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Prevalence and Control of Avian Metapneumovirus Infection in Poultry Farms of Kazakhstan

Urzhan Omarbekova, Assylbek Mussoyev*, Aspen Abutalip, Indira Mussayeva, Ryskeldi Bazarbayev

Department of Biological Safety, Kazakh National Agrarian Research University, Almaty 050010, Republic of Kazakhstan

Corresponding Author Email: mussoyevassylbek@gmail.com

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ABSTRACT

The purpose of this study was to assess the prevalence and impact of preventive measures to reduce metapneumovirus infection (MPVI) in poultry in Kazakhstan. The study was conducted during 2024 on 12 poultry farms located in the northern and southern regions of the country, encompassing a total monitored population of over 50,000 birds. Polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) were employed as diagnostic methods. For molecular diagnostics, tracheal washings, cloacal swabs, and faecal samples from 240 age-stratified poultry were analysed using reverse transcription polymerase chain reaction (RT-qPCR). Serological monitoring was conducted by ELISA on serum samples from 100 birds per farm. Vaccinated and unvaccinated poultry served as contemporaneous control groups under comparable management conditions to evaluate vaccination and biosafety measures. PCR detected viral ribonucleic acid in 72% of poultry under two months of age ($\chi^2 = 21.9$, df = 2, $p < 0.05$), indicating high susceptibility of young poultry to infection. Immunoenzyme analysis confirmed the presence of antibodies in 65% of poultry ($\chi^2 = 18.3$, df = 1, $p < 0.05$), which enabled an effective assessment of the immune status of the population after vaccination. Vaccination with Hipraviar SHS demonstrated high efficacy, reducing the incidence of infection by an average of 50% among vaccinated poultry compared to unvaccinated poultry ($\chi^2 = 24.6$, df = 1, $p < 0.05$). Moreover, reduced severity of symptoms and shorter duration of illness were observed among vaccinated poultry. Additional biosecurity measures, such as regular disinfection of facilities and equipment, reduced poultry crowding rates, and increased veterinary control, reduced infection rates by up to 25% in poultry farms where these measures were implemented ($\chi^2 = 19.6$, df = 1, $p < 0.05$). An integrated approach, including accurate diagnosis, effective vaccination, and strict sanitary measures, proved effective in controlling MPVI, substantially reducing not only the prevalence of the disease but also the associated economic losses. These findings provide a practical, evidence-based framework for regional disease control and demonstrate that comprehensive prevention strategies can substantially mitigate economic losses in Kazakhstan's poultry industry by improving flock health, productivity, and epidemiological stability.

1. INTRODUCTION

Avian metapneumovirus infection (MPVI) remains a significant global threat to the poultry industry, leading to substantial economic losses due to reduced productivity, impaired reproductive performance, and increased mortality. The causative agent, avian metapneumovirus (AMPV), primarily affects the respiratory tract and reproductive organs of poultry, with particularly severe consequences in young birds. Despite advances in diagnostics, vaccination, and biosecurity, MPVI continues to circulate endemically and episodically in many poultry-producing regions worldwide, indicating persistent shortcomings in existing control strategies.

A consistent finding across international studies is the heightened susceptibility of young poultry to AMPV infection. Jesse et al. [1] demonstrated that birds under two months of age are particularly vulnerable, with rapid viral transmission and pronounced clinical manifestations. These findings underscore the epidemiological importance of early-life infection. However, they largely focus on disease occurrence and pathogenicity, without addressing how integrated preventive strategies can mitigate infection under field conditions. As a result, the translation of such findings into effective, farm-level control programmes remains limited. Accurate and timely diagnosis is a cornerstone of MPVI control. Molecular methods, particularly polymerase chain reaction (PCR), have been shown to be highly sensitive for early detection of viral RNA in infected birds. Amirgazin et al. [2] confirmed the diagnostic value of PCR in identifying subclinical and early-stage infections, which is essential for outbreak containment. Nevertheless, molecular detection alone does not provide information on population-level immunity or post-vaccination status. The limited integration of serological tools, such as enzyme-linked immunosorbent assay (ELISA), restricts comprehensive monitoring of virus

circulation and immune protection within flocks.

Vaccination is widely recognised as a key preventive measure against MPVI. Kariithi et al. [3] reported that vaccination can reduce disease incidence by 40-60%, highlighting its potential to significantly limit viral spread. However, vaccine efficacy varies depending on viral strains, vaccination schedules, and regional production systems. Importantly, most available studies assess vaccine performance in specific geographic or experimental contexts, with little consideration of region-specific conditions. This lack of localised evaluation is particularly relevant for countries such as Kazakhstan, where poultry production systems, biosecurity practices, and epizootological patterns may differ from those described in the existing literature. Beyond vaccination, biosecurity and sanitary measures play a crucial role in reducing the risk of MPVI introduction and spread. Orynbayev et al. [4] demonstrated that improved hygiene, routine disinfection, and reduced stocking density significantly decrease the likelihood of viral outbreaks. However, these findings were not specifically adapted to the operational realities of poultry farms in Kazakhstan, leaving uncertainty regarding the feasibility and effectiveness of such measures under local conditions. Consequently, there remains a need to evaluate how biosecurity interventions interact with vaccination and diagnostics in real production settings.

