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# SEROLOGICAL EVIDENCE OF AVIAN PARAMYXOVIRUS-2 INFECTION IN BACKYARD AND COMMERCIAL POULTRY BIRDS IN SAUDI ARABIA

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## ABSTRACT

Serological survey was conducted to detect avian paramyxovirus serotype-2 (APMV-2) antibodies in commercial and backyard bird flocks, using Enzyme-linked immunosorbent assay (ELISA) and haemagglutination inhibition (HI) test. Sera were collected from 212 commercial and 56 backyard birds. Age of commercial birds ranged from one-day old to 62 weeks. In the backyard birds, seroprevalence of APVM-2 was 71.42 and 78.57%, whereas this seroprevalence was 52.35 and 60.84% using the HI test and the ELISA, respectively in commercial poultry birds. No antibodies against APMV-2 were detected in 1-5 days old chicks with either test. The HI test showed the highest positive samples (P<0.05) of APMV-2 in 19-35 days age group (58.33%), followed by age group 11-18 weeks (51.35%) and 25-62 weeks (47.05%). Similarly, ELISA also showed the highest positive samples of APMV-2 (68.75%) in 19-35 days age group (P<0.05), followed by age group 11-18 weeks (62.16%) and 25-62 weeks (56.86%). In conclusion, this study indicated the presence of antibodies to APMV-2 among backyard and commercial poultry birds in Saudi Arabia.

**Key words**: Avian paramyxovirus, serological survey, ELISA, HI test.

## INTRODUCTION

Avian paramyxoviruses (APMVs) comprise nine serotypes (Alexander *et al*., 1983). APMV-1 is the cause of Newcastle disease (ND) which is characterized by respiratory problems and drop in egg production, while APMV-2 causes mild respiratory problems and drop in egg production (Alexander, 1997). The other APMVs cause respiratory disease in a variety of birds (O'Loan *et al*., 1989).

The first isolation of APMV-2 was recorded in Yucaipa, California, USA during 1956 (Bankowski *et al.,* 1960). Afterward, antibodies to APMV-2 were detected around the world (Alexander *et al*., 1983). In spite of vaccination against the most important respiratory diseases such as ND and infectious bronchitis (IB) in most commercial chicken flocks, respiratory problems appear in these flocks. Other respiratory viruses that do not have commercial vaccines available may cause these symptoms. The APMV-2 could be one of these viruses that cause such symptoms. The present paper describes the prevalence of APVM-2 among the backyard and commercial poultry farms in Saudi Arabia.

## MATERIALS AND METHODS

### Collection of samples

Blood samples were collected from 212 birds belonging to commercial chicken farms and 56 backyard birds from Qassium area, Saudi Arabia during the period from 2007 to 2008, serum was extracted and stored at -20ºC till further analysis. The age of commercial chicken ranged from one day to 62 weeks, and they were divided into four groups viz. 1-5 days, 19-35 days, 11-18 weeks and 25-62 weeks. The age of backyard birds was not known.

### Preparation of hyperimmune serum

The APMV-2 (Yucaipa, 59) was obtained from Food Animal Health Research Program (FAHRP), Wooster, Ohio, USA. The virus was passaged three times in specific pathogen free (SPF) chicken embryos inoculated via the allantoic route. The *in vitro* passage history of the APMVs prior to use in this study was not known. Virus titrations were expressed as the mean embryo infective dose ($EID_{50}$) and calculated by the method described by Reed and Muench (1938). This virus was used for the preparation of haemagglutination and ELISA antigens and for hyperimmune serum.

Chickens, 30 week-old, were used to produce antisera to APMV-2. Purified viruses were inactivated using 0.1% β-propiolactone for 2 hours at 37ºC. Chickens were inoculated with APMV-2 virus three times at two weeks intervals. In the first inoculum, 0.5 ml of the inactivated purified virus was mixed with an equal volume of Freund's complete adjuvant. In the second and third inocula, 0.5 ml inactivated purified virus was mixed with an equal volume of Freund's incomplete adjuvant. Ten days after the last inoculation, the chickens were bled, the serum was harvested and heat inactivated at 56ºC for 30 minutes.

**Haemagglutination (HA) and Haemagglutination inhibition (HI) tests**

The HA and HI tests were used for the identification of the APMV-2 (Beard, 1989). Briefly, allantoic fluids from chicken embryos inoculated with APMV-2 were used for HA and HI tests. The diluted-serum constant-virus method was used for the HI test with 4 HA units of virus for each dilution (Alkhalaf and Saif, 2003). Positive and negative controls were kept with each test.

The HA and HI tests were performed following the procedure described earlier (Thayer and Beard, 1998). Briefly, in HA test, the allantoic fluid harvested 48-72 hours after egg inoculation with APMV-2 virus was collected. After making two fold dilutions in saline, a 0.5% suspension of chicken erythrocyte was added. A similar procedure was performed using allantoic fluid from an egg that was not injected APMV-2. After HA activity observation in the fluid from injected eggs, HI test was performed in microtiter plate using prepared APMV-2 hyperimmune serum and APMV-2 negative chicken serum to determine if APMV-2 was the cause of agglutination.

