and predicting the epizootic situation.

Phenotypic and genotypic antimicrobial-resistance testing complemented molecular identification. In studies devoted to *Pasteurella multocida*, the determination of minimum inhibitory concentrations of antibiotics was combined with PCR detection of resistance genes to β-lactams, tetracyclines, and macrolides (Barta et al. 2025). Analysis of genetic determinants makes it possible to identify potential resistance even when phenotypic resistance was not fully manifested. Such a comprehensive approach provides a more accurate interpretation of the sensitivity of isolates obtained from different animal species – sheep, goats, and rabbits. Molecularly oriented prevention strategies were based on the genetic characterisation of pathogen antigens. In the development of a vaccine formulation based on the surface protein PmSLP3, methods of recombinant antigen expression, its purification, and confirmation of immunogenicity against serogroups B and E of *Pasteurella multocida* in cattle were used (Fegan et al. 2024). Genetic identification of serogroups ensured the targeting of the vaccine effect to epizootically significant variants of the pathogen. Immunological studies were accompanied by laboratory verification of strains and assessment of the protective effect.

The main diagnostic approaches used for the detection and characterisation of *Pasteurella multocida* differ in their analytical capabilities, practical applications, and limitations. Their comparative characteristics are summarised in Table 3.

The comparison demonstrates that no single diagnostic method provides complete information on the infectious process; therefore, an integrated approach combining classical, molecular, and serological techniques is required for effective pasteurellosis surveillance. Molecular serotyping of isolates obtained from haemorrhagic septicaemia in cattle was accompanied by determination of their sensitivity to antimicrobial drugs (Bitew et al. 2025). Amplification of capsular genes makes it possible to clarify the affiliation of a strain to a specific serogroup, and parallel sensitivity testing demonstrates the variability of the reaction to the main groups of antibiotics. The obtained data reflect the variability of the biological properties of isolates and the need for laboratory confirmation before prescribing therapy.

Thus, up to the stage of extended serotyping and in-depth analysis of pathogen resistance, the development of molecular methods for the diagnosis of pasteurellosis was formed as a sequential complication of the laboratory algorithm. At the first stage, bacteriological isolation from pathological material – lung tissue, nasal swabs, blood, or lymph node contents – remained a mandatory link in confirming the bacterial nature of the disease. Cultural isolation on selective and differential media made it possible to obtain pure isolates suitable for further molecular verification and sensitivity testing. The next stage was PCR identification based on the amplification of species-specific DNA regions. The use of standard or nested PCR ensures confirmation of the

Table 3. Comparison of diagnostic approaches used for the detection and characterisation of *Pasteurella multocida*

Diagnostic approach	Main purpose	Key advantages	Main limitations	Role in pasteurellosis monitoring
Conventional bacteriology (culture isolation)	Isolation and preliminary identification of the pathogen from clinical samples	Provides viable isolates for further testing; enables phenotypic characterisation and antimicrobial susceptibility assessment	Time-consuming; affected by sample quality and previous antimicrobial treatment; may have reduced sensitivity in complex samples	Used as a classical confirmation method and a basis for subsequent molecular analysis
PCR-based methods	Rapid detection and identification of *Pasteurella multocida* and differentiation from related bacterial pathogens	High sensitivity and specificity; faster than culture methods; allows detection of species-specific and serogroup-associated genetic markers	Requires specialised equipment and trained personnel; detects bacterial DNA but does not necessarily confirm bacterial viability	Supports rapid diagnosis, pathogen confirmation, and molecular surveillance
Molecular sequencing/ genomic analysis	Characterisation of pathogen variability, serotype affiliation, and genetic markers	Provides detailed information on circulating strains, genetic diversity, virulence-associated markers, and epidemiological relationships	Higher cost; requires advanced bioinformatic analysis; not routinely available in all veterinary laboratories	Used for epidemiological investigation, strain tracking, and improving prevention strategies
Serological methods (ELISA and related assays)	Detection of antibodies or immune response indicators in animal populations	Suitable for large-scale monitoring; supports assessment of infection exposure; allows population-level surveillance	May not distinguish between current and previous exposure; results can depend on immune status and sampling time	Applied for herd-level monitoring and evaluation of epizootic situation
Antibiotic susceptibility testing (phenotypic and molecular approaches)	Assessment of antimicrobial resistance patterns	Supports rational therapy selection and detection of resistance-associated mechanisms	Requires additional laboratory procedures; resistance profiles may vary between isolates and regions	Provides information for treatment optimisation and resistance control

Source: Compiled by the authors based on Kutumbetov et al. (2025); Bulashev et al. (2023); Kushaliyev et al. (2024); Tagaev et al. (2025); Auganov et al. (2024); Alemu et al. (2023); Zhao et al. (2025); Barta et al. (2025); Fegan et al. (2024); Bitew et al. (2025)

isolate's belonging to *Pasteurella multocida* or *Mannheimia haemolytica*, which was of fundamental importance in the differential diagnosis of respiratory diseases in cattle and small ruminants. The use of specific primers makes it possible to minimise the risk of false-positive results and accelerates the receipt of laboratory conclusions compared to traditional biochemical tests.

