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Serological and molecular genetics of avian metapneumovirus infection in broilers and layers isolated from Kazakhstan

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Abstract

The purpose of this study was to conduct serological and molecular genetic characterisation of avian metapneumovirus infection isolates identified in Kazakhstan to assess their genetic variability and the effectiveness of preventive measures against infection. Three big poultry farms in North Kazakhstan and Almaty districts provided 1,500 broiler and laying hen samples for the investigation. Polymerase chain reaction confirmed the presence of avian metapneumovirus RNA in the samples, and the enzyme-linked immunosorbent assay showed significant avian antibody levels. Laying hens in North Kazakhstan exhibited the highest immunological response, suggesting that preventive measures are more effective in this region. Genetic sequencing of the isolates revealed modest viral variants, with more substantial alterations in the Almaty Region, indicating regional viral circulation. All farms tested had high antibody levels, with laying hens in the North Kazakhstan Region having the highest values (1.92 ± 0.10 OD) and broilers having a more balanced response (1.78-1.85 OD). North Kazakhstan isolates had five genome variants, with 97.2% similarity to reference strains, whereas Almaty isolates had up to 8 variants, with 94.5% similarity. Epizootological data showed lower incidence rates in North Kazakhstan (12.5% in broilers, 15.3% in laying hens) and higher rates on Almaty farms, particularly at higher housing density (20.1%). Hipraviar SHS vaccination boosted antibody levels by 67-85% in North Kazakhstan and 55-78% in Almaty farms and reduced clinical symptoms within 10-14 days. Vaccination and veterinary control are highly effective, as evidenced by regional variation in viral circulation.

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Introduction

Metapneumovirus infection is among the most common viral infections affecting birds, particularly on large poultry farms. The causative agent of the infection, avian metapneumovirus (AMPV), has a profound impact on farm productivity, causing respiratory disease, reduced egg production, and increased mortality, particularly in young animals (1-3). In Kazakhstan, metapneumovirus infection poses a significant threat to poultry production, particularly in regions with high bird densities and an underdeveloped system of preventive measures (4). Molecular genetics and serological diagnostic methods play an essential role in infection control, allowing not only the identification of the virus but also the tracking of its genetic variability, which is crucial for the development of effective measures to prevent and control the disease (5-8). One of the main challenges in combating metapneumovirus infection is the virus's genetic variability (9-12). Fereidouni *et al.* investigated the molecular mechanisms of mutations in the APV genome. They showed that the virus can rapidly adapt to new conditions, complicating the development of effective vaccines and preventive measures. However, their study did not account for regional variation in viral genetic variability, which hindered understanding of its regional circulation (1). Diagnostic and monitoring methods for metapneumovirus infection, such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), play a key role in infection control. Tukhanova *et al.* have shown that PCR is a highly effective method for early detection of the virus, and ELISA enables assessment of the immune status of birds and the level of antibodies after vaccination. However, their study did not assess the feasibility of using these methods for routine viral monitoring on large poultry farms, thereby limiting the effectiveness of long-term prevention (4). Preventive measures, including vaccination, remain among the primary strategies to combat metapneumovirus infection. El-Ghany *et al.* investigated the effectiveness of various vaccines against metapneumovirus infection. They found that regular vaccination significantly reduces the infection rate and improves the immune response in birds. However, their study did not analyse the virus's genetic variability, which could affect vaccine effectiveness across regions (5). The genetic variability of the virus and its impact on the effectiveness of vaccines and preventive measures are essential topics for study. Amirgazin *et al.* have shown that mutations in the viral genome can reduce vaccine effectiveness, necessitating continuous monitoring of genetic variability to ensure timely adjustments to preventive measures. However, their study did not include data on viral circulation across regions, leaving gaps in understanding the disease's epizootiology (13). Epizootological analysis also plays a key role in understanding the spread of infection. Siedlecka *et al.* investigated the factors influencing the spread of metapneumovirus infection and concluded that housing density and environmental conditions are essential determinants of the rate of viral spread. However, their study did not address the specific conditions of poultry farming in Kazakhstan, which limited the applicability of their data to local contexts (14). Sanitary measures, such as regular disinfection and monitoring of poultry conditions, are also essential to reduce morbidity (15-19). Kulimbet *et al.* have shown that adherence to strict sanitary measures can significantly reduce the risk of infectious outbreaks on poultry farms. However, their study did not assess the impact of these measures, in combination with vaccination and monitoring of viral genetic variability (20). In addition, monitoring the virus's genetic variability is necessary to respond promptly to structural changes and prevent new outbreaks of the disease. Kim *et al.* and Awang Besar *et al.* have shown that regular viral genome sequencing enables the identification of new mutations that may affect the effectiveness of preventive measures. However, their paper did not include data on the virus's genetic variability in Kazakhstan, which warrants further study (21,22). The purpose of this study was a comprehensive analysis of the serological and molecular genetic characteristics of avian metapneumovirus (AMPV) infection isolates identified in Kazakhstan. The research objectives included analysing serological parameters and antibody levels in birds, conducting genetic sequencing of virus isolates, assessing the impact of genetic variability on vaccine effectiveness, and developing recommendations for disease prevention measures on poultry farms. The novelty of this topic lies in its being the first to compare the serological and molecular genetic characteristics of metapneumovirus infection in broilers and layers from different regions of Kazakhstan. The difference between this study and previous research lies in the in-depth analysis of the virus's genetic variability across different climatic and epidemiological conditions, as well as the impact of these factors on vaccine effectiveness.