Monitoring the immune status of poultry populations is essential for assessing the effectiveness of preventive measures. Tamehiro et al. [5] showed that ELISA-based monitoring provides reliable insights into antibody dynamics following vaccination. Nonetheless, gaps remain regarding long-term immunity, optimal revaccination intervals, and their practical implications for disease control at the farm level. These uncertainties complicate the design of sustainable immunoprophylaxis programmes. The economic consequences of MPVI further emphasise the need for effective, integrated control strategies. Ball et al. [6] reported productivity losses of up to 30-50% during MPVI outbreaks, substantially increasing production costs. Despite this clear economic burden, existing studies rarely evaluate how combined diagnostic, vaccination, and biosecurity measures can jointly reduce both disease prevalence and associated financial losses.

Recent research has highlighted the potential benefits of integrating molecular and serological diagnostics. El-Ghany [7] and Kalkayeva et al. [8] demonstrated that the combined use of PCR and ELISA enhances surveillance and control of AMPV infection. However, these studies largely focus on methodological effectiveness rather than assessing the real-world impact of such integrated approaches on disease incidence under field conditions.

Taken together, the available literature underscores the global importance of MPVI while revealing a critical research gap: the lack of comprehensive, locally adapted assessments of integrated control measures in Kazakhstan. In particular, there is insufficient evidence on how diagnostics, vaccination, and biosecurity interact to reduce MPVI prevalence and severity in poultry farms operating under regional epizootological and production conditions.

Therefore, the aim of the present study was to assess the prevalence of MPVI and to evaluate the effectiveness of preventive measures in reducing MPVI among poultry in Kazakhstan. The specific objectives were to determine regional virus prevalence, assess the diagnostic performance of PCR and ELISA, and analyse the impact of vaccination and biosecurity measures on infection incidence and clinical severity in poultry populations.

2. MATERIALS AND METHODS

2.1 Study design and farm selection

The study was conducted at the Laboratory of Molecular Virology and Antiviral Biotechnology of the Kazakh National Agrarian Research University of the Republic of Kazakhstan from January to December 2024. The objects of the study were poultry farms in the northern and southern regions of Kazakhstan, where MPVI of poultry had been previously registered or where farms were threatened with the danger of infection.

The study encompassed 12 poultry enterprises, including industrial poultry farms and private farms specialising in commercial meat and egg production, with a cumulative flock size exceeding 50,000 birds under epidemiological surveillance. To ensure representativeness of regional epizootological conditions, farms were selected from both northern and southern regions of Kazakhstan, with a greater number of farms included from the south to reflect the higher density of poultry production in that area. Specifically, the northern regions comprised one industrial poultry farm and three private farms, whereas the southern regions included three industrial poultry farms and five private farms.

Housing systems varied between regions and farms. In the northern regions, two farms employed intensive cage housing systems, while two farms utilised floor-based housing. In the southern regions, four farms practised cage housing, three used floor housing systems, and one private farm operated an extensive free-range production system. All farms raised productive poultry breeds intended for either meat or egg production, with flock sizes ranging from small private holdings to large-scale industrial units.

2.2 Sample collection and processing

The reported total population of over 50,000 birds refers to the combined flock size of all participating farms. Laboratory investigations were performed on representative subsets of this population. For molecular diagnostics, samples were collected from 240 birds stratified by age: 100 birds younger than 2 months, 80 birds aged 2-4 months, and 60 birds older than 4 months. For serological analysis, blood samples were obtained from 100 birds per farm to assess population-level immune status. Sampling was non-random and targeted, based on the presence of clinical signs or suspicion of infection, with additional stratification by age group to support epizootological analysis.

Several approaches were employed to collect epidemiological data and diagnose infection. Samples for laboratory analysis were collected from sick and suspected infected poultry. Samples for examination included tracheal washings, cloaca swabs, and faeces. Samples were collected using sterile probes and tubes (Thermo Fisher Scientific, USA) and immediately transported to the laboratory under cold chain conditions (at 4°C).

2.3 Laboratory diagnostics (RT-qPCR and ELISA)

Diagnostics of MPVI were performed using real-time

reverse transcription polymerase chain reaction (RT-qPCR). Commercial RT-PCR reagents and AMPV-specific primers (Qiagen, Germany) were used. Amplification targeted a conserved region of the viral N gene using the following primers: forward 5′-AGCTTCTACGACAACGGAAA-3′ and reverse 5′-TGTGGTGAAGTCCATGTTGC-3′, generating an amplicon of 116 bp. PCR cycling conditions consisted of reverse transcription at 50°C for 30 min, initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 15 s and annealing/extension at 60°C for 60 s. Reactions were performed using an Applied Biosystems 7,500 real-time PCR system (Thermo Fisher Scientific, USA). Samples with a cycle threshold (Ct) value ≤35 were considered positive, while samples with Ct values >35 or undetermined were considered negative.

Serological confirmation of MPVI was performed using a commercial IDEXX Antibody Test Kit (IDEXX Laboratories, USA) in accordance with the manufacturer's instructions. Blood samples were collected from the wing vein of 100 birds at each farm, allowed to clot, and centrifuged at 2,000 × g for 10 min to obtain serum. Serum samples were analysed using a single-dilution indirect ELISA, in which sera were diluted 1:500 and added to antigen-coated microplate wells. After incubation and washing steps, horseradish peroxidase conjugated anti-chicken IgG and substrate solution were applied. Optical density (OD) was measured at 450 nm using a microplate reader.

Interpretation of results was performed using the cut-off criteria provided by the kit manufacturer. Samples exceeding the positive threshold defined by IDEXX were considered seropositive, while samples below this threshold were considered seronegative. For comparative and statistical analysis, ELISA results are presented as mean OD_{450} values ± standard deviation, reflecting relative antibody reactivity under single-dilution conditions rather than absolute antibody titres.