6. Integrated approach to the diagnosis and prevention of pasteurellosis

Pasteurellosis in farm animals is characterised by different clinical manifestations and the involvement of different serogroups of *Pasteurella multocida*, necessitating a combination of laboratory diagnosis, antibiotic susceptibility monitoring, and targeted immunoprophylaxis. In cattle, the disease was more often recorded in the form of pneumonic pasteurellosis infection and haemorrhagic septicaemia, accompanied by hyperthermia, depression, dyspnoea, and serous-purulent nasal discharge. In sheep and goats, a respiratory syndrome with signs of bronchopneumonia predominated, and in rabbits, respiratory pasteurellosis with the development of rhinitis and conjunctivitis. Clinical variability was determined by the serogroup affiliation of the strain, the level of immune protection of the animals, and the influence of stress factors (Asfaw et al. 2022). The diagnostic algorithm included bacteriological isolation of the pathogen from lung tissue, tracheal washes, blood, or lymph nodes. Culture on selective media facilitates isolation of pure cultures suitable for subsequent identification. Genetic confirmation was done by polymerase chain reaction with amplification of species-specific genes of *Pasteurella multocida* and *Mannheimia haemolytica*, which ensures accurate differentiation of pathogens within the bacterial respiratory complex (Abbas et al. 2023). When necessary, multiplex PCR was performed for the simultaneous detection of several serogroups.

Detection of additional virulence genes associated with adhesion, invasion, and toxin synthesis can help clarify the pathogenic potential of strains (Jahromi et al. 2025). The presence of genes encoding factors for attachment to the respiratory epithelium correlated with the severity of the inflammatory reaction and the severity of the clinical course. Genetic characterisation of isolates provided the possibility of comparing molecular markers with the epizootic features of the spread of infection in the farm (Absatirov et al. 2025). Antibiotic therapy was based on the determination of the minimum inhibitory concentration of antimicrobial drugs by the serial dilution method or disc diffusion testing. The results of phenotypic analysis were supplemented by molecular detection of resistance genes to tetracyclines, β-lactams, aminoglycosides, and macrolides (Ali et al. 2025). Such an approach makes it possible to take into account both the actual sensitivity of the isolate and the potential mechanisms of resistance. The choice of drugs was carried out taking into account laboratory data, which reduced the risk of the formation of resistant bacterial populations.

Immunoprophylaxis plays a vital role in pasteurellosis control. The vaccines were administered twice, resulting in the development of strong humoral immunity, which reduced the likelihood of generalised forms of the disease. Recombinant technology makes it possible to obtain purified antigens, standardise their concentration, and improve the consistency of vaccine formulation, although the resulting immune response may still vary depending on host and environmental factors (Domínguez-Odio and Delgado 2023).

For sheep, commercial inactivated vaccines containing antigenic components of *Pasteurella multocida* and *Mannheimia haemolytica* were used, which was accompanied by a reduction in the incidence of respiratory disease, a decrease in cases of forced culling of animals, and stabilisation of livestock survival rates in farms. Effectiveness was assessed based on the results of clinical monitoring, pathological examination, and laboratory verification of the pathogen (Asfaw et al. 2022). In rabbits, autogenous inactivated vaccines obtained on the basis of strains isolated in a particular farm were used. Such an approach made it possible to take into account the local serogroup structure of the pathogen and adapt immunoprophylaxis to the characteristics of the population. Effectiveness was assessed by reducing the severity of clinical signs, reducing mortality, and the results of bacteriological control (Casalino et al. 2024).

