Abdou Nagy *et al., J. of Virol. Sci.*, Vol. 4: 8-14, 2018
ISSN: 1685-1687

Journal of Virological Sciences

RESEARCH

Seroprevelance of Avian Metapneumovirus in Egyptian Chicken and Duck Flocks with a Reference on Economic Impact

Abdou Nagy[1], Fatma Abdallah[1], Kareem Sweed[2], Ahmed Salama[3], Mohamed Omar[4]

ABSTRACT

Background: Avian metapneumovirus (aMPV) is one of common respiratory pathogens affects poultry and can cause economic losses, particularly when accompanied with secondary pathogens.

Aim: This study aimed to perform seroprevalence of aMPV for non-vaccinated chicken and duck flocks from six provinces of Egypt (Ash Sharqiyah, Ismailia, Qalyubia, Menufia, Sohaq and North-Sinai) and determine its economic effect.

Methods: Serum samples were collected from non-aMPV vaccinated apparently healthy 40 chicken (23 broilers and 17 layers) and 8 duck (6 Pekin and 2 Muscovy) flocks and then, tested with commercial indirect aMPV ELISA.

Results: Five out of 23 broiler, 6 out of 17 layer, 1 out of 6 Pekin duck and 1 out of 2 Muscovy duck flocks showed positive results. On the other hand, the results showed significant difference in the net profits as a measure of economic impact between infected and non-infected flocks in different provinces.

Conclusion: The results denote that chicken and duck flocks in Egypt were exposed to field strains of aMPV. For our knowledge, aMPV antibodies are detecting in Pekin and Muscovy duck flocks for first time in Egypt.

Keywords: Avian metapneumovirus, Seroprevalence, Net-profit, Duck, Chicken.

BACKGROUND

Avian metapneumovirus (aMPV) is one of the important respiratory virus infections affecting poultry with impairing the production of eggs in both turkeys and chickens (Stuart, 1989; Giovanardi *et al*., 2014). The aMPV belongs to the genus *Metapneumovirus*, subfamily *Pneumovirinae* within the family *Paramyxoviridae* (Pringle, 1998). The aMPV is an enveloped virus with single-stranded, linear, non-segmented, negative-sense RNA genome (Lamb and Kolakofsky, 2001). Only subtypes (A, B, C, and D) have been documented according to genetic and antigenic analysis of the G attachment gene (Cook and Cavanagh, 2002). In the Middle East, the aMPV subtypes A and B are mainly prevalent (Banet-Noach *et al*., 2005). The aMPV is the cause of turkey rhinotracheitis, which is an acute respiratory tract infection in all ages of the turkeys. The aMPV is also associated with the swollen-head syndrome (SHS) in chicken broilers and its breeders (O'Brien, 1985; Gough *et al*., 1994) and with losses in the production of egg in layers (Sugiyama *et al*., 2006). Nevertheless, it has been showed that aMPV isolates in chicken are closely related to turkey isolates based on antigenic level (Cook *et al*., 1993a). Moreover, the aMPV infectious particles and RNA have been demonstrated in mallard and Muscovy ducks, pheasants, sparrows, starlings, guinea fowls, blue-winged teal and Canada geese (Picault *et al*., 1987; Gough *et al*., 1988; Toquin *et al*., 1999; Shin *et al*., 2000; Catelli *et al*., 2001; Bennett *et al*., 2002). Another study reported that Pekin ducks inoculated with aMPV of turkey origin were not able to develop clinical symptoms of disease, but they played a role as non-clinical carriers of the virus (Shin *et al*., 2001). The aMPV diagnosis is based on serology, virus isolation and polymerase chain reaction (Stuart, 1989; Gough, 2003). The successful of aMPV isolation from birds displaying severe chronic symptoms is rarely and from chickens may be more difficult than

Correspondence: mm.fatma@yahoo.com
Full list of author information is available at the end of the article

from turkeys for unclear causes (Naylor and Jones, 1993). Serology is the most common method of diagnosis of aMPV infections, particularly in non-vaccinated chicken and turkey flocks (Cook, 2000a).