2.4 Data collection and epizootological analysis

Epizootological analysis included estimation of disease prevalence among different age groups of poultry. Each farm was studied for risk factors such as poultry crowding rate, housing conditions, sanitary standards, and frequency of veterinary inspection. ArcGIS software (produced by Esri, USA) was used to construct an epizootological map, which enabled visualisation of outbreak foci and assessment of the dynamics of MPVI spread (Figure 1).

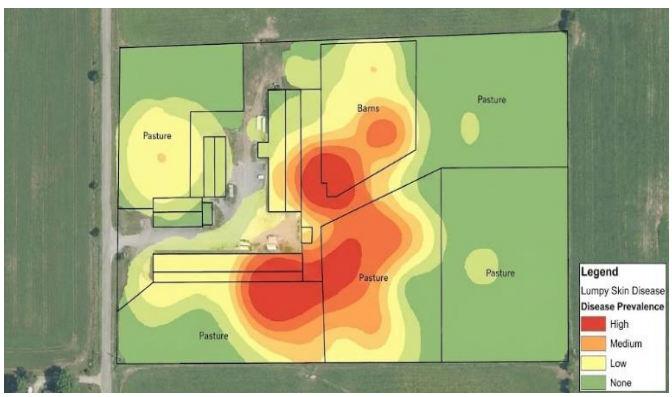

Figure 1. Epidemiological map showing the distribution of MPVI cases on the farms, constructed using ArcGIS

2.5 Interventions: Vaccination and biosecurity measures

Prevention and control measures included vaccination of poultry using live dry vaccine against MPVI, Hipraviar SHS (produced by Amer (Girona), Spain). The vaccine was administered intranasally or by aerosolised coarse spray in poultry between 1 and 4 weeks of age, depending on the age group. Vaccination efficacy was assessed by serological antibody monitoring using ELISA. In addition to vaccination, feed quality was monitored through regular sampling for analysis of nutrient content and microbiological parameters. Unvaccinated poultry were maintained as contemporaneous control groups within the same farms as vaccinated birds, ensuring comparable housing, management, and biosecurity conditions for valid comparative analysis. Water quality was checked weekly, and physicochemical properties such as pH, nitrate contamination levels, and the presence of pathogens were analysed. Biosecurity was ensured by strictly controlling access to the poultry houses, regular disinfection of equipment and holding areas, and implementing protocols to prevent contact with wild poultry. Vitamins and minerals were introduced into the diet by adding premixes to the feed, calculated based on the needs of different age groups of poultry.

Within the framework of preventive measures, sanitary control at poultry farms was also strengthened, including disinfection of premises and equipment. Disinfectants based on quaternary ammonium compounds (Ecolab, USA) were used. Disinfection measures were performed on a regular basis in combination with other measures such as vaccination, improvement of housing conditions (feed and water quality control), and introduction of vitamins and minerals into the diet. The integrated approach was aimed at maximising protection of the poultry and preventing the spread of infection.

2.6 Statistical analysis

The results of the study were processed using SPSS Statistics software (version 27, manufactured by IBM, USA). Student's t-test was employed to evaluate differences in infection rates and the effectiveness of preventive measures, Pearson's method was used to analyse correlations, and the chi-square (χ^2) test was applied to assess associations between categorical variables. The level of statistical significance was set at $p < 0.05$.

2.7 Ethical considerations

All manipulations with poultry were performed according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [9] and the Universal Declaration on Animal Welfare [10].

3. RESULTS

3.1 Epidemiological survey results: Infection rate, regional, and age distribution

MPVI detected in several regions of Kazakhstan showed significant variation in prevalence among poultry farms. In southern regions, such as Zhambyl and Turkestan (formerly South Kazakhstan) regions, infection rates were higher than in northern regions. Among poultry under two months of age, 68%

were infected, suggesting a significant susceptibility of young poultry to infection. Poultry from two to four months of age showed a lower infection rate of 45%, which may be caused by the development of immunity after vaccination or natural resistance (Table 1 and Figure 2).

Poultry crowding rate proved to be a key factor influencing the spread of the virus. In farms where the rate exceeded 25 birds per square metre, infection spread much faster and led to major outbreaks. Under such conditions, 70% of poultry was infected ($\chi^2 = 20.8$, df = 1, $p < 0.05$). Neglect of sanitary standards exacerbated the situation, and on farms where regular veterinary inspection was not performed, outbreak rates were as high as 55% ($\chi^2 = 17.4$, df = 1, $p < 0.05$). In contrast, farms with regular weekly veterinary inspection showed a markedly lower infection rate of only 20% ($\chi^2 = 19.1$, df = 1, $p < 0.05$). This confirms the significance of regular veterinary inspections and strict adherence to housing standards in preventing epidemics. All proportions were compared using Pearson's χ^2 test; differences were considered statistically significant at $p < 0.05$.

