# SEROLOGICAL SURVEY ON AVIAN PNEUMOVIRUS INFECTION IN COMMERCIAL POULTRY FARMS IN SAUDI ARABIA

AN Alkhalaf

Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P. O. Box 1482 Buraydah Saudi Arabia. analkhalaf@yahoo.com

## Summary

The avian metapneumovirus (AMPV), previously called Avian pneumovirus (APV), is the etiologic agent of turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) in turkeys and chickens, respectively. It causes respiratory diseases in young birds and a drop in egg production in breeder flocks. These clinical signs have been observed in different countries and found to be similar, but different names were given to the disease, which reflects the complexity of the etiology when associated with other pathogens. Serological tests such as enzyme-linked immunosorbent assay (ELISA) and virus neutralization test (VN) test are the most commonly used methods to diagnose the APV infection. Therefore, this study was conducted to detect the presence of APV antibodies in commercial poultry farms using ELISA and VN in Saudi Arabia. Eighty seven chicken serum samples were collected from several commercial poultry farms in Al-Qassium area, Saudi Arabia in a period from 2007 - 2008. The serum samples were collected from birds of various ages (from one-day old to 62 weeks of age). Antibodies to APV were detected in 50% (8 out of 16) in both ELISA and VN in farms 11 - 18 weeks of age only. The total positive samples were 8/87 (9.2%) of all examined samples. In conclusion, this study indicates the presence of antibodies to APV among 11 - 18 weeks old commercial poultry farms in Saudi Arabia.

**Key Words:** Avian pneumovirus, ELISA, Virus neutralization test, Chicken.

## Resumen

El metapneumovirus aviar (AMPV), anteriormente llamado pneumovirus aviar (APV), es el agente etiológico de la rinotraqueitis del pavo (TRT) y del síndrome de cabeza hinchada (SHS) en pavos y pollos, respectivamente. Este virus causa enfermedades respiratorias en aves jóvenes y una caída en la producción de huevos en planteles de reproductoras. Estos signos clínicos han sido observados en diferentes países y se le ha dado diferentes nombres a esta enfermedad, lo que refleja la complejidad de la etiología cuando se asocia con otros agentes patógenos. Pruebas serológicas como la prueba por inmunoabsorción ligado a enzimas (ELISA) y la prueba de virus neutralización (VN) son los métodos comúnmente utilizados para diagnosticar la infección por APV. Por lo tanto, se realizó un estudio para detectar la presencia de anticuerpos de APV en granjas avícola comerciales utilizando ELISA y VN en Arabia Saudita. Se tomaron ochenta y siete muestras de suero de aves de diferentes edades (desde un día a 62 semanas de la edad), provenientes de varias granjas avícolas comerciales en el área de Al-Qassium, Arabia Saudita entre 2007 y 2008. Sólo se detectaron anticuerpos contra APV en el 50% de las muestras (8 de 16), tanto por ELISA como por VN, en granjas con edades de 11 - 18 semanas. Las muestras positivas fueron 8/87 (9,2%) de todas las muestras revisadas. En conclusión, este estudio indica la presencia de anticuerpos contra APV entre 11 y 18 semanas de vida en granjas avícola comerciales de Arabia Saudita.

**Palabras Clave:** Pneumovirus aviar, ELISA, Virus neutralización, Pollos.

## Introduction

Avian pneumovirus (APV) is the causative agent of turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) in turkeys and chickens, respectively (Buys *et al.*, 1989). The APV belongs to the family paramyxoviridae which has two subfamilies, the paramyxovirinae and pneumovirinae (Regenmortel *et al.*, 2000). The first isolate of APV was obtained in South Africa in 1979 (Buys *et al.*, 1989) and later the virus was detected in other countries around the world (Alexander, 1997). One APV serotype has been identified and within this serotype there are three subgroups designated A, B, and C based on molecular and antigenic differences (Cook *et al.*, 1999; Harlow & Lane, 1988; Seal, 2000).