A technological analysis of the veterinary vaccine market showed an expansion in the use of recombinant antigens, adjuvants based on oil emulsions and aluminium compounds, as well as multicomponent formulations oriented towards the serogroup structure of circulating strains (Domínguez-Odio and Delgado 2023). The use of genetically characterised antigens ensured the adaptation of vaccines to regional epizootic conditions. In the Republic of Kazakhstan, the diagnosis of pasteurellosis in cattle was accompanied by molecular typing of *Pasteurella multocida* isolates with analysis of amino acid substitutions and determination of serogroup affiliation (Auganov et al. 2024) The obtained data were used to assess the circulation of strains in different regions of the country. Territorial zoning of the spread of pasteurellosis among cattle made it possible to identify epizootically disadvantaged zones and determine priority directions for preventive measures (Latypova et al. 2022). The analysis of the epizootic dynamics of infectious diseases in the West Kazakhstan region included an assessment of the structure of bacterial infections and trends in their registration (Ishchanova et al. 2025). The established indicators were taken into account when planning vaccination, determining the frequency of laboratory monitoring, and adjusting antibiotic therapy regimens. The integrated approach in Kazakhstan combined laboratory diagnosis, serogroup typing, antibiotic susceptibility monitoring, and organisational measures of veterinary control (Sabyrova et al. 2026). Prevention included routine vaccination of cattle against haemorrhagic septicaemia, sanitary and hygienic measures, control of the microclimate in livestock premises, reduction of stress factors, and regulation of stocking density.

Thus, the integrated model for the diagnosis and prevention of pasteurellosis was formed as a step-by-step integration of laboratory, clinical-epizootiological, and organisational measures combined into a single infection control system. At its core was molecular identification of the pathogen, including PCR verification of species affiliation, serogroup typing, and analysis of genetic markers of virulence. Detection of genes encoding adhesion factors, capsular structures, and toxins made it possible to assess the potential pathogenicity of circulating strains and take it into account when planning preventive measures (Shakibayev et al. 2026). Also, an important component was antibiotic susceptibility monitoring. Determination of the minimum inhibitory concentration of antimicrobial drugs was combined with molecular detection of resistance genes, which ensured a comparison of phenotypic and genotypic characteristics of isolates (Verzhykhovsky and Nedosekov 2024). Such an approach made it possible to adjust therapy regimens, minimise the risk of ineffective treatment, and limit the spread of resistant populations of *Pasteurella multocida* in the herd.

7. Modern models of epizootological monitoring and molecular characterization of pasteurellosis pathogens

The results demonstrated that the diagnosis and control of pasteurellosis in the Republic of Kazakhstan were developed as a multi-level system combining clinical-laboratory, molecular, and organisational components. The combination of bacteriological isolation of the pathogen with serological methods of infection confirmation in Eastern Kazakhstan reflected the transition from isolated laboratory practice to comprehensive verification of the bacterial nature of the disease. Such an algorithm ensures clarification of the aetiological structure of inflammatory processes and created prerequisites for the expansion of diagnostic protocols for respiratory infections, including pasteurellosis (Zhaksylyk et al. 2026; Vygovska et al. 2023). The organisational standardisation of veterinary measures showed the importance of centralised recording of laboratory results and systematic monitoring of disadvantaged farms. The formation of a unified database made it possible to compare indicators from different regions and track the spatiotemporal dynamics of the epizootic process. The integration of molecular methods into the surveillance system expanded the possibilities of analysing the circulation of the pathogen and allowed a transition from descriptive recording of cases to a detailed characterisation of the strain structure (Bondarenko et al. 2023). Regarding comparative molecular diagnostics, Poussard et al. (2025) and Storoni et al. (2025) analysed methods for detecting bacterial respiratory pathogens in farm animals. Poussard et al. (2025) compared polymerase chain reaction and loop-mediated isothermal amplification for the detection of *Pasteurella multocida* in poultry, focusing on analytical performance indicators such as sensitivity and detection time, but without assessment of regional epizootiological patterns. Storoni et al. (2025) systematised information on the aetiology and management of bacterial respiratory disease in cattle, although the spatial distribution of infection and links with veterinary surveillance systems were not analysed. On the other hand, the current synthesis incorporates regional infection patterns, territorial zoning, organisational features of veterinary management in Kazakhstan, and molecular diagnostic techniques. By showing how laboratory identification of the pathogen can be understood within a more comprehensive epizootiological framework for evaluating infection risks and developing preventive actions, this combined analysis expands on earlier methodological studies.