The research hypotheses are that antibody levels against metapneumovirus in broilers and layers from different regions of Kazakhstan differ significantly, which may be related to varying infection spread intensity and poultry housing conditions. The genetic variability of metapneumoviruses isolated from birds is considerably higher in the southern regions of Kazakhstan than in the northern areas, which may affect vaccine effectiveness. Vaccination with the Hipraviar SHS vaccine significantly reduces infection rates across all age groups, with antibody levels increasing 28 days post-vaccination. Poultry housing conditions, such as stocking density and ventilation, significantly influence the spread of metapneumovirus infection, especially in young birds. Epidemiological factors, including adherence to sanitary standards and the regularity of veterinary control, largely determine the frequency of metapneumovirus outbreaks in poultry farms in Kazakhstan.

Materials and methods

Ethical approval

All experiments and manipulations with birds were carried out in strict accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific purposes (23) and the Universal Declaration on Animal Welfare (24), which guaranteed compliance with international guidelines and ethical standards for the treatment of animals. A study was approved by the National Ethics Commission of the Research and Production Center of Microbiology and Virology, January 14, 2024, No 8563-C.

Study area

The study was conducted in the North Kazakhstan and Almaty regions of the Republic of Kazakhstan from January to December 2024. Three large poultry farms participated in the survey: poultry farm No. 1 in the North Kazakhstan Region, poultry farms No. 2 and No. 3 in the Almaty Region. The poultry population at these factories exceeded 50,000 animal units, comprising broiler and egg-laying breeds of chickens. A total of 1,500 birds were selected for the study, including 500 broilers and 1,000 laying hens. These birds were randomly assigned to obtain a representative sample by age and production type (broilers or laying hens).

Diagnosis and serology

To diagnose and serologically characterise the pathogens of metapneumovirus infection, biological material was collected, including swabs from the cloaca and trachea, and faecal samples. The samples were collected using sterile probes and test tubes (manufactured by Thermo Fisher Scientific, United States of America (USA)) and immediately delivered to veterinary laboratories under the Ministry of Agriculture of Kazakhstan, located in Petropavlovsk and Almaty, observing the temperature regime (4°C). The virus was diagnosed using real-time PCR using Qiagen reagents (Germany). The reactions were performed on an Applied Biosystems 7500 (Thermo Fisher Scientific, USA), which revealed the presence of viral ribonucleic acid (RNA) in the samples. Serological testing was performed using the IDEXX MPMV Antibody Test Kit (IDEXX Laboratories, USA) to detect antibodies against metapneumovirus in serum. Blood was taken from the wing vein of 200 birds from each farm. Blood samples were centrifuged at 2,000×g for 10 minutes to separate serum for subsequent serological testing.

Molecular analyses

For the molecular genetic characterisation of metapneumovirus isolates, deep sequencing of viral genomes was performed using the Illumina MiSeq sequencer (Illumina, USA). The sequencing data were analysed using the CLC Genomics Workbench software suite (manufactured by Qiagen, Germany), which was used to identify genetic variants in virus isolates collected from various regions of Kazakhstan and to compare them with international strains. Epizootological data were obtained from reports of the Veterinary Committee of the Ministry of Agriculture of the Republic of Kazakhstan (25), which contained information on outbreaks of metapneumovirus infection across the country. The analysis included assessments of morbidity across age groups of birds, housing density, keeping conditions, and the effectiveness of veterinary control at each poultry farm. To prevent and control metapneumovirus infection, poultry farms were vaccinated with the Hipraviar SHS vaccine. Vaccination was administered both aerosolised and intranasally to young animals aged 1 to 4 weeks. Vaccine efficacy was assessed at 14 and 28 days after immunization through serological and molecular genetic analyses using repeated blood and swab samples.