Turkeys and chickens, of any age, are known to be natural hosts of APV. The APV causes respiratory diseases in young birds and a drop in egg production in breeder flocks (Alexander, 1997; Cook *et al.*, 1999; Jones *et al.*, 1988). Although, all ages of turkeys are susceptible to APV, the severity of the disease varies. Alexander (1997) reported that the disease was more severe in day old poults than 6-week-old turkeys. The clinical disease is characterized by nasal and ocular discharge, swollen infraorbital sinuses and sneezing (Jones *et al.*, 1986; McDougall & Cook, 1986, Wyeth *et al.*, 1986). These clinical signs have been observed in different countries and found to be similar but different names were given to the disease, which reflects the complexity of the etiology when associated with secondary infections. Serological tests such as enzyme-linked immunosorbent assay (ELISA) and virus neutralization (VN) test are the most commonly used methods to diagnose the APV infection. The VN test is performed in a variety of systems, including TOC, CEF, or Vero cells. Although, ELISA and VN test show similar sensitivity (Cook *et al.*, 1999), ELISA is the most commonly used assay (Chettle & Wyeth, 1988; Eterradossi *et al.*, 1995, O’Loan *et al.*, 1989). Therefore, serological survey was conducted to detect avian pneumovirus (APV) antibodies in commercial poultry farms using ELISA and VN test in Saudi Arabia.

## Materials & Methods

### Samples

Eighty seven serum samples were collected from different commercial chicken farms flocks in Qassium area, Saudi Arabia in a period from 2007- 2008. Serum samples were collected from birds showing respiratory symptoms, with different ages ranging from one day to 62 weeks old.

### Antigen preparation for ELISA

The preparation of APV antigen for ELISA was similar to that described (Chettle & Wyeth, 1988) with some modifications. Briefly, the supernatant of infected Vero cells was collected and treated by centrifugation at 100,000x g for three hours at 4 C (Beckman L7-55 ultracentrifuge, rotor SW 41. Palo Alto, CA). The pellets were resuspended in PBS (pH 7.2) and placed on a sucrose gradient (35% and 55%) and centrifuged at 100,000x g for three hours at 4 ºC. The virus was harvested from the sucrose interphase and diluted with PBS (pH 7.2), then centrifuged at 100,000x g for three hours at 4 ºC. The concentration of the virus protein was determined by electrophotometry at wavelengths of 260 and 280 nm (Harlow & Lane, 1988). A negative control of Vero cells was treated the same as the APV-infected Vero cells. The ELISA procedures were performed as described by O’Loan *et al. (*1989). Positive control sera were prepared in specific pathogen free (SPF) turkeys that were inoculated with inactivated virus.

### Virus neutralization (VN) test

The procedure for conducting the VN test was described by O’Loan *et al.* (1989) with some modification as follows: the serum samples were serially diluted two fold in serum free tissue culture medium (MEM) starting from 1:10 up to 1: 1280. A volume of 50 μl containing 100 tissue culture infective dose50 (TCID50) of APV (Minnesota/turkey/2a/97) was added to an equal volume of each serum dilution contained in sterile 96-well flat-bottom plates (Corning Incorporated, Corning, NY). A volume of 50 μl of the virus/serum mixture of each dilution was transferred to a duplicate of monolayer of Vero cells contained in 96-well flat-bottom plates. The cells were incubated at 37 ºC for 5-6 days and checked every day for cytopathic effect (CPE) consisting of large syncytial formation and rounded cells.

## Results & Discussion

Turkeys and chickens, of any age, are known to be natural hosts of APV. The APV causes respiratory diseases in young birds and a drop in egg production in breeder flocks (Alexander, 1997; Cook *et al.*, 1999; Jones *et al.*, 1988). Although, all ages of turkeys are susceptible to APV, the severity of the disease varies. Serological tests such as ELISA and VN test are the most commonly used methods to diagnose the APV infection. Baxter-Jones *et al.* (1989) reported the detection of antibodies to APV five days after the clinical signs appeared using VN test in chicken embryo fibroblast (CEF) then the antibody titer declined by days 13 after the appearance of clinical sings.

