Detection Of Avian Paramyxovirus-3 Antibodies In Backyard And Commercial Chicken Farms In Saudi Arabia Using Enzyme-Linked – Immunosorbent Assay And Hemagglutination Inhibition Tests

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ABSTRACT

Serological survey was conducted to detect avian paramyxovirus type-3 (PMV-3) antibodies in commercial and backyard chicken flocks using Enzyme-linked immunosorbent assay (ELISA) and hemagglutination inhibition (HI). Two hundred and twelve chicken serum samples were collected from several commercial farms and back yard bird-flocks in Saudi Arabia. The serum samples were collected from birds with different ages (from one-day old to 62 weeks). Results from samples collected from back yard birds showed that 35.70% (20 out of 56) were positive using the HI test and 44.46% (25 out of 56) were positive using the ELISA respectively. Antibodies to PMV-3 were also detected in 41.02% (64 out of 156) and 85 54.48% (85 out of 156) of serum samples collected from commercial farms using the HI and the ELISA tests respectively.

From this study it could be concluded that antibodies to PMV-3 are widespread among backyard and commercial chicken farms in Saudi Arabia.

INTRODUCTION

Avian viruses that belong to the family paramyxoviridae are the cause of respiratory and reproductive diseases in several avian species. The family paramyxoviridae has been reclassified into two subfamilies, the paramyxovirinae and pneumovirinae.

The subfamily pneumovirinae contains two genera, pneumovirus and metapneumovirus. The subfamily paramyxovirinae contains three genera, Respirovirus, Rubulavirus, and Morbillivirus. The avian paramyxoviruses belong to the genus Rubulavirus *(1)*. The new classification is based on morphologic criteria, the organization of the genome, the biologic activities of the proteins, and the sequence relationship of the encoded proteins *(2)*.

It has been reported that there are some cross relationships between avian paramyxoviruses serotypes *(3)*. Minor cross relationships between PMV-1, 3, 4, 7, 8, and 9 and between PMV-2 and 6 was found *(4)*. However, the relationship between PMV-1 and PMV-3 appears to be closer and more important than the others *(3,5)*. In addition, chickens infected with PMV-3 viruses may be protected against challenge with virulent NDV strain *(6)*. Hemagglutination inhibition (HI) antibodies to PMV-3 viruses may be detected in turkeys and chickens showing high vaccine-induced titers to NDV, and ND-vaccinated birds infected with PMV-3 viruses show a rise in HI titer to both viruses *(7,8)*.

Antibodies to PMV-3 were consequently detected in several European countries *(3)*. This study was conducted to detect the possibility of the presence of PMV-3 antibodies among backyard and commercial chickens in Saudia Arabia using HI and ELISA tests.

MATERIAL AND METHODS

Samples

Two hundred and twelve serum samples were collected from different commercial chicken farms with different ages ranging from one day–62 weeks of age and from backyard chickens. Serum samples kept in refrigerator until used.

Virus

The PMV-3 was obtained from Y. M. Saif at Food Animal Health Research Program (FAHRP) Wooster, Ohio. The virus was

passaged three times in specific pathogen free (SPF) chicken embryos, which were inoculated via the allantoic route. The *in vetro* passage history of the PMVs prior to use in this study is not known. Virus titrations were expressed as the mean embryo infective dose (EID_{50}) and calculated by the method previous described ***(9)***.

This virus was used for preparation of hemagglutination and ELISA antigens and for preparation of hyperimmune serum.

Preparation of hyperimmune serum

Thirty week-old chickens were used to produce antiserum to PMV-3. Purified viruses were inactivated using 0.1% β-propiolactone for 2 hours at 37 °C. chickens were inoculated three times at two weeks intervals. In the first inoculum, 0.5 ml of the inactivated purified viruses was mixed with an equal volume of Freund's complete adjuvant. In the second and third inocula, 0.5 ml inactivated purified viruses was mixed with an equal volume of Freund's incomplete adjuvant. Ten days after the last inoculation, the chickens were bled and the sera were collected and heat inactivated at 56° C for 30 minutes was carried out.

Hemagglutination (HA) and Hemagglutination inhibition tests

The HA and HI tests were used for identification and detection of the avian PMV-3 as described earlier ***(10)***. Allantoic fluids, from chicken embryos inoculated with avian PMV-3 were used. Chicken red blood cells (RBCs) were used for both tests. The diluted-serum constant-virus method was used for the HI test with 4 HA units of virus for each dilution. Positive and negative controls were used with each test.

Antigen preparation for ELISA test

The preparation of avian PMV-3 antigen for ELISA was similar to that previously described ***(11)***. Negative controls of allantoic fluids from SPF chicken embryos were treated the same way. The ELISA procedures were performed as described earlier ***(12)***. For each ELISA plate positive and negative controls were set for each test. The ELISA cutoff point for the PMV-3 is the mean of the negative controls plus three stander standard a deviations (SD).

Statistical analysis was carried out as previously described ***(13)***.

RESULTS AND DISCUSSION

There are several serological tests that could be used to diagnose the paramyxoviruses infection in birds. These tests include, agar gel precipitin, single radial immunodiffusion, single radial hemolysis, virus neutralization (VN) test in chick embryos, HA and HI tests, and enzyme linked immunosorbent assays (ELISA) ***(3)***.

Good correlation has been reported between ELISA and the HI tests which are the most commonly used serologic tests to diagnose these infections.

In this study, the results of the ELISA and HI tests are depicated in table 1.

The total positive samples that tested were 84 / 212 (39.62%) and 110 / 212 (51.80%) using HI and ELISA, respectively. Antibodies to PMV-3 were detected in 20 out of 56 samples examined (35.71%) and 25 out of 56 samples examined (44.64%) in backyards birds using HI and ELISA, respectively. Twenty samples of one day old that were test were all negative using HI and ELISA. The highest positive ratio 60.40% using HI test and 72.90% using ELISA test were observed among broiler farms 19- 35 days old compared with 43.24% and 62.16% using HI and ELISA test respectively in rearing farms (11- 18 weeks of age) and 37.25% and 52.94 % in layer farms.

In earlier studied it was found that no antigenic relationships were detected between APM-2, ND, APM-3 and APM-7 ***(14)***. Therefore the antibodies detected in the examined birds will be attributed to infection with PMV-3 virus among these farms.

In conclusion, the presence of antibody against PVM-3 in flock yard, broilers and layer farms in Saudia Arabia as indicated by

positive ELISA, and HI test could be attributed to infection with PMV-3 virus that result in appearance of mild respiratory signs. However, Virus isolation is important to diagnose the infection as well as characterization of the infecting strain *(3)*.

Table 1: Serological survey against PMV-3 virus in serum samples collected from backyard and commercial poultry farms in Saudia Arabia using HI and ELISA test.

Birds	Age	Number of samples	Positive samples	
			HI	ELISA
Back yard	Weeks- months	56	20/56 (35.71%)	25/56 (44.64%)
Commercial	1- 5 days	20	0	0
	19- 35 days	48	29/48 (60.40%)	35/48 (72.90%)
	11- 18 weeks	37	16/37 (43.24%)	23/37 (62.16%)
	25- 62 weeks	51	19/51 (37.25%)	27/51 (52.94%)
Total		212	84/212 (39.62%)	110/212 (51.80%)

ELISA: Enzyme-linked immunosorbent assay
HI : Hemagglutination inhibition test

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