In HI test, the allantoic fluid containing APMV-2 virus was used that was previously used as HA antigen. The amount of antigen used in each well test was 8 HA unit. A 0.5% suspension of chicken RBCs was used and prepared by adding 0.5 ml of packed washed RBCs cells to 100 ml of PBS at pH 7.0-7.2. The tested serum samples were heated at 56°C for 30 minutes in a water bath to inactivate complements. Atypical β procedure (Diluted-Serum-Constant-virus) was performed in 96 well round bottomed microtiter plates. After making serial dilutions of the tested serum, antigen was added, incubated and 0.5% chicken erythrocyte suspension was added. The plates were left at room temperature until the known HI- positive wells exhibited a tight, well-circumscribed button of unagglutinated, sedimented erythrocytes. HI titer was recorded as the reciprocal of the highest dilution of serum at which there was complete inhibition of haemagglutination.

**Antigen preparation for ELISA**

The APMV-2 antigen for ELISA was prepared as described earlier (Chettle and Wyeth, 1988). Negative controls of allantoic fluids from SPF chicken embryos were treated the same way. The ELISA was performed as described by O'Loan *et al.* (1989). For each ELISA plate, positive and negative controls were set for each test. The ELISA cutoff point for the APMV-2 was defined as the mean of the negative controls plus three standard deviations.

**Data analysis**

Data thus collected were analysed by Chi square test to know: i) the statistical difference of APMV-2 prevalence in various age groups in commercial poultry and ii) the difference of APMV-2 prevalence between commercial and backyard poultry.

## RESULTS AND DISCUSSION

This study was conducted to survey the presence of antibodies against APMV-2 virus in poultry farms in Saudi Arabia using ELISA and HI tests. The overall frequencies of positive samples were 52.35 and 60.84% as determined by using HI and ELISA, respectively in commercial poultry birds (Table 1).

**Table 1: Serological survey of APMV-2 virus in commercial poultry birds in Saudi Arabia using HI and ELISA tests**

| Age | Number of samples | Number (%) of positive samples | |
|---|---|---|---|
| | | HI | ELISA |
| 1-5 days | 20 | 0 | 0 |
| 19-35 days | 48 | 28 (58.33) | 33 (68.75) |
| 11-18 weeks | 37 | 19 (51.35) | 23 (62.16) |
| 25-62 weeks | 51 | 24 (47.05) | 29 (56.86) |
| Total | 212 | 111 (52.35) | 129 (60.84) |
| Chi square value | | 10.399 | 12.240 |
| P value | | 0.015 | 0.007 |

Overall, HI and ELISA showed 52.35 and 61.84% positive cases of APMV-2 infection in commercial poultry birds in the present study (Table 1). Statistical analysis of both HI (P=0.015) and ELISA (P=0.007) tests in commercial poultry birds showed significant difference in prevalence of APMV-2 between birds of various age groups. No antibodies against APMV-2 were detected in 1-5 days old chicks with either test. The HI test showed the highest positive samples of APMV-2 in 19-35 days age group (58.33%), followed by age group 11-18 weeks (51.35%) and 25-62 weeks (47.05%). Similarly, ELISA showed the highest positive samples of APMV-2 (68.75%) in 19-35 days age group, followed by age group 11-18 weeks (62.16%) and 25-62 weeks (56.86%). Age groups excluding 1-5 days showed non-significant difference in the positive cases of APVM-2 in the either test compared.

Antibodies against APMV-2 were detected in 71.42 and 78.57% backyard poultry birds using HI and ELISA test, respectively. Non significant difference was found while comparing these HI and ELISA tests in each category of poultry (Fig. 1).

Seroprevalence of APMV-2 has been reported as 42.9 and 15% in China (Zhang *et al*., 2007) and USA (Warke *et al*., 2008), respectively. Variation in the seroprevalence could be due to differences in the age or breed of the birds used in different studies. Moreover, restricted feeding may also result in low immune response against Newcastle disease (Mahmood *et al*.,

2007). Cross antigenic relationships between APM-2 and ND, APM-3 and APM-7 were studied earlier and no antigenic relationships were detected between these viruses (Alkhalaf and Saif, 2003). However, there were low cross reactions among the avian PMVs using the HI test (Alexander *et al.,* 1983; Box *et al*., 1988). Therefore, the presence of antibodies detected in the examined birds could be attributed to infection with APMV-2 virus.

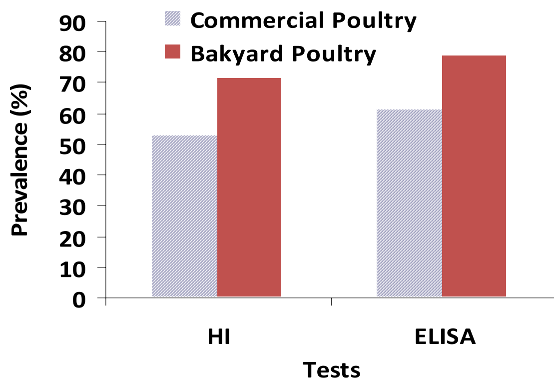


**Fig. 1: Comparison of HI and ELISA tests used for serological survey of APMV-2 virus in commercial and backyard poultry.**

The APMV-2 causes moderate respiratory disease and decline in egg production in mature birds. Several reports have suggested that viruses belonging to the same APMV-2 serotype are present around the world (Alexander, 1986) and serological surveys of poultry in the United States showed that the APMV-2 was more frequently infecting turkeys than chickens (Bradshaw and Jensen, 1979). The respiratory signs like increased respiration, respiratory distress, gasping, coughing, etc. were persisting in the poultry birds of the present study, although they had been vaccinated against the most important respiratory diseases. The cause of these symptoms might be pathogens against which vaccination has not been done.

In conclusion, the presence of antibodies against APVM-2 in backyard and commercial poultry birds in Saudia Arabia, as indicated by positive ELISA and HI test, could be attributed to infection with APMV-2 virus that resulted in appearance of mild respiratory signs.

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