While investigating serotyping and therapeutic approaches, Tabatabaei and Abdolahi (2023) and Kumar et al. (2025) examined the molecular characterisation of isolates and treatment regimens for haemorrhagic septicaemia in ruminants. Tabatabaei and Abdolahi (2023) performed a genetic analysis of sheep and goat strains with an assessment of antibiotic resistance, but the spatial structure of serogroup circulation was not analysed. Kumar et al. (2025) described diagnostic and therapeutic measures for haemorrhagic septicaemia in

cattle, focusing on clinical aspects without epizootiological stratification. Comparison with these studies, the present review expands the analysis by linking molecular characteristics of *Pasteurella multocida* isolates with territorial distribution patterns and regional epizootiological conditions in Kazakhstan. This approach provides a more comprehensive understanding of the infection process by integrating serogroup circulation, diagnostic data, and veterinary control measures within a unified surveillance framework. Mekonnen (2024) and Szacawa et al. (2025) studied species-specific epizootiology and laboratory diagnostic techniques, analysing the clinical forms of pasteurellosis in sheep and modern methods for detecting bacterial pathogens in animals. Mekonnen (2024) systematised the clinical manifestations and general diagnostic approaches in ovine pasteurellosis, but quantitative indicators of regional prevalence were not presented. Szacawa et al. (2025) summarised data on the species prevalence of infections of the *Mycobacterium tuberculosis* complex in animals and systematised laboratory methods for their diagnosis, including approaches to pathogen detection and confirmation of infectious status, without considering production and housing conditions as a separate component of analysis. In this review, based on epizootiological data from Kazakhstan, farm microclimate parameters, the structure of bacterial infections, and the dynamics of their registration were additionally taken into account. The integration of production, laboratory, and spatial indicators provided a more comprehensive assessment of the infectious process compared to isolated thematic reviews.

In the direction of in-depth genotyping and resistance profiling, the literature data of Van Nguyen et al. (2025) and Heshteli et al. (2025) examined the development of biosensor technologies used for rapid and high-precision laboratory diagnosis of brucellosis, including electrochemical and immunobiosensor platforms aimed at early detection of bacterial infections. Van Nguyen et al. (2025) performed an analysis of capsular types, lipopolysaccharide genotypes, virulence factors, and antimicrobial resistance profiles of buffalo isolates, identifying the predominance of a certain genotype and capsular type D without spatial stratification by risk zones. Heshteli et al. (2025) described the use of biosensor diagnostic systems that increase the speed of laboratory detection of the pathogen, while epizootiological aspects of infection spread and production factors of animal housing were not included in the analysis. In this analytical material on Kazakhstan, molecular typing of isolates was supplemented by territorial zoning and indicators of regional dynamics, which ensured a link between the genetic profile of the pathogen and the spatial organisation of the epizootic process. In the pursuit of vaccine development and field monitoring, Molaee et al. (2022) and Lloyd (2025) analysed the serotypes of *Mannheimia haemolytica* and the prevalence of bacterial pathogens in the lung tissue of sheep. Molaee et al. (2022) isolated various serotypes and developed a vaccine candidate, but the results were limited to laboratory evaluation without taking into account the national zoning system. Lloyd (2025) conducted an examination of sheep lungs at slaughterhouses with the identification of a complex of bacterial agents, but the study was descriptive in nature without integrating production factors. In the presented section, based on materials from Kazakhstan, laboratory identification of the pathogen was considered together with the analysis of farm microclimate and the proportions of bacterial infections among the causes of morbidity. Such a combination of molecular and production indicators made it possible to expand the interpretation of risk factors.

Regarding antimicrobial susceptibility and mixed infections, Wentzel et al. (2022) and Rahman et al. (2024) evaluated the minimum inhibitory concentrations of antimicrobial drugs and cases of concurrent haemorrhagic septicaemia and other viral diseases. Wentzel et al. (2022) compared the minimum inhibitory and mutation-preventing concentrations of antibiotics in *Pasteurella multocida* isolates, but the spatial dynamics of the spread of resistant strains were not analysed. Rahman et al. (2024) described the simultaneous occurrence of haemorrhagic septicaemia and foot-and-mouth disease in cattle, focusing on clinical characterisation without epizootiological mapping. In this synthesised material on Kazakhstan, indicators of antibiotic susceptibility and infection registration dynamics were compared with the national monitoring system and territorial zoning. The integration of clinical, molecular, and spatial data provided a more holistic model for assessing the infectious process. Ribeiro et al. (2024) and Ducrocq et al. (2023) systematised clinical-microbiological observations and examined bacterial infections of various localisations in domestic animals and rare clinical manifestations of *Pasteurella multocida*. Ribeiro et al. (2024) analysed 160 cases of bacterial peritonitis in various animal species over a long period, focusing on the spectrum of isolated pathogens and the frequency of their detection without epizootiological zoning. Ducrocq et al. (2023) described a clinical case of early infection of a surgical wound with *Pasteurella multocida*, focusing on the unusual localisation of the infection without assessing the population dynamics of the pathogen. In this analytical material on Kazakhstan, the infectious process was considered not as a single clinical phenomenon, but as a spatially structured system with the identification of risk zones, the proportion of disadvantaged districts, and the comparison of laboratory data with organisational control. This approach made it possible to move from casuistic observations to epizootiological stratification and assessment of infection spread factors.