In this study, results of serological survey on APV in commercial poultry farms with different ages using ELISA and VN tests were summarized in table 1. It was observed that the antibody against APV were detected in 50% (8/16) in both ELISA and VN of serum samples collected from 11 - 18 weeks old chickens. No antibody was detected in younger or older poultry. This result disagrees with the finding of Alexander (1997), who reported that the disease was more severe in day old chicks than 6-week-old chickens.

## Conclusion

This study indicates the presence of antibody against APV in commercial poultry farms with age range from 11 - 18 weeks of age in Saudi Arabia.

**Table 1:** Serological survey against avian pneumovirus (APV) in serum samples collected from commercial poultry farms in Saudi Arabia using VN and ELISA test

|  |  |  |  |
| --- | --- | --- | --- |
| **Age** | **Number of samples** | **Positive samples (%)** | |
| **VN** | **ELISA** |
| **1 - 12 days old** | 15 | 0 | 0 |
| **19 - 35 days old** | 27 | 0 | 0 |
| **11 - 18 weeks old** | 16 | 8 (50%) | 8 (50%) |
| **25 - 35 weeks old** | 13 | 0 | 0 |
| **45 - 62 weeks old** | 16 | 0 | 0 |
| **TOTAL** | 87 | 8 (9.2%) | 8 (9.2%) |

ELISA: Enzyme-linked immunosorbent assay. VN: Virus Neutralization assay.

## References

Alexander DJ. 1997. Newcastle Disease and other avian paramyxoviridae infections. In: diseases of poultry, 10th ed. Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM (eds). Iowa state University press, Ames, Iowa. Pp. 541-569.

Baxter-Jones C, Grant M, Jones RC, Wilding GP. 1989. A comparison of three methods for detecting antibodies to turkey rhinotracheitis virus. Avian Pathol. 18: 91-98.

Buys SB, Preez JH, Els HJ. 1989. The isolation and attenuation of a virus causing rhinotracheitis in turkeys in South Africa. Onderstepoort J. Vet. Res. 56: 87-98.

Chettle NJ & Wyeth PJ. 1988. Turkey rhinotracheitis: detection of antibodies using an ELISA test. Br. Vet. Journal 144: 282-287.

Cook JKA, Huggins MB, Orbell SJ, Senne DA. 1999. Preliminary antigenic characterization of an avian pneumovirus isolated from commercial turkeys in Colorado, USA. Avian Pathol. 28: 607-617.

Eterradossi N, Toquin D, Guittet M, Bennejean G. 1995. Evaluation of different turkey rhinotracheitis viruses used as antigens for serological testing following live vaccination and challenge. J. Vet. Med. Series B. 42: 175-186.

Harlow E & Lane D. 1988. Antibodies: A laboratory manual. Cold Spring Harbor, N.Y. Cold Spring Harbor laboratory. Pp. 673.

Jones RC, Baxter-Jones C, Wilding GP, Kelly DF. 1986. Demonstration of a candidate virus for turkey rhinotracheitis in experimental inoculated turkeys. Vet. Rec. 199:599-600.

Jones RC, Williams RA, Baxter-Jones C, Savage CE, Wilding GP. 1988. Experimental infection of laying turkey with rhinotracheitis virus: distribution of virus in the tissues and serological response. Avian Pathol. 17: 841-850.

McDougall JS & Cook JKA. 1986. Turkey rhinotracheitis: preliminary investigation. Vet. Rec. 119: 206-207.

O’Loan CJ, Allan G, Baxter-Jones C, McNulty MS. 1989. An improved ELISA and serum neutralisation test for the detection of turkey rhinotracheitis virus antibodies. J. Virology methods. 25: 271-282.

Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB. 2000. Virus Taxonomy classification and nomenclature of viruses, seventh report of the international committee on taxonomy of viruses. Pp. 549-561.

Seal BS. 2000. Avian pneumovirus and emergence of a new type in the United satiates of America. Animal Health Research Reviews 1(1): 67-72.

Wyeth PJ, Gough RE, Chettle NJ, Eddy R. 1986. Preliminary observation on a virus associated with turkey rhinotracheitis. Vet. Rec. 119: 139.