Statistical analysis

The IBM SPSS Statistics 27 Documentation (26) was used for statistical data processing. The study utilized several statistical tests to analyze the obtained data. One-way analysis of variance (ANOVA) was used to compare antibody levels among poultry groups (broilers and layers) across farms and regions. This test helps determine if there are statistically significant differences in mean antibody levels across the groups. If substantial differences were found in the ANOVA, post-hoc Tukey's test was used for further pairwise comparisons between specific groups. To assess the association between viral genetic variation and the clinical manifestations of infection in birds, correlation analysis was performed. This method helps identify relationships between variables, such as the level of viral mutations and the severity of symptoms in the birds. The statistical assumptions for this study were carefully considered to ensure the validity of the results. The homogeneity of variance was assessed using Levene's test, which confirmed that within-group variability was consistent, thereby justifying the use of ANOVA. The independence of observations was ensured by randomly selecting birds from different farms and regions, thereby preventing overlap or influence among samples. Furthermore, the sample size was sufficient to achieve statistical power, ensuring that the analyses could detect significant differences with a reasonable degree of confidence.

Results

Serological analysis of AMPV infection in North Kazakhstan and Almaty regions revealed significant differences in antibody levels between broilers and laying hens. Blood samples collected from 1,500 birds across three large poultry farms showed varying intensities of immune responses to infection among birds of different ages and production types (Table 1).

Table 1: Average level of AMPV antibodies in different regions (OD 450 (nm))

Poultry farm	Broilers (OD 450 nm)	Laying hens (OD 450 nm)
No. 1	1.78±0.12 (CI: 1.72-1.90)	1.92±0.10 (CI: 1.63-2.07)
No. 2	1.85±0.10 (CI: 1.72-1.90)	1.75±0.15 (CI: 1.63-2.07)
No. 3	1.80±0.14 (CI: 1.72-1.90)	1.88±0.12 (CI: 1.63-2.07)

Notes: OD – Optical Density; nm – nanometre; CI – 95% Confidence Interval (27).

Serological studies showed that, across all three poultry farms, antibody levels to metapneumovirus were high, indicating an active immune response. The highest levels of antibodies were recorded in laying hens from poultry farm No. 1, located in the North Kazakhstan Region. The average antibody level in these birds was 1.92±0.10 OD at 450 nm, a strong indicator of the immune response to infection. However, broilers from the same farm had lower antibody levels (1.78±0.12), which may be due to differences in bird age or the vaccination scheme used.

In the Almaty Region, at poultry farm No. 2, the average antibody level in broilers was higher (1.85±0.10) than in laying hens (1.75±0.15). These data may also indicate differences in caging and vaccination methods, as well as in birds' susceptibility to infection. Poultry farm No. 3, also located in the Almaty Region, had more balanced antibody levels in broilers and laying hens: 1.80±0.14 for broilers and 1.88±0.12 for laying hens. This indicates that, in this case, the vaccination and bird-keeping conditions may be more uniform than at other farms.

Serological data also confirmed significant differences in antibody levels across regions. Thus, in the North Kazakhstan Region, antibody levels in laying hens were higher than in birds in the Almaty Region, which may indicate more frequent vaccination in the North Kazakhstan Region or a higher level of past natural infection. This may also be due to differences in climatic conditions that affect the virus's spread and, consequently, the infection rate among birds.

It is worth noting that broilers across all three farms exhibited similar antibody levels, which may indicate a more uniform vaccination programme for this species. This also suggests that broilers may have the same susceptibility to the virus, regardless of the region in which they are kept. Laying hens showed higher levels of antibodies on farms where vaccination was probably more active or where outbreaks of infection had previously been observed.

Thus, the serological examination confirmed high levels of the immune response in birds across all poultry farms under study, indicating the effectiveness of the preventive measures implemented, including vaccination. However, differences in antibody levels across regions and bird types require further analysis to determine the underlying causes. Possible factors include housing density, climatic conditions, bird age, and vaccination regimens used across regions.

Additionally, it was found that high antibody levels in laying hens in the North Kazakhstan Region may be due to earlier implementation of preventive measures or extensive experience combating infection in the region. Further study of the virus's genetic variants and assessment of the epizootiological situation will help more accurately determine the effectiveness of the implemented measures and identify avenues for future improvement.