Table 1. Methopneumovirus infection rates by region and age group

Region	Poultry Under 2 Months	Poultry Aged 2-4 Months	Poultry Over 4 Months	χ^2 (df)	p-value
Northern	52% (52/100)	38% (38/100)	15% (15/100)	18.7 (df=2)	<0.05
Southern	68% (68/100)	45% (45/100)	20% (20/100)	22.4 (df=2)	<0.05

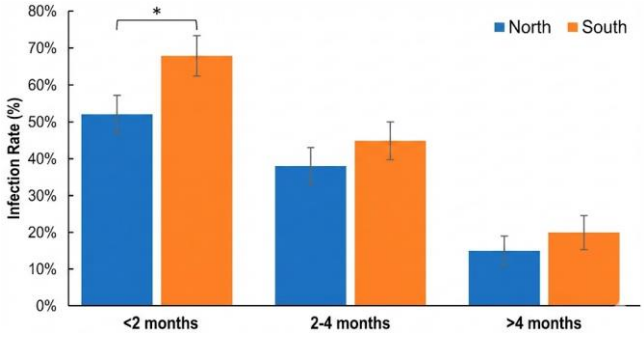

Figure 2. Methopneumovirus infection rates by region and age group

Regional differences also played a major role in the spread of infection. In the south of Kazakhstan, particularly in Zhambyl and Turkestan regions, more outbreaks were concentrated due to the high poultry crowding rate at farms and less strict enforcement of sanitary regulations. In northern regions, such as Kostanay and Pavlodar regions, the epidemic situation was more stable, but the lack of regular control and preventive measures also contributed to the spread of the virus. This suggests that regional factors, including climatic conditions, poultry crowding rate, and level of control, considerably influence the level of the epidemic.

The age of poultry was found to be one of the crucial factors affecting vulnerability to the virus. Young poultry under two months of age were found to be the most vulnerable group to MPVI. The mortality rate among young poultry was 30% greater compared to older poultry ($\chi^2 = 16.7$, df = 1, $p < 0.05$), suggesting that young poultry is more susceptible to infection and that additional preventive and protective measures are needed for this age group. Recovery time in infected poultry was also longer, resulting in considerable losses to farms. This particularly affected farms where outbreaks were more frequent. Losses were associated with both increased treatment costs and a drop in poultry productivity. Notably, older poultry was more resistant to infection, confirming the necessity of additional protection for young birds.

3.2 Risk factor analysis: Stocking density, sanitary conditions, and infection rate

Epizootological analysis revealed that high poultry crowding rate and poor sanitary control are key risk factors. On farms where planting densities did not exceed Kazakhstan's mandatory norms – 10-12 birds per square metre for young poultry and 7-9 poultry per square metre for adult poultry – and where veterinary inspections were conducted every fortnight, the number of outbreaks was reduced by 45% ($\chi^2 = 21.3$, df = 1, $p < 0.05$). These findings suggest the need for stricter sanitary standards, including reducing the crowding rate to 8-10 birds per square metre for young poultry and 6-8 for adult poultry, and increasing the frequency of veterinary inspections to weekly inspections, so that potential outbreaks can be promptly identified and the necessary measures taken to prevent them.

Young poultry proved to be the most vulnerable group, which makes it mandatory to vaccinate and strengthen preventive measures during this critical period. Data from the epizootological map revealed that farms applying integrated measures such as regular veterinary inspections, improved sanitation, and compliance with planting density showed less susceptibility to virus outbreaks, resulting in an average 35% reduction in mortality ($\chi^2 = 18.9$, df = 1, $p < 0.05$). This substantially reduced economic losses associated with poultry morbidity and mortality.

Thus, the greatest outbreaks were observed in young poultry, and the intensity of spread was directly related to housing density and the level of veterinary control. Effective prevention of outbreaks requires strict enforcement of sanitary regulations and regular veterinary monitoring, especially in regions with high poultry crowding rates [11]. These measures will help to reduce the risks of infection and economic losses caused by MPVI.

3.3 Comparison of diagnostic methods: PCR versus ELISA

MPVI was diagnosed using two key methods: RCR and ELISA. Both methods were highly effective in identifying infection in poultry in the farms under study. PCR aimed at detecting virus RNA gave a positive result in 72% of poultry, with the diagnosis confirmed by clinical symptoms (Table 2). This method proved to be particularly accurate for early diagnosis, as it detected the virus even in poultry with minimal or no symptoms.

PCR enabled accurate detection of the presence of the virus even in cases where clinical symptoms were minimal. This was particularly significant in the younger age groups, where infection rates were highest. Among poultry under two months of age, 72% of PCR results were positive, while among poultry between two and four months of age, positive results were observed in 56% of cases, indicating the high sensitivity of the method. Older poultry showed the lowest infection rate of 30%,

which may be attributed to increased immune defence. Statistical comparison of PCR positivity rates between age groups using Student's t-test demonstrated statistically significant differences ($p < 0.05$), confirming the age-dependent susceptibility to MPVI.

Table 2. Detection of aMPV RNA by real-time PCR in different age groups

Poultry Group	Samples	Positive PCR Results	Negative PCR Results	χ^2 (df)
Poultry under 2 months	100	72	28	21.9 (df = 2)
Poultry aged 2-4 months	80	45	35	21.9 (df = 2)
Poultry over 4 months	60	18	42	–

To confirm the PCR results, an ELISA was performed to detect antibodies to metapneumovirus. This method helped to assess the level of immune response of poultry to the infection. ELISA was positive in 65% of poultry ($\chi^2 = 18.3$, df = 1, $p < 0.05$), which was consistent with PCR results and indicated the high efficiency of ELISA as an additional diagnostic method. However, ELISA was less accurate in detecting early stages of infection, as antibodies appear later after infection. Notably, among the PCR-positive samples, 85% were also positive by ELISA, confirming a high degree of agreement between the two methods ($\chi^2 = 12.4$, df = 1, $p < 0.05$). This cross-validation further supports the reliability of both methods in diagnosing MPVI, although ELISA may still be limited in early detection when antibodies are not yet present.