There is limited data about the epidemiology of aMPV in the Middle East, particularly Egypt. This limitation might be due to the big emphasis placed on monitoring other major poultry respiratory viral infections such as avian influenza (AI), Newcastle disease (ND) and infectious bronchitis (IB). The Egyptian aMPV strains published to date belong to the subtype B and were detected in turkeys under accession numbers HQ677586 and JX647840 in the molecular database (Mahmoud *et al*., 2008). Recently, another study reported circulation of aMPV strain of subtype A in turkeys in Egypt without evidence for virus isolation, and the reported virus sequences were submitted to the GenBank database under accession numbers KJ196272 and KJ196273 (Abdel-Azeem *et al*., 2014). To our knowledge, there is very limited data about aMPV seroprevalence in different avian species other than turkeys such as commercial chickens and ducks from different locations in Egypt. Therefore, this study was conducted to survey the presence of aMPV neutralizing antibodies in non-vaccinated apparently healthy chicken and duck flocks using commercially available indirect ELISA test and compare the economic impact on the net profits of infected and non-infected flocks.

MATERIALS AND METHODS

Samples collection and Ethical statement:

A total of 460 sera samples were collected from apparently healthy non-aMPV vaccinated; 23 chicken broiler flocks (n= 214 samples), 17 chicken layer flocks (n= 168 samples), 6 Pekin duck flocks (n= 52 samples) and 2 Muscovy duck flocks (n= 26 samples). All flocks except duck flocks had histories of vaccination against other pathogens particularly AIV, NDV and IBV. The surveillance was conducted in six Egyptian provinces (Ash Sharqiyah, Ismailia, Qalyubia, Menufia, Sohaq and North-Sinai) for the period of 2016-2017. Mean body weight gains were recorded for broiler chicken and duck flocks, as well as, records for rate of egg production for layer flocks also was recorded. All sera samples were further processed and heat inactivated for 30 minutes at 56 °C in water bath. Serum samples were obtained from different chicken and duck flocks in the present study were approved ahead by the Animal Ethical Committee of the Faculty of Veterinary Medicine, Zagazig University, Egypt.

Analysis of Sera samples by ELISA:

The presence of antibodies against aMPV in collected serum samples was evaluated by Avian Rhinotracheitis Antibody Test Kit (BioChek, Holland) following the manufacturer's instructions. Briefly, sera samples were diluted 1:500 by adding 1 µl serum sample to 499 µl of sample diluent. One positive control and one negative control serum (100 µl of each) were used in each run. A volume of 100 µl of diluted serum samples were added to aMPV antigen coated wells and incubated for 1 hour (h) at room temperature. Later, all wells were washed with washing buffer (300 µl /well) four times and dehydrated using absorbent paper. A volume of 100 µl of conjugate (anti-chicken IgG labelled with alkaline phosphatase enzyme) was added to each well and incubated for 1 h at room temperature for detection of chicken antibodies. In order to make the kit suitable for detection of duck antibodies, we replaced the anti-chicken IgG labelled with alkaline phosphatase enzyme (provided with the kit) with Anti-duck IgG labelled with alkaline phosphatase enzyme (not provided with the kit). After incubation, all wells were washed and dehydrated as aforementioned. A volume of 100 µl of substrate reagent was added to each well and incubated for 30 minutes at room temperature in dark. The reaction was stopped by

adding to all wells 100 µl of stop solution. The final results were recorded using ELISA Micro-plate reader (Stat Fax 3200, USA) as optical density values at 450 nm.

The calculation of S/P ratio was done using this equation:

$$\frac{\text{Mean of test Sample absorbance - Mean of negative control absorbance}}{\text{Mean of positive control absorbance - Mean of negative control absorbance}}$$

Calculation of antibody titre was done using this equation:

Log_{10} titre $=1.0(\log_{10}S/P) + 3.52$
Titre range =1655 or less is considered negative while 1656 or greater is considered positive.

Economic and statistical analysis:

The net profit from investigated flocks was analysed using economic measures as the total costs and total returns for all studied flocks were calculated according to (Fardos, 2009). The net profits for broiler flocks (LE/chick), layer flocks (LE/ dozen) and duck flocks (LE/ duck) were calculated according to (Omar, 2003). Variation in positive rate among different provinces was analysed using One-way ANOVA in GraphPad Prism version 7.0 (GraphPad Software Inc., CA USA, http://www.graphpad.com/scientific-software/prism/). The variable responses were subjected to comparisons for all pairs by using the Tukey-Kramer test. While, the Student t-test was made for pairwise mean comparisons between each two different provinces. A *p value* of ≤ 0.05 was considered as a significant difference.

