

## SEROLOGICAL ASPECTS OF AVIAN METAPNEUMOVIRUS INFECTION IN KAZAKHSTAN

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### Abstract

Avian metapneumovirus (AMPV), formerly known as avian pneumovirus (APV) is epizootic agent of turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) in turkeys and chickens. The infection primarily affects the upper respiratory tract of young birds (broilers), while also decreases egg production of adult hens. Thus, the development of infection in susceptible birds of any age can cause serious economic losses.

The purpose of this study is to test serums from broilers and hens for the presence of antibodies against the avian metapneumovirus. In this series of studies 317 serum samples taken from one 1 day to 75 weeks old birds were tested. Thus, on the basis of serological tests of blood serum and of chicken flocks and broilers, we had a preliminary diagnosis on the presence of avian metapneumovirus infection. Serological studies of unvaccinated against avian metapneumovirus infection bird flocks using the ELISA method showed antibody titers on average at  $22\ 859 \pm 4133$ . Avian metapneumovirus infection in birds was accompanied by a decrease in egg production of chicken flocks by 8.0 - 12.8%.

**Key words:** Rhinotracheitis of turkeys, swollen head syndrome, bird's metapneumovirus, seropositivity, antibodies.

### Introduction

Avian metapneumovirus (AMPV), formerly known as avian pneumovirus (APV) is epizootic agent of turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) in turkeys and chickens. The infection primarily affects the upper respiratory tract of young birds (broilers), while also decreases egg production of adult hens. Thus, the development of infection in susceptible birds of any age can cause serious economic losses.

In recent years, early unknown infectious disease was observed among chicken population of Kazakhstan poultry farms, mainly of respiratory character, later diagnosed (Assanov et al., 2012; Assanov et al., 2012) as an avian metapneumovirus infection of poultry (AMPV). The first signs of rhinotracheitis were observed in 1970 in South Africa (Buys et al., 1980), while on the European continent it was firstly discovered in 1981 in France in turkeys (Buys et al., 1989). Later the disease spread rapidly in the UK, France, Spain, Germany, Italy, the Netherlands, Israel and Asia (Jing et al., 1993).

Causative agent is avian metapneumovirus, an RNA virus of the *Paramyxoviridae* family, Metapneumovirus genus (Pedersen et al., 2000).

Virus genome is presented by the linear not segmented molecule of not infectious RNA and contains 8 genes. There are four subtypes of the metapneumovirus of birds: A, B, C and D. Viruses of subtypes A and B are spread in Europe, Asia, Africa, Southern and Northern America whereas this virus of a subtype C circulates mainly among turkeys in the USA Cook J.K.A., 2000. The metapneumovirus of birds of subtype D has been revealed only once in

France (Bäyon-Auboyer et al., 2000).

The problem of antigenic structure of the metapneumovirus besides the big theoretical value now represents also essential practical interest in the period of mass vaccination against this illness and also at studying of replication of vaccinal and epizootic strains of a virus in an organism and cell cultures and their spread among birds.

According to our observation, clinical symptoms of metapneumovirus infection in chickens are characterized by rhinitis, conjunctivitis, swollen feathers and infraorbitals, while individual chickens have complete complex of symptoms of swollen head syndrome (SHS). Such symptoms are little observed in adult hens, but the infection development is characterized by the reduction of egg production, lower average daily increase and feed conversion ratio deterioration. In addition, immunosuppression caused by pneumovirus makes poultry sensitive to other agents including conditionally pathogenic microflora.

Urgency of the problem lies in the fact that up to now, epizootic peculiarities of avian metapneumovirus infection, diagnosis and serotyping of epizootic virus strains as well as effectiveness of preventive maintenance methods were not studied in Kazakhstan.

The purpose of this study is to test serums from broilers and hens for the presence of antibodies against the avian metapneumovirus. In this series of studies 317 serum samples taken from one day to 75 weeks old birds were tested.