In the study, antibody levels were greater in vaccinated poultry, suggesting the success of vaccination. Vaccinated poultry showed greater antibody levels compared to unvaccinated poultry, confirming the efficacy of the metapneumovirus vaccine. PCR and ELISA complement each other, providing a comprehensive approach to diagnostics. PCR was particularly useful for early detection of the virus, while ELISA allowed monitoring antibody levels after vaccination and assessing the immune status of the poultry.

The diagnostic results also revealed the prevalence of the virus in different age groups. Young poultry, as shown by PCR, were the most susceptible to infection, confirming the necessity of early diagnostics and vaccination. At the same time, antibody levels were greater in older poultry, suggesting the development of an immune response after infection or vaccination.

3.4 Evaluation of vaccine immunisation efficacy

Vaccination is the primary method of prevention of MPVI, which poses a major threat to poultry farms. The hypervariable SHS vaccine was selected for the study of vaccination efficacy and administered in poultry between one and four weeks of age. The vaccine was administered intranasally or via aerosol spray, which enabled coverage of many flocks in a brief period. Vaccination efficacy was assessed based on antibody levels and infection rates among vaccinated and unvaccinated poultry, and in relation to the age of the poultry (Table 3).

Antibody responses measured by ELISA are presented in Table 3 as mean OD_{450} values ± standard deviation (SD). Vaccinated poultry demonstrated significantly higher ELISA seroreactivity compared with unvaccinated controls across all age groups. In poultry under two months of age, the mean OD_{450} was 1.85 ± 0.12 in vaccinated birds versus 0.55 ± 0.08 in unvaccinated birds ($p < 0.05$). Similarly, in poultry aged 2–4 months, vaccinated birds showed a mean OD_{450} of 1.65 ± 0.15 compared with 0.72 ± 0.10 in unvaccinated birds ($p < 0.05$). In poultry over four months of age, ELISA values were also significantly higher in vaccinated birds (1.42 ± 0.18) than in unvaccinated birds (0.90 ± 0.12; $p < 0.05$).

Table 3. ELISA seroreactivity (OD_{450}) measured using a single-dilution method on Day 14 after vaccination, by poultry group

Poultry Group	Mean ELISA OD_{450} in Vaccinated Poultry (Single Dilution)	Mean ELISA OD_{450} in Unvaccinated Poultry (Single Dilution)
Poultry under 2 months	1.85 ± 0.12	0.55 ± 0.08
Poultry aged 2-4 months	1.65 ± 0.15	0.72 ± 0.10
Poultry over 4 months	1.42 ± 0.18	0.90 ± 0.12

Note: Data are presented as mean optical density at 450 nm (OD_{450}) ± standard deviation (SD). All proportions were compared using Pearson's χ^2 test; differences were considered statistically significant at $p < 0.05$.

These statistically significant differences indicate a robust vaccine-associated serological response. Although ELISA OD values represent assay signal intensity rather than a linear measure of antibody concentration, the consistent separation between vaccinated and unvaccinated groups confirms the immunogenic effect of the Hipraviar SHS vaccine under the applied testing conditions.

Analysis of the incidence of infections among vaccinated and unvaccinated poultry confirmed the high efficacy of vaccination. Vaccinated poultry under two months of age showed a significantly lower frequency of infections – only 15% of the entire group were infected ($\chi^2 = 26.1$, df =1, $p < 0.05$) with the virus, whereas in unvaccinated poultry of the same age, the infection rate reached 65% ($\chi^2 = 26.1$, df = 1, $p < 0.05$). In the group of poultry from two to four months of age, the results were analogous: vaccinated poultry was infected in 20% of cases, while among unvaccinated poultry, the rate reached 55%. In older poultry, the incidence of infection in vaccinated poultry was 12%, whereas among unvaccinated poultry, infection affected 35% of the group.

These findings demonstrate that vaccination not only increases antibody levels but also considerably reduces the incidence of disease, especially in young poultry. Vaccination helps to prevent not only infection but also mortality in young poultry, which is of great significance in maintaining farm stability and productivity [12-14].

It was also noted that vaccinated poultry had faster recovery from infection than unvaccinated poultry. The duration of illness was shorter in vaccinated poultry by an average of 25% ($\chi^2 = 14.8$, df = 1, $p < 0.05$) compared to unvaccinated poultry. Symptoms in vaccinated poultry were less severe, suggesting that vaccination not only helped to reduce the incidence of infection, but also alleviated symptoms in case of infection, reducing the need for additional costs for treatment and care

of sick poultry.

Importantly, vaccination not only protects the flock from disease but also improves the overall health of the poultry. Vaccinated poultry showed more stable performance, were less sick, and recovered more quickly from the disease. This is particularly significant for poultry farms, where maintaining a strong level of poultry health directly affects the economic efficiency of production. The introduction of vaccines enabled farms to reduce losses and increase resistance to infectious diseases.

Regular monitoring of antibody levels after vaccination also revealed that vaccination created lasting immunity in birds. Antibody levels were measured fortnightly for six months after vaccination, and the findings showed that antibody levels stayed consistently high throughout this period. This suggests the need for revaccination only as immune defence declines, which was usually observed after six months. This approach maintains a strong level of protection in the herd and minimises the risks of re-infection outbreaks.