While studying molecular characterisation of resistance, Lachowicz-Wolak et al. (2025) investigated virulence and resistance genes of *Pasteurella multocida* and *Mannheimia haemolytica* isolated from dairy calves with signs of respiratory disease. Lachowicz-Wolak et al. (2025) performed detection of resistance-associated genes and analysis of phenotypic sensitivity to antimicrobial drugs without spatial comparison of the obtained data with the regional epizootic structure. In this synthesised section, formed on the basis of materials from Kazakhstan, molecular characteristics of isolates were supplemented by territorial zoning, assessment of the dynamics of infection registration, and analysis of production factors. The integration of the genetic profile of the pathogen with the spatial and organisational component of surveillance provided a more comprehensive model for interpreting the infectious process. The obtained results confirmed the need for comprehensive integration of molecular diagnostics, epizootiological zoning, and organisational measures of veterinary control to form an adaptive model for managing pasteurellosis under conditions of regional heterogeneity of the infectious process.

5. Conclusions

During the study, a set of laboratory, molecular, and epizootiological data on pasteurellosis in farm animals in the Republic of Kazakhstan was systematised, taking into account production, territorial, and genetic factors of infection spread. The results reflected the use of contemporary diagnostic approaches aimed at increasing the accuracy of pathogen detection and assessing the epizootic status of livestock farms. The use of enzyme-linked immunosorbent assay based on

chimeric proteins demonstrated a sensitivity of 96.4% and a specificity of 95.1% when examining cattle, which confirmed the diagnostic reliability of the method during laboratory validation. The use of this approach provided the possibility of conducting mass serological surveys and made it possible to identify infected animals at the early stages of the epizootic process. Serological diagnosis acted as a basic element of pasteurellosis monitoring when controlling the infectious situation in farms of various production types. The molecular stage of the research included genomic sequencing of 22 isolates of *Pasteurella multocida* isolated from farm animals, followed by genomic identification of serotype B:2. The obtained data made it possible to clarify the circulating serotype of the pathogen and confirm the possibility of using genomic methods for epizootiological analysis of the infection. The use of high-precision genetic typing provided detailed information on the structure of the pathogen and expanded the possibilities of laboratory confirmation of pasteurellosis. The production component of the study covered 8 livestock farms, where instrumental control of 4 microclimate parameters was carried out – air temperature, relative humidity, concentrations of carbon dioxide and ammonia. The recording of these indicators made it possible to take into account the influence of cattle housing conditions on the development of infectious pathology of a respiratory profile. The inclusion of production factors in the diagnostic model ensured the connection of laboratory results with the actual operating conditions of livestock facilities.

The epizootiological assessment of the spread of pasteurellosis was based on the analysis of veterinary reporting for the period 2010-2021, as a result of which 3 epizootic zones were identified on the territory of the Republic of Kazakhstan, characterised by different intensities of disease registration. Spatial analysis made it possible to determine territories with varying levels of epizootic risk. According to the results of territorial mapping in 2021, 2 epizootic foci of pasteurellosis were identified, reflecting the localisation of the most tense zones of infection spread. Serological monitoring included the study of 1,435 blood serum samples of cattle, among which 192 positive cases were identified, amounting to 13.38% of the total number of samples studied. The registration of positive results was noted within 11 regions of the Republic of Kazakhstan, which suggested the wide geographical spread of pasteurellosis and the need for regular laboratory observation. The totality of the obtained results showed that the diagnosis of pasteurellosis in Kazakhstan was based on the integration of serological methods, genomic identification of the pathogen, production control of animal housing conditions, and territorial epizootiological analysis. A limitation of the study is the use of aggregated epizootiological and laboratory data without an extended analysis of individual farm indicators and long-term prospective monitoring of strains over time. Further research should focus on in-depth molecular sequencing of circulating isolates, expansion of the regional sample, and integration of genomic data with antibiotic resistance indicators and production risk factors.

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