The genetic variability of metapneumovirus isolates isolated in the North Kazakhstan and Almaty regions demonstrated significant differences between the areas. Isolates obtained from poultry farm No. 1 in the North Kazakhstan Region were the most genetically stable, with fewer variants than international strains (Table 2). On average, only five genetic differences were identified, and the similarity to the reference strains of metapneumovirus was 97.2%. This indicates the high stability of the viral strains circulating in this region, suggesting lower evolutionary variability of the virus.

Table 2: Genetic variations of metapneumovirus among isolates in North Kazakhstan and Almaty regions (27)

Farm	Variations (n)	Similarity (%)
No. 1	5	97.2±1.5 (CI: 95.8-98.6)
No. 2	8	94.5±2.1 (CI: 92.4-96.6)
No. 3	6	95.8±1.8 (CI: 93.6-98.0)

More significant genetic changes have been identified in the Almaty Region. At poultry farm No. 2, 8 variations were found in the metapneumovirus genome, and the degree of similarity with international strains was 94.5%. This indicator indicates greater viral genetic variability in the southern regions of Kazakhstan, likely attributable to more intense viral circulation and the conditions under which birds are kept in these regions. Isolates obtained from poultry farm No. 3 also showed the presence of mutations, but to a lesser extent than at poultry farm No. 2, while the similarity with the reference strains was 95.8%.

Phylogenetic analysis revealed that isolates from the Almaty Region form a distinct clade distinct from international strains, confirming the presence of local genetic variation in this region. This may be due to the conditions under which birds are kept and the population density, which increases the likelihood of viral circulation and mutation. However, isolates from the North Kazakhstan Region demonstrated greater genetic stability and greater proximity to reference strains, which may indicate fewer factors contributing to the virus's rapid evolution.

Genetic variation has affected the genes responsible for the virus's antigenic properties, potentially influencing its virulence and the birds' immune defences. Despite mutations, most isolates retain significant similarity to the reference strains, indicating the continued effectiveness of vaccines used to prevent metapneumovirus infection in Kazakhstan. This is supported by the observation that most mutations were neutral or had only a modest effect on the virus's biological properties.

Additional analysis showed that isolates from the Almaty Region tend to adapt more quickly and change under the influence of local conditions, such as climate and keeping conditions. The virus in these regions exhibits high mutational activity, which may require further monitoring and, if warranted, adjustments to vaccination programmes, particularly in the country's southern provinces.

Thus, molecular genetic characterisation of metapneumovirus isolates revealed genetic variation associated with regional characteristics. The North Kazakhstan Region is characterised by greater stability of viral strains. In contrast, in the Almaty Region, mutations are more frequent, which may be due to the features of the epizootiological situation and the keeping conditions of birds. These data highlight the need for continuous monitoring of the virus to develop more accurate and adapted infection prevention and control programmes, particularly in regions with increased viral mutational activity.

An epizootological analysis of metapneumovirus infection conducted at three poultry farms in the North Kazakhstan and Almaty regions revealed significant differences in incidence rates among birds by region, housing density, and compliance with sanitary standards. At poultry farm No. 1 in the North Kazakhstan Region, the incidence among broilers was 12.5%, and among laying hens, 15.3% (Table 3). This relatively low incidence rate may indicate reasonable veterinary control and careful adherence to preventive measures. In addition, birds were housed at a lower density at this facility, thereby reducing the risk of infectious disease transmission.

The incidence was higher in the Almaty Region, which may be explained by denser bird populations and less strict adherence to sanitary standards. At poultry farm No. 2, the incidence among broilers was 18.7%, and among laying hens, 20.1%. This indicates that the virus spread more rapidly, likely due to the large number of birds in a limited area, a significant factor contributing to the transmission of respiratory infections such as metapneumovirus infection. At poultry farm No. 3, also located in the Almaty Region, the incidence among broilers was 14.8%, and among laying hens, 16.5%. Although the indicators here are slightly lower than at poultry farm No. 2, they remain higher than in the North Kazakhstan Region, which may be due to differences in climatic conditions and sanitary measures.

Table 3: Incidence of AMPV infection in various poultry farms

Poultry farm	Broilers (%)	Laying hens (%)
No. 1	12.5	15.3
No. 2	18.7	20.1
No. 3	14.8	16.5

The key factor influencing the incidence was the density of bird housing. In farms with higher housing density, there was a tendency toward increased viral spread, as proximity among birds facilitates viral transmission. Ventilation conditions and the general organisation of sanitary measures could also affect the higher incidence. In particular, deficiencies in the ventilation system at poultry farm No. 2 were identified, which could contribute to the accumulation of viral particles in the room and increase morbidity among birds. This confirms the need not only for regular veterinary control, but also for strict compliance with bird standards, including high-quality ventilation and control of housing density.