Thus, vaccination against MPVI is an indispensable tool in controlling the spread of the virus on poultry farms. The introduction of the vaccine into the system of preventive measures helped to reduce mortality among poultry by an average of 35%, which substantially reduced the economic losses of farms. Vaccination also improved overall poultry health and productivity, making it a crucial element in the management of poultry farms.

Prevention of metapneumovirus infection requires a comprehensive approach including vaccination, strict sanitary measures, improvement of poultry housing conditions, and regular veterinary control [15, 16]. In the present study, all the above measures were applied and were highly effective in preventing the spread of the virus. Improvements in housing conditions included optimising housing density, ensuring constant access to fresh drinking water, improving ventilation, and introducing a fortified diet with vitamins and minerals. Vaccination was the mainstay of prevention, but it is vital to note that success in controlling infection was also conditioned by strict adherence to sanitary standards and improved housing conditions. These comprehensive measures resulted in a 40% reduction in the incidence of the disease (χ^2=20.2, df=1, $p<0.05$), confirming their high efficacy.

3.5 Evaluation of biosafety measures effectiveness

One of the crucial measures was the strengthening of sanitary control on farms where poultry were kept. Regular disinfection of premises and equipment using quaternary ammonium compounds disinfectants (Ecolab, USA) was an essential factor in preventing the spread of the virus among birds. As presented in Table 4 and Figure 3, the frequency of sanitation directly affected bird infection rates: farms with regular weekly disinfection revealed a considerable reduction in infection rates.

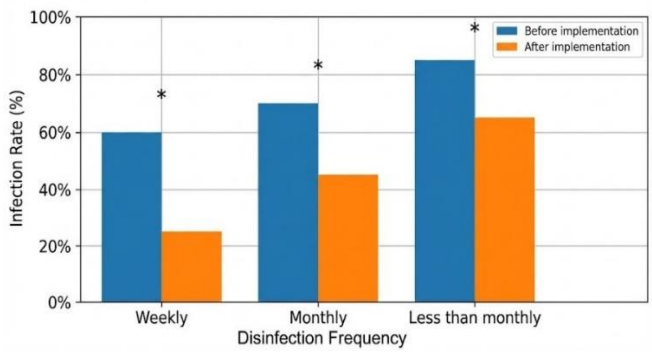

Figure 3. Longitudinal effect of sanitation frequency on MPVI rates within the same poultry farms before and after implementation of enhanced sanitary measures

Table 4. Longitudinal effect of sanitation frequency on metapneumovirus infection (MPVI) rates within the same poultry farms before and after implementation of enhanced sanitary measures

Disinfection Frequency	Infection Rate Before Implementation of Measures (%)	Infection Rate After Implementation of Measures (%)	χ^2 (df)
Weekly	60%	25%	19.6 (df = 1)
Once a month	70%	45%	20.8 (df = 1)
Less than once a month	85%	65%	18.3 (df = 1)

Note: Data represent paired before-and-after observations collected from the same farms. Differences in infection rates were analysed using Pearson's χ^2 test for paired proportions. All reductions observed after implementation of sanitary measures were statistically significant at $p < 0.05$.

Weekly disinfection showed the most pronounced results, reducing the infection rate from 60% to 25% (χ^2 = 19.6, df = 1, $p < 0.05$). This indicates that regular sanitation is an essential aspect of MPVI prevention. Statistical analysis using Student's t-test confirmed that the reduction in infection rates after the implementation of weekly disinfection was statistically significant compared to less frequent sanitation schedules ($p < 0.05$). Disinfection was performed simultaneously with other measures such as vaccination and improved housing conditions, providing an integrated approach to prevention. On farms where disinfection was less frequent than once a month and not combined with other preventive measures, infection rates stayed high at 65%, suggesting that measures were not sufficiently efficacious when applied infrequently and in isolation. These data emphasise the need for regular disinfection in combination with other preventive measures as a critical element in the prevention of infectious diseases in poultry.

An essential factor in the control of metapneumovirus was the improvement of poultry housing conditions, specifically the reduction of crowding rates. On farms where the poultry crowding rate exceeded 25 birds per square metre, the risk of spreading the infection was much greater, complicating the control over the disease. Reducing the crowding rate to 15-20 birds per square metre substantially reduced infection rates, as the improved conditions helped to reduce poultry stress and improve overall health. Reducing the crowding rate also enabled better ventilation of the premises, which reduced the spread of aerosol particles that contribute to virus transmission. The study also involved free-range farms where the incidence of disease was 50% lower compared to conventional, densely crowded farms, suggesting a positive effect of free-range on

reducing the risk of spreading infection.

Regular veterinary inspection also proved to be an indispensable element in the system of preventive measures. The introduction of regular weekly inspections enabled the prompt identification of infected poultry, its isolation from the rest of the stock, and the prevention of further spread of infection. This approach resulted in a 40% reduction in mortality and an average 35% reduction in farm economic losses, which confirmed the significance of regular monitoring and prompt intervention in case of disease detection.

Restricting access of unauthorised persons to farms and ensuring staff hygiene were vital elements of the biosecurity system. All farm staff working with poultry were required to follow strict hygiene rules, including the use of protective clothing and footwear, and regular disinfection of hands and equipment every 2 hours. These measures prevented the possible introduction of infection from outside and considerably reduced the risk of spreading the virus to other farms. The introduction of strict biosecurity measures at the specified frequency was an essential step in preventing cross-infections and protecting the herd from new outbreaks.

