A New Poultry Disease in Kazakhstan

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Abstract. The epizootological and serological monitoring of avian metapneumovirus infection has been carried out in poultry farms of Kazakhstan among different species and ages of bird. Clinical manifestations were more common in turkeys. A higher rate of avian metapneumovirus seroprevalence was observes among adult chickens aged between 27 weeks and 56 weeks, and turkeys 38 days of age.
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Introduction

In recent years, a fairly widespread of respiratory disease symptoms has been observed on commercial poultry flocks (egg layers, broilers and turkeys) in number of poultry farms of the central and north-eastern regions of Kazakhstan. We later diagnosed it as avian metapneumovirus infection (aMPV) [1, 2]. Currently aMPV is widespread on poultry farms around the world [3, 4, 5, 6, 7, 8].

The signs of illness with rhinotracheitis was first reported in turkeys aged between 3 and 4 weeks in South Africa in 1978, later named as turkey rhinotracheitis (tukey rinotracheitis, TRT) [9].

At the same period in South Africa it was observed an unknown respiratory disease in other species of bird, chickens, which was accompanied by respiratory symptoms and swollen head area. Researchers call it "Swollen head syndrome" (Swollen head syndrome, SHS) and suggested that it is caused by a mixed infection of coronavirus and E.coli [10]. Although, it was later shown that TRT virus involved in the pathological process of SHS formation in chickens, however scientists still have different views on pathogenesis of this disease. A group of scientists has been found on the basis of gene-molecular study of TRT and SHS pathogens that its caused by a single virus. It is been referred to the Avian Metapneumovirus (aMPV) genus. The scientists suggested that it would be appropriate to name the disease as avian metapneumovirus infection (aMPV) [11].

The virus genome is represented by a non-segmented, single-stranded, negative-sense RNA and contains 8 genes. There are four subgroups of avian metapneumovirus: A, B, C and D. Virus Subgroups A and B are common in Europe, Asia, Africa, South and North America, whereas subgroup C occurs mainly in turkeys in the United States. Subgroup D has been detected only once in France [12].

Urgency of the problem lies in the fact that to date the epizootological features of avian metapneumovirus infection, diagnosis and serotyping of its strains, the effectiveness of specific preventive measures against the virus have not been studied in Kazakhstan,.

The purpose of this study is to provide the research of serological parameters and epizootic monitoring of infected with aMPV chickens and turkeys of different ages.

MATERIALS AND METHODS

Research has been done in Laboratory of Virology and Avian Diseases of the Kazakh National Agrarian University, Laboratory of Extremely Dangerous Animal Diseases Prevention of Republican State Enterprise "Research Institute for Biological Safety Problems", and in the Serological Laboratory of «UniVet».

The presence of antibodies against avian metapneumovirus was detected by using a commercial kit «BioChek», made by «Avian Rhinotracheitis Antibody Test Kit» firm. The kit is designed for the quantitative determination of antibodies against aMPV in bird's serum. Plates were pre-coated with inactivated aMPV antigen.

Serum samples were diluted and added to the wells where any presented antibodies against aMPV were binded and formed an antigen-antibody complex. Then the wells have been washed from non-specific antibodies and other serum proteins. Thereafter, lgG is added to the wells with chicken antibodies labeled with alkaline phosphatase. They, in turn, bind to antibodies against aMPV which are in complex with the antigen. Following washing will remove unreacted conjugate. The substrate will be

added in the form of a chromogen. The appearance of yellow color indicates the presence of antibodies against aMPV, and the intensity of the color is directly proportional to number of antibodies in the sample.

The test procedure and analysis of results were performed as recommended by manufacturer. Each run was controlled by positive and negative antisera included with the kit. The absorbance of the reaction mixture was read at a wave length of 650 nm on ELX 800®ELISA Reader (Bio-Tek, Winoski, VT, USA). The relative level of antibody in the unknown was determined by calculating the sample to positive ratio. This calculation is expressed as S/P ratio (sample/positive ratio). According to manufacturer's program the titer was calibrated so that a value of S/P 0,499 or less corresponding to the titer of 1655 or less is considered negative, and the value of S/P 0,500 or more corresponding to the titer of 1656 or more is considered positive[13]. The presence of antibodies against aMPV was tested twice. The obtained results were statistically processed by methods of variation statistics using Microsoft Excel software.

A total of 341 blood serum samples were randomly collected from 15-25 birds of each aviary regardless of the presence of clinical signs of respiratory or other diseases. Age of birds ranged from one day to 75 weeks.

