Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

Short Communication

Serological Survey on Avian Pneumovirus Infection in Commercial Poultry Farms in Saudi Arabia

A. N. Alkhalaf

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

ARTICLE HISTORY

Received: December 12, 2009
Revised: January 11, 2010
Accepted: February 11, 2010

Key words:
Avian pneumovirus
Chickens
ELISA
Saudi Arabia
Virus neutralization test

ABSTRACT

This study was conducted to detect the presence of avian pneumovirus (APV) antibodies in commercial poultry farms using Enzyme-linked immunosorbent assay (ELISA) and virus neutralization test (VN). Eighty seven chicken serum samples were collected from several commercial poultry farms in Al-Qassium area, Saudi Arabia during 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) by both ELISA and virus neutralization (VN) test at farms in birds of 11-18 weeks of age only. The total positive samples were 8/87 (9.2%) of all examined samples. In conclusion, this study indicated the presence of antibodies to APV among 11-18 weeks old commercial chicken at farms in Saudi Arabia.

To cite this article: Alkhalaf AN, 2010. Serological survey on avian pneumovirus infection in commercial poultry farms in Saudi Arabia. Pak Vet J, 30(2): 124-125.

INTRODUCTION

The avian metapneumovirus (AMPV), previously called avian pneumovirus (APV) or turkey rhinotracheitis virus (TRTV), is a member of the *Paramyxoviridae* family, *Pneumovirinae* subfamily, within the new genus *Metapneumovirus* (Ferreira *et al*., 2009). 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 first isolate of APV was obtained in South Africa in 1979 (Buys and Preez, 1980) 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 as A, B, and C based on molecular and antigenic differences (Cook *et al*., 1999; Seal, 2000).

Turkeys and chickens of any age are known to be natural hosts of APV. The virus causes respiratory diseases in young birds and a drop in egg production in breeder flocks (Alexander, 1997; Cook *et al*., 1999). 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 occular discharge, swollen infraorbital sinuses and sneezing (Buys and Preez, 1980; McDougall and Cook, 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 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 Tracheal Organ Cultures, Chicken Embryo Fibroblast or Vero cells. However, enzyme-linked immunosorbent assay (ELISA) and VN test show similar sensitivity (Cook *et al*., 1999) but the ELISA is the most commonly used assay (Chettle and Wyeth, 1988; Eterradossi *et al*., 1995). Therefore, a serological survey was conducted to detect avian pneumovirus (APV) antibodies in commercial poultry farms using ELISA and virus neutralization (VN) test.

MATERIALS AND METHODS

Samples

A total of 87 serum samples were collected from different commercial chicken flocks in Qassium area, Saudi Arabia during 2007-2008. Serum samples were collected from birds with different ages, ranging from one day to 62 weeks (Table 1).

Antigen preparation for ELISA

The preparation of APV antigen for ELISA was similar to that described earlier (Chettle and Wyeth, 1988) but 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), placed on a sucrose gradient (35 and 55%) and centrifuged at 100,000x g for three hours at 4°C. 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°C. The concentration of the virus protein was determined by electrophotometry at wavelengths of 280 and 260 nm (Harlow and Lane, 1988). A negative control of Vero cells was treated in the same way as the APV-infected Vero cells. The ELISA procedures were performed as described earlier (McDougall and Cook, 1986). Positive control sera were prepared in specific pathogen free (SPF) turkeys that had been inoculated with inactivated virus.

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

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

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

Virus neutralization (VN) test

The procedure for conducting the VN test described earlier (McDougall and Cook, 1986) was followed but with some modification. 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 dose$_{50}$ ($TCID_{50}$) 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, USA). 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°C for 5-6 days and were examined every day for cytopathic effect (CPE) consisting of large syncytial formation and rounded cells.

RESULTS AND DISCUSSION

In this study, results of serological survey on APV among commercial poultry farms with different ages using ELISA and VN test are summarized in Table 1. It was observed that the antibodies against APV were detected in 50% (8/16) of serum samples collected from birds aged 11-18 weeks. No antibody was detected in younger or older age group. These results disagree with the findings of Alexander (1997), who reported that the disease was more severe in day old poults than 6-week-old turkeys. According to Nishikori *et al*. (2008), swollen heads caused by AVP was more prevalent in four-week-old chicks. This study indicated the presence of antibodies against APV in commercial poultry with age ranging from 11 to 18 weeks in Saudia Arabia.

Acknowledgement

Funds provided for this research by the Qassim University, Buraydah, Saudi Arabia is highly acknowledged.

REFERENCES

Alexander DJ, 1997. Newcastle disease and other avian paramyxoviridae infections. In: Diseases of Poultry, 10th Ed, BW Calnek, HJ Barnes, CW Beard, LR McDougald and YM Saif, (eds). Iowa State University Press, Ames, Iowa, USA, pp: 541-569.

Buys B and JH Preez, 1980. A preliminary report on the isolation of a virus causing sinusitis in turkeys in South Africa and attempts to attenuate the virus. Turkeys, 28: 36-46.

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

Chettle NJ and PJ Wyeth, 1988. Turkey rhinotracheitis: detection of antibodies using an ELISA test. British Vet J, 144: 282-287.

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

Eterradossi N, D Toquin, M Guittet and G Bennejean, 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.

Ferreira HL, FR Spilki, MMAB dos Santos, RS de Almeida and CW Arns, 2009. Comparative evaluation of conventional RT-PCR and real-time RT-PCR (RRT-PCR) for detection of avian metapneumovirus subtype A. Cienc Rural, 39: 1445-1451.

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

Nishikori T, N Hirai, K Sawada, K Osaka and N Ouchi, 2008. Attempts to control swollen-head syndrome in a broiler farm. J Japanese Soc Poult Dis, 43(4): 219-227.

Seal BS, 2000. Avian pneumovirus and emergence of a new type in the United States of America. Anim Hlth Res Rev, 1: 67-72.