Additionally, epizootological monitoring using ArcGIS software enabled accurate tracking of infection foci and rapid response to their occurrence. The construction of epizootological maps involved analysing the geographical location of farms, poultry crowding rate, wind directions, and transport routes between farms. This helped to identify probable routes of transmission, such as spread via aerosol particles downwind or the movement of infected vehicles and equipment between farms. This approach enabled farmers and veterinary services to introduce prompt preventive measures such as traffic restrictions and additional disinfection of equipment, preventing the spread of infection to neighbouring farms.

Control of feed and water quality also helped to improve the overall resistance of poultry to infection. The poultry diet with additional vitamins and minerals was supplemented on a monthly basis, which helped to strengthen their immunity and reduce susceptibility to infectious diseases. The quality of water used to water poultry was checked weekly to detect possible contamination and prevent the spread of infections through water sources. Regular checks and adjustments helped to maintain the health of the flock and minimise the risks of disease outbreaks.

Thus, an integrated approach including vaccination, regular disinfection, veterinary control, biosecurity, and improved housing conditions demonstrated strong effectiveness in reducing MPVI. The application of these measures substantially reduced mortality and economic losses on farms. This approach will continue to be a valuable tool for maintaining poultry health and preventing outbreaks of infectious diseases.

4. DISCUSSION

4.1 Epidemiological characteristics of metapneumovirus infection in Kazakhstan: Comparison with global and regional data

A key epidemiological finding was the markedly higher susceptibility of birds up to two months of age. This pattern is biologically plausible and not explained by “age” alone. First, young birds have an immature innate and adaptive immune system: mucosal barriers and local respiratory immunity are still developing, which reduces early interferon-mediated control and delays effective virus clearance. Second, maternal antibodies can be a double-edged factor in early life. While they may partially protect very young chicks, their titres decline over time. The “window”, when maternal antibodies are too low to protect, yet still sufficient to interfere with an optimal response to infection or vaccination, can increase vulnerability. Third, young poultry are often exposed to pronounced management-related stressors (brooding temperature fluctuations, regrouping/mixing, transportation, vaccination handling, high ammonia levels from litter, and nutritional transitions). Stress can suppress immune responses via neuroendocrine pathways, increasing both susceptibility and clinical severity [17]. Finally, in high-density settings, young birds experience higher contact rates and aerosol exposure, which increases infectious dose and accelerates transmission chains – an effect that becomes particularly consequential for a predominantly respiratory pathogen. Therefore, the high prevalence in the youngest group supports Martínez-Espinoza et al. [18] and provides a mechanistic rationale for prioritising protection during the first weeks of life, as also emphasised in farm-level prevention discussions by Lupini et al. [19].

The discrepancy with García-García et al. [20] can be interpreted through differences in population structure and management conditions (including stocking density, ventilation quality, hygiene regimes, and immunoprophylaxis timing). Even when “young age” is comparable, infection pressure may differ: lower stocking density, better air exchange, and stricter sanitation reduce effective exposure, which can yield apparently lower susceptibility. Conversely, where crowding is high and sanitary control is inconsistent, young birds, already immunologically disadvantaged, are more likely to become infected and to develop more severe disease. Thus, the present findings reinforce that risk is shaped by the interaction of host age, immune status (including maternal antibody dynamics), and farm-level stressors rather than by age in isolation.

Regarding regional differences, the higher outbreak propensity in the southern regions (e.g., Zhambyl and Turkestan) is most plausibly linked to management and contact structure, not solely to climate. Ardiçli et al. [21] similarly associate higher circulation of respiratory viruses with intensive production settings and crowding. In the present context, a higher concentration of farms can increase between-farm connectivity (shared personnel, equipment, vehicles, and service providers), making consistent inspections and uniform sanitation more difficult to maintain [22, 23]. Jesse et al. [24] report regional contrasts that may reflect environmental and production differences. However, our data indicate that stocking density and hygiene standards likely have a stronger direct influence on transmission than climate alone, because they determine both infectious dose and frequency of effective contacts within and between flocks.

Epidemiological analysis revealed that southern regions of Kazakhstan, such as Zhambyl and Turkestan, were more prone to outbreaks than northern regions, which may be associated with higher poultry farm density, increased crowding rates, and less stringent sanitary control. Although some regional differences in infestation levels have been linked to climatic conditions, the findings of the present study indicate that virus spread is influenced primarily by poultry density and hygiene standards rather than by climate alone. These results

underscore the importance of implementing comprehensive preventive measures, including regulation of stocking density and enhancement of sanitary conditions, to mitigate the risk of viral transmission across regions.

Differences in the researchers' findings and the presented study may be related to the conditions under which the poultry is kept in various farms, as well as approaches to vaccination and sanitation. The data from the present study confirmed that the density of poultry and strict control of sanitary standards are key factors affecting the spread of the virus, especially among young poultry. Under conditions of high crowding rate and poor sanitation, the virus spreads much faster, resulting in greater infection and mortality rates in poultry.

4.2 Applicability and limitations of diagnostic methods

The analysis of metapneumovirus circulation on poultry farms in Kazakhstan confirmed that combining RT-qPCR (for early detection of viral RNA) with ELISA (for assessment of post-infection/post-vaccination serostatus) provides a more complete picture of both active virus circulation and flock immunity. In line with Cho et al. [25] and Salles et al. [26], PCR showed high diagnostic value for early infection, which is consistent with our finding of viral detection in 72% of birds younger than two months. The agreement with Lupini et al. [19] and Tucciarone et al. [27] further supports that molecular detection is particularly informative at the stage when clinical signs may be mild or non-specific, whereas ELISA becomes most useful once an antibody response has developed.