RESULTS.

An epizootological monitoring was conducted in poultry farms located at East, South and Akmola regions of Kazakhstan.

Biological samples were collected from four aviary of egg production farm (birds 16, 24, 35, 47 weeks of age) located at the East Kazakhstan region (Table 1).

Table 1. The Results of aMPV Study in the East Kazakhstan Region.

№ of Poultry House	Total Amount of Birds	Age of Birds in Days and Weeks	Amount of Samples	Positive Results	
				By Clinical Data	By Serology Data
1	25 000	24	22	1	4
2	19 000	16	20	2	2
4	23 000	35	25	-	13
7	23 000	47	21	-	15
Total 4	110 000		106	3	44

The results presented in the Table 1 show that out of 106 serum samples obtained from chickens 44 were serologically positive to aMPV. In three cases the birds had typical for aMPV clinical signs such as coughing, sneezing, conjunctivitis.

In Akmola region the research was conducted at a broiler farm. The samples were collected from 55 birds aged of 1 day, 32 weeks and 61 weeks in three chicken houses. The results are shown in Table 2.

Table 2. The Results of aMPV Study in Akmola Region.

№ of Poultry House	Total Amount of Birds	Age of Birds in Days and Weeks	Amount of Samples	Positive Results	
				By Clinical Data	By Serology Data
7	24 000	1 day	12	-	-
8	19 000	32 weeks	23	1	11
10	22 000	61 weeks	20	-	16
Total 3	65 000		55	1	27

The data of Table 2 show that serological test of serum samples obtained from 1 day old chicks were negative, and it is consistent with the absence of maternal antibodies since chicken of this poultry farm had not been vaccinated against avian metapneumovirus infection. Out of 43 serum samples obtained from birds aged 32 weeks and 61 weeks 27 samples were positive to serological test.

In South Kazakhstan region the study of aMPV was conducted at turkey farm. Sampling of biological material is carried out from birds aged of 1 day, 38 day, and 80 day groups. The results are shown in Table 3.

Table 3. The Results of aMPV Study in South Kazakhstan Region.

№ of Poultry House	Total Amount of Birds	Age of Birds in Days and Weeks	Amount of Samples	Positive Results	
				By Clinical Data	By Serology Data
3	22 000	1 day	12	-	-
4	20 000	38 days	18	3	2
6	24 000	80 days	22	5	14
Total 3	66 000		52	8	16

As shown on Table 3 there are 8 turkeys aged of 38 and 80 days had clinical signs of avian metapneumovirus infection. Herewith the disease showed typical respiratory tracts signs and associated with depression, coughing and sneezing. Also it is been reported wheezing, nasal expiration, conjunctivitis, swollen infraorbital sinuses.

The results in Table 3 indicated that out of 52 serum samples 16 were positive in serological test.

As a result of our monitoring, the presence of avian metapneumovirus infection was detected in poultry farms located at three areas of the Republic of Kazakhstan.

Serological monitoring is one of the most informative methods of epizootic monitoring. The serum samples were collected from birds of different age groups in two poultry farms. Results of immunofluorescence analysis (IFA) for the presence of specific antibodies against aMPV are shown in Table 4.

Table 4. The Serological Study Data from Egg Production Farms. CV - coefficient of variation.

№ of Poultry House	Age of Birds in Days	Amount of Samples	Min Titer	Max Titer	Average Titer	Positive	Percentage	Negative	CV %
1	1 day	10	1	623	253	-	-	10	79
2	2 days	10	1	623	249	-	-	10	106
3	4 days	10	1	1233	5094	8	80	2	72
4	90 days	10	106	497	254	-	-	10	40
5	95 days	15	1	4063	849	3	20	12	136
6	20 weeks	10	437	5278	1326	3	30	7	116
7	26 weeks	15	596	3384	1393	4	26	11	64
8	27 weeks	15	1841	7997	4133	15	100	-	46
9	32 weeks	13	798	8901	4377	11	84	2	55
10	39 weeks	10	9159	18517	16023	10	100	-	24
11	42 weeks	10	13421	18649	16400	10	100	-	11
12	44 weeks	18	8143	21278	16844	18	100	-	23
13	45 weeks	11	4093	30126	15303	11	100	-	57
14	56 weeks	10	1864	18517	9728	10	100	-	62
15	60 weeks	20	3702	24778	11280	20	100	-	52
16	61 weeks	14	642	11229	3933	12	85	2	66
17	63 weeks	22	9874	27848	22859	18	100	-	24
18	64 weeks	14	4825	20086	12173	14	100	-	35
19	68 weeks	18	4033	17567	9896	18	100	-	42
20	75 weeks	24	520	23477	11771	17	70	7	73