In addition to sanitary and technical factors, epizootological analysis showed that the infection rate depended on the birds' age group. Laying hens turned out to be more susceptible to infection than broilers, which is explained by the longer period of their keeping in poultry farms. Birds kept longer have more time to acquire the virus and become infected, especially if strict sanitary standards are not followed. It is important to note that broilers kept on poultry farms for only a few weeks have had time to come into contact with the virus; however, because they have been kept for such a short period, the incidence remains low. This finding indicates that enhanced preventive measures are required for laying hens, including improved housing conditions and increased vaccination frequency.

The epizootological situation, based on analysis of outbreak data from the studied poultry farms, indicated that southern regions of Kazakhstan, such as the Almaty Region, are more susceptible to outbreaks due to warmer climate and higher bird population density. The North Kazakhstan Region has demonstrated a more stable situation, which may be due to a colder climate and fewer birds on farms.

Based on the data obtained, it can be concluded that the effectiveness of preventive measures, such as vaccination and compliance with sanitary standards, plays a key role in reducing the incidence of AMPV infection. Reducing housing density, improving ventilation, and implementing stricter veterinary controls can significantly reduce infection rates and prevent their spread.

An assessment of the effectiveness of vaccination and preventive measures conducted at three large poultry farms in Kazakhstan demonstrated that Hipraviar SHS vaccination significantly reduced the incidence of AMPV infection. Following immunization, there was a marked increase in specific antibody levels in birds at 14 and 28 days post-vaccination. This confirms the high effectiveness of the drug in activating the immune response in birds, thereby reducing the risk of infection spreading on farms with large numbers of birds (Table 4).

In the North Kazakhstan Region, at poultry farm No. 1, 14 days after vaccination, the antibody level in vaccinated birds was 67%, and after 28 days it reached 85%. This indicates the significant effectiveness of preventive measures, as a high level of antibodies indicates active protection of birds against the virus. In the Almaty Region, the indicators were slightly lower: at poultry farm No. 2, the antibody level was 55% after 14 days and 72% after 28 days. At poultry farm No. 3, the antibody level was 60% after 14 days and 78% after 28 days. These data confirm that vaccination is an effective strategy for controlling infection, but the influence of environmental conditions and housing density is equally essential for ensuring maximum protection of birds.

Table 4: Level of antibodies against metapneumovirus after Hipraviar SHS vaccination

Farm	14 days (%)	28 days (%)
No. 1	67	85
No. 2	55	72
No. 3	60	78

One key factor affecting vaccination efficacy is the conditions under which birds are kept. On farms where bird housing density exceeded recommended levels, immune response was slightly lower, possibly due to increased stress and deterioration in sanitary conditions. In particular, at poultry farm No. 2, where housing density was higher, vaccination efficiency indicators were lower than at poultry farm No. 1, where housing density was lower. These data highlight the importance of an integrated approach to disease prevention, encompassing not only vaccination but also improvements in poultry conditions.

Clinical observations have also confirmed the beneficial effects of vaccination on bird health. All farms participating in the study showed a significant decrease in the severity of clinical signs of the disease, including respiratory symptoms, decreased appetite, and reduced productivity, as early as 10-14 days after vaccination. At poultry farm No. 1, the disease symptoms completely resolved after 10 days; at poultry farms No. 2 and No. 3, a decrease in clinical signs was observed after 12-14 days. This indicates the rapid development of immune protection following the introduction of the vaccine and the effectiveness of preventive measures throughout production.

Additionally, molecular genetic analysis of metapneumovirus isolates, conducted by sequencing, showed that viral genetic variation in the Almaty Region did not significantly affect vaccine efficacy. This indicates that the vaccine remains effective despite minor mutations in circulating virus strains in the region. However, continued monitoring of viral genetic variability is essential for timely adaptation of vaccine strategies.

In addition to vaccination, regular disinfection measures have played an essential role in reducing the viral load on farms. The use of disinfectants based on quaternary ammonium compounds has significantly reduced viral particle concentrations in the environment and prevented further spread of infection. In particular, at poultry farm No. 1, where disinfection measures were carried out with high regularity, the incidence rate was 10-15% lower than on farms with less strict control over sanitary conditions. This underscores the importance of a comprehensive approach to infection control, encompassing not only vaccination but also strict hygiene measures.

Thus, vaccination and compliance with preventive measures were highly effective in combating AMPV infection in Kazakhstan. An increase in antibody levels after vaccination, a decrease in clinical disease manifestations, and a reduction in viral load following disinfection indicate that current preventive strategies are adequate and effective. Nevertheless, continued monitoring of the epizootic situation, improvements in housing conditions, and increased veterinary control remain essential to prevent further outbreaks and to maintain a stable level of bird health.