Differences in reported PCR performance across regions, e.g., the variability noted by Kariithi et al. [28], can reasonably be explained by pre-analytical and analytical factors rather than by geography alone. In the present study, standardisation of sampling (use of sterile instruments, maintenance of cold chain, and prompt processing) likely reduced degradation of viral RNA and minimised inhibitors, improving reproducibility. In addition, the use of updated reagents/primers and optimised amplification protocols could reduce false-negative results compared with studies relying on older primer sets or less controlled sample handling. This interpretation is consistent with the broader observation that accurate surveillance requires not only a sensitive assay, but also strict control of sampling quality and transport conditions, as also implied by the need for "comprehensive approaches" highlighted in work discussing methodological variability [29].

4.3 Vaccine efficacy analysis (in comparison with vaccine package insert data and other studies)

The observed ~50% reduction in morbidity/incidence among vaccinated birds can be explained by how live aMPV vaccines prime protective immunity in the respiratory tract. Vaccination (Hipraviar SHS) likely induces (i) mucosal immune responses that limit initial replication in the upper airways, (ii) systemic antibody responses that neutralise virus and reduce viraemia-like dissemination, and (iii) immunological memory that accelerates secondary responses upon exposure. Importantly, vaccine protection often manifests less as complete sterilising immunity and more as reduced viral replication and shedding. This lowers the probability of onward transmission and decreases the proportion of birds developing clinically evident disease. This mechanism is consistent with the broader evidence that vaccination reduces disease incidence and severity under field conditions [3], and it aligns with the reduced severity and shorter duration of illness documented in vaccinated groups in our study.

The need for revaccination suggested by Mernizi et al. [30] is also mechanistically coherent: as antibody levels and mucosal protection wane over time, susceptibility can rise again, particularly in older birds with ongoing exposure pressure, which supports implementing antibody monitoring (ELISA) to optimise booster timing.

4.4 Cost-effectiveness and feasibility of biosafety measures

Finally, the ~25% reduction in infection associated with strengthened biosecurity can be interpreted through "key control points" that interrupt the main transmission routes (aerosol/droplet spread within houses and mechanical introduction between houses/farms). The most critical control points are: (1) access control and separation of "clean" and "dirty" zones (restricting entry, dedicated clothing/footwear, hand and equipment disinfection), which reduces virus introduction by personnel; (2) systematic cleaning and disinfection of premises and equipment on a strict schedule (with attention to correct disinfectant concentration and contact time), which decreases environmental contamination and the infectious dose; (3) stocking density management and ventilation optimisation, which reduce stress, ammonia irritation of respiratory mucosa, and aerosol concentration – thereby lowering transmission efficiency; (4) all-in/all-out or cohorting strategies with prompt isolation of symptomatic birds identified during regular veterinary inspections, which shortens infectious contact networks; and (5) prevention of contact with wild birds and control of vectors/fomites (vehicles, crates, tools), which limits cross-farm spread. When these control points are implemented together, they act synergistically: reduced introduction risk, reduced within-house amplification, and earlier interruption of outbreaks. This provides a practical explanation for why sanitary measures combined with management improvements and veterinary control produce measurable reductions in infection and mortality, consistent with the integrated effect discussed in the study and supported by related evidence on combined preventive approaches [31-33].

4.5 Significance of integrated prevention and control strategies and implications for local practice

In this context, the development and application of a multi-component vaccination strategy, particularly in high-risk areas, is essential. Vaccination not only limits virus circulation but also reduces disease severity in infected flocks, while its combination with regular sanitary monitoring substantially decreases economic losses by lowering mortality rates and improving overall poultry productivity [34-36].

Prospects for further research on MPVI include a better understanding of the factors that influence the duration of the immune response after vaccination to determine the best timing of revaccination. More research is necessary to develop vaccination programmes adapted to various age groups and housing conditions of poultry. A vital area is the study of the genetic variability of the virus, which will help in the development of new, more effective vaccines that can factor in possible mutations of the virus and its adaptation to varying environmental conditions.

Limitations of the study included possible variation in disinfection methods and sanitation practices between different farms, which may affect the overall outcome. It is vital to further investigate the best timing of vaccination and frequency of sanitation checks to further reduce infection rates.

5. CONCLUSIONS

The findings of the present study confirmed the high efficacy of comprehensive measures for the prevention and control of MPVI at poultry farms in Kazakhstan. Considerable reduction of infection was achieved through vaccination, improved housing conditions, and strict observance of sanitary norms. Vaccination, especially in young poultry, was highly effective: antibody levels among vaccinated poultry were almost three times higher than in unvaccinated poultry. Furthermore, the incidence of disease among vaccinated poultry was reduced by 50%, which significantly reduced mortality and economic losses to poultry farms.

Sanitation measures, such as regular weekly disinfection, also demonstrated their significance. On farms with regular sanitation, infection rates decreased by 20-35%, emphasising the need for strict sanitation to control the spread of infection. An equally significant factor was the reduction in the poultry crowding rate to the recommended level, which helped to reduce the spread of the virus and improve the condition of the poultry.

The use of PCR and ELISA methods for diagnosis enabled early detection of infection and monitoring of the immune defence in poultry after vaccination. PCR, due to its high accuracy, became the key method for early diagnosis, while ELISA was used to monitor antibody levels after vaccination.

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NOMENCLATURE

APV	Avian metapneumovirus
MPVI	Metapneumovirus infection
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
SHS	Swollen head syndrome