### Materials and Methods

The work was carried out in 2011-2012 in the laboratory of virology and bird illnesses of

Kazakh National Agrarian University, laboratory on prophylaxis of special dangerous illnesses of animals of Republican State Enterprise “Scientific research institute of problems of biological safety” and serological laboratory of Univet Limited partnership.

The following materials were used:

317 samples of chicken blood serum have been sampled. For serological testing a set for detection of antibodies to agent of metapneumovirus infection of birds BioCek, manufactures of firm “Avian Rhinotracheitis Antibody Test Kit” (Holland) has been used. Procedure of test and the analysis of results were made according to recommendations of the manufacturer (Svanova Biotech, Lyon, France).

The positive and negative control of antiserum has been used in each period. Absorption has been read on length of a wave of 650 nanometers on ELX 800 ® ELISA reader (Bio-Chek, Winoski, VT, USA). The relative level of antibodies has been defined by calculation of the sample to positive (S/P) ratio. Serum samples with S/P ratio are more 0.2 (titres more than 396), in re-testing it is considered positive of AMPV. Statistical processing was conducted using the student’s t-test.

Samples from each poultry yard with 18-25 birds were taken randomly. Blood samples were taken regardless of whether there are any signs of respiratory or other clinical disease in the herd. A total of 317 blood samples were taken from 1 day to 75 week old hens. The presence of antibodies against avian metapneumovirus in each serum sample was tested twice using immune-enzymatic analysis, which was able to identify antibodies against A, B and C subtypes of avian metapneumovirus.

For comparison, serological studies were conducted in three different poultry farms and in different age groups.

### Results and Discussion

The results of this study may indicate the possible involvement of avian metapneumovirus in respiratory diseases that are observed in chickens in Kazakhstan. Its prevalence has to be investigated in other parts of the Republic. It meets the information of different authors in other countries. According to Gharaibeh SM et al., (2007) in 100% of cases in Jordan it has been confirmed by ELISA method that birds have positive antibodies to AMPV. In Poland

Table 1

**These serological studies in poultry farms No. 1**

Number of subgroups	Age in days and weeks	Number of samples	Min titer	Max titer	Mean titer	Positive	%	Negative	CV%
1	1 day	10	1	623	253	-	-	10	79
2	90 days	10	106	497	254	-	-	10	40
3	20 weeks	10	437	5278	1326	3	30	7	116
4	26 weeks	15	596	3384	1393	4	26	11	64
5	32 weeks	13	798	8901	4377	11	84	2	55
6	61 weeks	14	642	11229	3933	12	85	2	66
7	64 weeks	14	4825	20086	12173	14	100	-	35

CV- coefficient of variation.

Table 2

**These serological studies in poultry farms No. 2**

Number of subgroups	Age in days and weeks	Number of samples	Min titer	Max titer	Mean titer	Positive	%	Negative	CV%
1	2 day	10	1	623	249	-	-	10	106
2	90 day	10	228	1477	650	-	-	10	66
3	20 weeks	10	480	5517	1226	1	10	9	124
4	27 weeks	15	1841	7997	4133	15	100	-	46
5	32 weeks	13	1454	12924	5671	13	100	-	48
6	45 weeks	11	4093	30126	15303	11	100	-	57
7	56 weeks	10	1864	18517	9728	10	100	-	62
8	60 weeks	20	3702	24778	11280	20	100	-	52
9	68 weeks	18	4033	17567	9896	18	100	-	42

CV- coefficient of variation.

Table 3

**These serological studies in poultry farms No. 3**

Number of subgroups	Age in days and weeks	Number of samples	Min titer	Max titer	Mean titer	Positive	%	Negative	CV%
1	4 day	10	1	1233	5094	8	80	2	72
2	95 day	15	1	4063	849	3	-	12	136
3	27 weeks	15	2182	20464	11472	15	100	-	60
4	39 weeks	10	9159	18517	16023	10	100	-	24
5	42 weeks	10	13421	18649	16400	10	100	-	11
6	44 weeks	18	8143	21278	16844	18	100	-	23
7	63 weeks	22	9874	27848	22859	18	100	-	24
8	75 weeks	24	520	23477	11771	17	-	7	73

CV- coefficient of variation.