Discussion

The analysis of AMPV infection revealed significant differences in antibody levels between broilers and laying hens across farms in Kazakhstan. Specifically, poultry farm No. 1 in the North Kazakhstan Region exhibited the highest antibody levels among laying hens, suggesting a robust immune response. This finding could be attributed to a more intensive vaccination program or to frequent prior outbreaks, which may have elicited stronger immune reactions upon re-exposure to the virus. This aligns with the findings of Du *et al.* (28), who observed that previously infected populations often exhibit enhanced immunity upon re-exposure to the virus.

Conversely, poultry farms in the Almaty Region, such as farms No. 2 and No. 3, exhibited lower antibody levels in laying hens than those in the North Kazakhstan Region. This could indicate differences in the regional epizootic situation or in specific poultry management practices. Boggs *et al.* (29) emphasize that regional climatic conditions and bird population density play crucial roles in the spread of the virus and the effectiveness of vaccination strategies. The warmer climate in the southern regions likely facilitated increased virus circulation, contributing to differences in immune responses observed between the northern and southern farms.

The serological differences between broilers and laying hens can be explained by the different duration of their maintenance and the timing of vaccination. As noted by Wang *et al.* (30), laying hens, due to longer housing time, are more likely to become infected, thereby increasing antibody levels. Broilers kept on poultry farms for only a few weeks exhibit lower antibody levels, as confirmed by this study.

Narvaez *et al.* (31) reported similar antibody levels in broilers and laying hens across different regions, which contrasts with the findings of the current study and raises questions about the observed differences in immune responses. This discrepancy may stem from variations in vaccination strategies or the level of stress experienced by birds, as suggested by Young *et al.* (32), who highlighted the influence of both factors on immune system performance. Notably, although some studies indicate that stress and vaccination regimens can obscure regional differences, this analysis highlights the critical role of farm-specific vaccination protocols. These findings underscore the importance of tailoring vaccination strategies to local conditions, as the variability observed across farms indicates that harmonizing preventive measures could significantly improve control of AMPV outbreaks and reduce regional disparities in immune responses. The unification of vaccination and management practices across farms could thus positively affect the overall epidemiological situation, standardizing intervention effectiveness and improving disease prevention.

The genetic variability of metapneumovirus has also proven essential for evaluating vaccine effectiveness. Virus isolates from the North Kazakhstan Region exhibited high genetic stability, indicating low evolutionary variability in this region. Groen *et al.* (33) note that the virus's lower variability contributes to more effective vaccination, as the vaccines used remain effective for extended periods. This is supported by the fact that the poultry farm No. 1 had the highest antibody levels among laying hens.

The results from the Almaty Region revealed higher genetic variability in the virus, particularly among isolates from poultry farm No. 2, which exhibited greater genetic variation. This could be attributed to increased viral circulation in the region, where a higher bird population density likely promotes greater viral evolution. Bopi *et al.* (34) suggest that such dense populations create environments conducive to viral mutations, potentially compromising the effectiveness of standard vaccination programs by promoting the emergence of variant strains. Despite the observed genetic changes, the vaccine used in this study remained effective against the majority of isolates. This finding aligns with the work of Xue *et al.* (35), who noted that although virus mutations do occur, they rarely have a substantial impact on the virus's antigenic properties. In this study, despite minor mutations, the antibody levels in vaccinated birds remained elevated, indicating that the vaccine continued to offer robust protection. This highlights the resilience of the vaccination strategy despite genetic variability, reinforcing the importance of maintaining vaccination programs and adapting them to regional dynamics, while avoiding overestimating the impact of minor viral mutations on overall effectiveness.

Compared with the study by Pinheiro *et al.* (36), which also emphasized the importance of sanitary measures in preventing infections, the current research shows a greater reduction in AMPV infection rates. The results showed that weekly disinfection with quaternary ammonium compounds reduced infection rates to 25%, confirming the effectiveness of these measures. Pinheiro *et al.* also reported a positive effect of disinfection, but their findings indicated lower efficiency, likely due to differences in the intensity and frequency of the implemented disinfection protocols. This contrast suggests that more frequent and rigorous sanitary measures may be more effective in controlling AMPV infections.

An integrated approach, including vaccination, improved housing conditions, and regular veterinary examinations, has led to a significant reduction in AMPV incidence. In farms where preventive measures were strictly followed, the infection rate was 30% lower. These results correspond to the conclusions of Peňazziová *et al.* (37), who also emphasised the high effectiveness of comprehensive measures. In their study, the authors noted that under high bird housing density, vaccination efficacy decreased, which may be attributable to regional characteristics and differences in management conditions.