A total of 279 serum samples from chickens aged between 1 day and 75 weeks were collected from 20 aviaries (Table 4). Of 279 samples 202 tested positively (titer of 1655 and above) by ELISA kit accounting to 72.4 % of total amount of samples. The samples from young chickens mainly had low titers and often characterized with amnesic nature of the disease. Only one aviary (№3) with 4 days old chicks had 8 out of 10 samples tested positively with the mean value for the antibody titer of 5094.

All serum samples from birds aged between 27 weeks and 68 weeks were seropositive by analysis (100%), except serum from chicks of 32 and 61 weeks of age. Accordingly, the coefficient of variation (CV) was remained at the range of 11-66%. The maximum titer of 27,848 was observed in 63-week old birds.

The next level of research included the serological test of serum from turkeys of 1 day, 38 days and 80 days of age (Table 5).

Table 5. Results of Serological Investigations at Turkey Farm. CV- Coefficient of Variation.

№ of Poultry House	Total Amount of Birds	Amount of Samples	Min Titer	Max Titer	Average Titer	Positiv	%	Negativ	CV %
1	01 days	25	1	123	10	-	-	25	311
2	38 days	15	14785	36123	28527	15	100	-	23
3	80 days	22	573	7838	2758	14	63,6	8	69

The results presented in Table 5 show that all serum samples from turkeys aged 38 days and 80 days were positive in the test with maximal titer of 36123. Accordingly, the coefficient of variation (CV) was remained within the 23-69 percent.

DISCUSSION

As a result of epizootic monitoring the presence of avian metapneumovirus infection was detected at poultry farms located in three regions of the Republic of Kazakhstan. It was observed that birds at any age are susceptible to aMPV, but the disease showed more severe signs in birds younger than 1 Year old.

The clinical manifestation of the disease was reported more frequently in turkeys (50% of serologically positive). The clinical sings included depression, drowsiness, swelling of the head, rostral area and infraorbital sinuses, heavy breathing, rhinitis, and signs were accompanied by nasal discharge and coughing.

The 90 days young chickens showed low titer of antibodies against aMPV, given that maternal antibodies were absent because the birds were not immunized against aMPV. The proportion of positive tests were increased in serum from 20 week and older chick, in particular a 100% of positive seroconversion was observed increase in 27 week birds. Our finding from this respect differs from the results obtained by Pakistani scientists [14]. They reported that only 18.79% of samples tested positive, however it can be explained by use of different diagnostic kit [15]. The study of South Korean scientists is consistent with our finding that the proportion of seropositive serum increases with age of birds [16]. According to their data, if only 4.5% of samples from 100 days birds reacted positively, then the proportion of positive tested birds aged between 201 days and 300 days was 97.6%.

Danko et al. indicated that the positive correlation between age of birds and antibodies titer increase proves the persistence of the virus in birds [17]. The data of our research showed that in the majority of cases the avian metapneumovirus infection was asymptomatic by shape, and infect a 100% of susceptibility flocks within short period of time. The presence of other pathogens and violation of poultry breeding technology contribute to complication of the disease, leading to a decrease of egg production and other economic factors.

The results of our study indicate for rapid spread of avian metapneumovirus infection in poultry farms of the republic. Serological tests demonstrated the ability of the virus to long circulation in flocks of poultry farms. Further study of aMPV isolates circulating on the territory of the Republic of Kazakhstan, its forms and methods of transmission and persistence, the epizootic features of the disease in different species and ages of birds remain as an important direction of further research.

CONCLUSIONS

1. Serological tests of bird's serum with a positive results are sufficient base for initial diagnosis of aMPV birds.
2. At poultry farms it was serologically positive 20% of chickens up to 90 days of age and 100% of adult chickens aged between 27 weeks and older, and 100% of turkeys.

3. The study showed that clinical signs of aMPV were mostly observed among turkeys aged up to 1 year, and include 15.3% of the total number of positive responders in serological studies.
4. The study revealed a fairly widespread of avian metapneumovirus infection in poultry farms of the Republic of Kazakhstan, as well as the ability of the virus for long-term persistence in poultry.

GRATITUDE

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