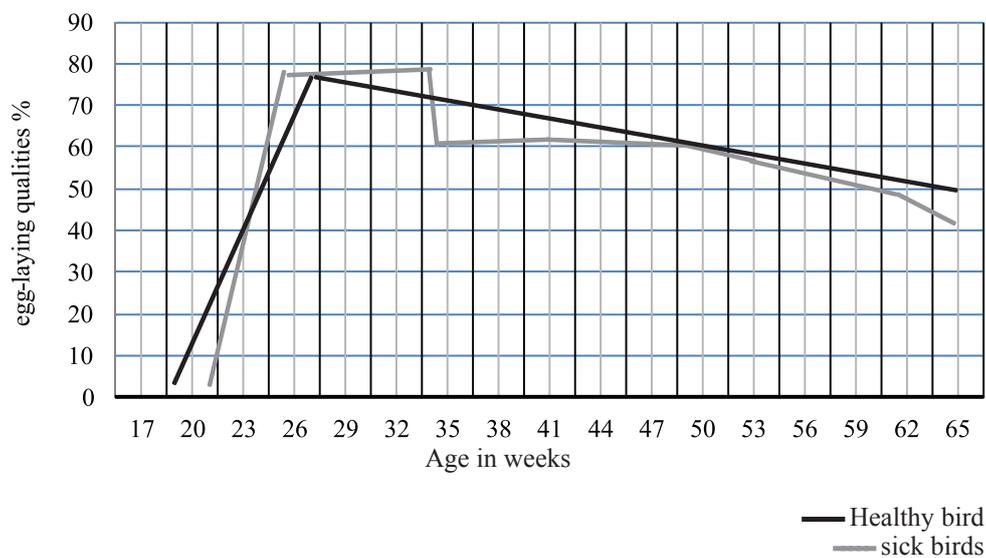


Figure 1. Chicken egg production curve unfavorable for AMPV.

according to Minta et al., (1995) 56.4% of birds have positive results.

As can be seen from Table 1 in young birds up to 90 days of age, specific antibody titers were minor, but in the poultry farm number 3 (Table 3) in 4-day old chicks 80% of the samples were positive.

Studies of blood sera of birds poultry number 1 showed that for 32-week old chicks antibodies were detected in 84% of individuals flocks, and at 64 weeks AMPV antibodies were found in 100% of cases.

The highest antibody titers were detected in the study of sera from birds in poultry farm No. 2, where the individuals of 27 weeks of age, 100% of the positive results.

Similar results were obtained in a study of sera from chickens in poultry farm No. 3. The average antibody titer to metapevmovirusny infection of birds was 22859, with 45% of birds antibody titers being

very high of more than 10,000. The maximum titre was 27848 at 63 weeks of chickens.

During these studies the egg production decrease was observed in adult hens. Egg production of 32 weeks old laying hens declined by 63.6%, which is below the accepted standard of 12.8%. When these hens reached 50 weeks, their egg production was at the standard level, but at the end of the observation period 60 weeks figure was lower than the norm by 80% Figure 1.

Birds in these poultry farms have not been vaccinated against AMPV, while the presence of antibodies in the serum of birds indicates the circulation of epizootic virus in bird flocks.

Thus, on the basis of serological tests of blood serum and of chicken flocks and broilers, we had a preliminary diagnosis on the presence avian metapneumovirus infection.

### Conclusions

Serological studies of unvaccinated against avian metapneumovirus infection chickens flocks using the ELISA method showed antibody titers on average

at  $22\ 859 \pm 4133$ . Avian metapneumovirus infection in birds was accompanied by a decrease in egg production of chicken flocks by 8.0 - 12.8%.

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