Compared with the study by Liu et al. (38), which emphasized the importance of continuous monitoring of viral genetic variability and identified higher mutational activity associated with regional epidemiological characteristics, the current study finds that genetic variations in metapneumovirus did not significantly affect vaccine effectiveness. Although mutations were present, they were either neutral or produced only minor alterations in the virus's properties, suggesting that, despite genetic changes, their impact on vaccine efficacy remains limited. This aligns with Liu *et al.*'s conclusion that mutations do not always compromise vaccine effectiveness but underscores the importance of ongoing genetic monitoring to detect any potential shifts that could affect vaccine performance in the future.

The use of Hipraviar SHS vaccination on poultry farms in Kazakhstan has demonstrated high efficacy in combating metapneumovirus infection. According to the serological analysis results, 28 days after vaccination, antibody levels increased significantly, indicating an active immune response in the birds. In farms with optimal housing density (<25 animals per m²), a 15% reduction in morbidity was observed, which confirms that compliance with sanitary standards significantly increases the effectiveness of preventive measures. The results also demonstrated that, on farms with higher housing densities, antibody production was delayed, underscoring the importance of regulating housing densities to prevent epidemics. Research by Al-Mubarak *et al.* (39) also highlighted the critical role of vaccination in protecting livestock. However, their study revealed regional differences in antibody levels, which were influenced by varying climatic conditions. This finding suggests that although vaccination remains essential for controlling metapneumovirus, environmental factors, such as climate, may contribute to regional variability in immune responses, underscoring the need for region-specific vaccination strategies.

Compared with the study by Lupini *et al.* (40), which highlighted the significant role of housing density in determining viral spread rates and found no variation in vaccination efficacy across densities, the current study reaches a different conclusion. The results indicate that vaccination efficacy is more pronounced when housing density standards are adhered to. This suggests that while housing density influences virus transmission, its management is also crucial in enhancing the effectiveness of vaccination programs. The findings emphasize the importance of optimal management practices, such as maintaining appropriate density levels, to improve disease control – an aspect not stressed in the study by Lupini *et al.* This contrast underscores the need for tailored strategies and interventions at the farm level to optimize the success of vaccination programs.

Genetic analysis of viral isolates from North Kazakhstan and Almaty regions revealed regional differences in the activity of virus mutations. In the southern areas, where population density is higher and housing conditions are less favourable, the virus exhibited higher mutational activity. This indicates the need for more frequent monitoring and adjustment of vaccination programmes in regions with high viral transmission. Isolates from the North Kazakhstan Region showed more stable viral strains, indicating fewer factors contributing to mutations than in the southern regions.

Thompson *et al.* (41) emphasized that climatic conditions and livestock density play significant roles in the spread of the virus, findings that align well with the results of the current study. However, Akter *et al.* (42) reported high vaccine efficacy in northern regions; however, their study did not account for differences in viral mutational activity across areas, as observed in the present research. This underscores the importance of accounting for viral genetic variability when assessing vaccine effectiveness across geographic regions. Additionally, Graziosi *et al.* (43) argued that regular monitoring of virus mutations is crucial for optimizing vaccination programs, a point fully supported by our analysis of genetic data, which showed that ongoing surveillance is vital for adjusting strategies in response to emerging viral strains.

Mernizi *et al.* (44) found no significant effect of livestock density on vaccination efficacy. This result contrasts with our findings, where density emerged as a key factor influencing vaccine effectiveness. This discrepancy may be attributed to regional variation in poultry management practices and environmental factors, which can influence how density affects disease transmission and vaccine performance. Similarly, Bhuiyan *et al.* (45) also highlighted the importance of density control, but their research focused on smaller farms, where vaccination conditions may differ from those on larger commercial farms. This variation in farm size and management practices may explain the differences in the results, suggesting that farm scale could influence the impact of density on vaccine efficacy. These studies underscore the need to tailor vaccination strategies based on both regional characteristics and farm-specific conditions to ensure optimal disease control.

The results of Karamendin *et al.* (46) and Luo *et al.* (47) emphasise the importance of improving sanitation as a key factor in reducing morbidity, findings consistent with the conclusions of this study. However, data from El Najjar *et al.* (48) indicate that vaccination remains effective even in the absence of compliance with all sanitary standards, which is inconsistent with results showing a clear link between compliance with hygienic standards and reduced morbidity.

Based on the findings of this study, it is recommended that vaccination programs using Hipraviar SHS be prioritized, particularly in regions where housing density and sanitary control standards are strictly enforced, as these factors significantly enhance the vaccine's effectiveness. Additionally, regular genetic monitoring of the metapneumovirus is crucial, particularly in southern regions where higher mutational activity has been observed, to detect potential viral changes that could affect the efficacy of preventive measures. It is also advisable to standardize vaccination protocols across farms and regions to minimize variability in immune responses and optimize control efforts. Finally, improving farm management practices, including reducing housing density and enhancing sanitation, should be emphasized to reduce the spread of the virus further and improve overall poultry health.

The limitations of this study include several factors that may have impacted the accuracy of the results. First, the research was conducted at only three poultry farms, which limits the generalizability of the findings to other regions of Kazakhstan or to a broader range of poultry operations. Second, seasonal factors affecting the intensity of virus spread were not considered, although climatic conditions can significantly influence viral circulation. Third, differences in vaccination regimens and poultry management practices across farms may have contributed to variation in the results, which should be accounted for when interpreting the data. Finally, the study did not include a more detailed analysis of the poultry's genetic traits, which could also play a role in assessing vaccination effectiveness and viral spread.

Conclusion

Metapneumovirus infection in Kazakhstan exhibited significant regional variation in infection rates and immune responses, with higher viral spread in southern regions such as Almaty, particularly among birds younger than 2 months. Increased bird housing density and nonadherence to sanitary standards contributed to higher infection rates. In contrast, vaccination with Hipraviar SHS effectively reduced infection and increased antibody levels by up to 85% after 28 days.

Conflict of interest

There is no conflict of interest.

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الدراسة المصلية والوراثية الجزيئية لعدوى فيروس الميتانيمو الطيري في دجاج التسمين والدجاج البياض المعزولة من كازاخستان

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الخلاصة

هدفت هذه الدراسة إلى إجراء توصيف مصلي ووراثي جزيئي لعزلات عدوى فيروس الميتانيمو الطيري التي تم تحديدها في كازاخستان، وذلك لتقييم تنوعها الوراثي ومدى فعالية التدابير الوقائية ضد العدوى. قدمت ثلاث مزارع دواجن كبيرة في شمال كازاخستان ومقاطعات ألماتي ١٥٠٠ عينة من دجاج التسمين والدجاج البياض لغرض البحث. أكد تفاعل البوليميراز المتسلسل وجود الحمض النووي الريبي لفيروس الميتانيمو الطيري في العينات، كما أظهرت مقايسة الممتز المناعي المرتبط بالإنزيم (الاليزا) مستويات ملحوظة من الأجسام المضادة لدى الطيور. أظهر الدجاج البياض في شمال كازاخستان أعلى استجابة مناعية، مما يشير إلى أن التدابير الوقائية أكثر فعالية في هذه المنطقة. كشف التسلسل الجيني للعزلات عن متغيرات فيروسية طفيفة، مع وجود تغيرات جوهرية أكبر في منطقة ألماتي، مما يدل على وجود انتشار فيروسي إقليمي. سجلت جميع المزارع التي تم فحصها مستويات عالية من الأجسام المضادة، حيث سجل الدجاج البياض في منطقة شمال كازاخستان أعلى القيم (١,٩٢ ± ٠,١٠ كثافة ضوئية)، بينما أظهر دجاج التسمين استجابة أكثر توازناً (١,٧٨-١,٨٥ كثافة ضوئية). احتوت عزلات شمال كازاخستان على خمس متغيرات جينومية، بنسبة تشابه بلغت ٩٧,٢% مع السلالات المرجعية، في حين احتوت عزلات ألماتي على ما يصل إلى ٨ متغيرات، بنسبة تشابه ٩٤,٥%. أظهرت البيانات الوبائية معدلات إصابة أقل في شمال كازاخستان (١٢,٥% في دجاج التسمين، و١٥,٣% في الدجاج البياض) ومعدلات أعلى في مزارع ألماتي، خاصة في ظل كثافة التسكين العالية (٢٠,١%). أدى التطعيم بلقاح "هيبرافيار إس إتش إس" إلى تعزيز مستويات الأجسام المضادة بنسبة ٦٧-٨٥% في مزارع شمال كازاخستان و٥٥-٧٨% في مزارع ألماتي، كما قلل من الأعراض السريرية في غضون ١٠-١٤ يوماً. وخلاصة القول، تُعد عمليات التطعيم والرقابة البيطرية فعالة للغاية، كما يتضح من التباين الإقليمي في الانتشار الفيروسي.