RESULTS:

Analysis of samples by aMPV indirect ELISA:

Five out of 23 broiler chicken flocks, 6 out of 17 layer chicken flocks, 1 out of 6 Pekin duck flocks and 1 out of 2 Muscovy duck flocks showed positive results (Table 1). The difference in the positive rates among chicken and duck flocks was not significant. On the other hand, the results showed that the net profit was significantly different ($P \leq 0.05$) between studied provinces for chicken (broiler, layer) and duck (Pekin, Muscovy) flocks (Table 2).

Table (1): Serological prevalence of aMPV in chicken and duck flocks.

Breeding type	Location (Province)	Age (weeks)	No of flocks	Positive flocks	
				No	%
Broiler chicken	Ash Sharqiyah	2-4	13	3	23.07
Broiler chicken	Sohag	2-4	10	2	20
Total			23	5	21.7
Layer chicken	Ash Sharqiyah	13-47	9	3	33.3
Layer chicken	North-Sinai	13-47	3	1	33.3
Layer chicken	Qalyubia	13-47	3	1	33.3
Layer chicken	Menufia	13-47	2	1	50
Total			17	6	35.2
Pekin duck	Ash Sharqiyah	12-13	6	1	16.7
Muscovy duck	Ismailia	12-13	2	1	50

Table (2): Difference in economic impact between infected and non-infected aMPV flocks.

Location (Province)	Net profit							
	LE/ chick (Broiler flocks)		LE/ dozen (Layer flocks)		LE/ duck (Pekin flock)		LE/ duck (Muscovy flock)	
	Net profit of infected flock	Net profit of non-infected flock	Net profit of infected flock	Net profit of non-infected flock	Net profit of infected flock	Net profit of non-infected flock	Net profit of infected flock	Net profit of non-infected flock
Sohag	3.87± 0.71	5.03± 0.11	-	-	-	-	-	-
North-Sinai	-	-	1.01± 0.23	1.90± 0.23	-	-	-	-
Qalyubia	-	-	1.27± 0.23	2.03± 0.23	-	-	-	-
Menufia	-	-	1.57± 0.71	2.13± 0.11	-	-	-	-
Ismailia	-	-	-	-	-	-	4.00± 0.22	5.23± 0.34
Ash Sharqiyah	3.23± 0.23	4.73± 0.33	1.23± 0.13	2.73± 0.33	3.90± 0.13	3.90± 0.12	-	-

Notes: Data are expressed as mean values ± SEM

DISCUSSION

In chickens, there is a strong suggestion that the aMPV is the etiological agent of swollen-head syndrome (SHS), which was reported in both broilers and broiler breeders. The aMPV diagnosis is based on serological assays, polymerase chain reaction and virus isolation (Stuart, 1989; Gough, 2003). Isolation of aMPV is scarcely successful from birds showing extreme chronic signs that are usually due to secondary infectious agents. Different serological assays have been developed to detect aMPV neutralizing antibodies include ELISA, virus neutralization test, and immunofluorescence assay (Stuart, 1989; Gough, 2003). This study is intended to present a report on the seroprevalence of aMPV neutralizing antibodies in non-aMPV vaccinated chicken and duck flocks from different provinces in Egypt using commercial ELISA kit. The results showed variable positive percentage, which is 21.7 %, 35.2%, 16.7% and 50% for tested (broiler, layer) chicken and (Pekin, Muscovy) duck flocks, respectively. However, this is low prevalence rate in contrast to other studies conducted in neighbouring countries such as Jordan where (Gharaibeh and Algharaibeh, 2007) reported that out of 38 chicken flocks tested by ELISA, 18 flocks (47.4%) were found to have positive antibody titre for aMPV, the positive flocks included 21.7, 75, and 100% of broilers, layers, and broiler breeders, respectively. As well as, the results are strengthened by Cook et al. (1993b), who suggested that chickens responded serologically to aMPV less well than turkeys, either to challenge with virulent virus or to vaccination with live-attenuated TRT vaccines. In addition to, aMPV may not be a primary pathogen in chickens, but always involved with other agents in a complex respiratory disease syndrome such as IB virus (Cook, 2000a). Both IB and aMP viruses are known to replicate in the epithelial tissue of the upper part of respiratory tract (Cook *et al.*, 1991; Catelli *et al.*, 1998; Cavanagh *et al.*, 1999; Cavanagh and Naqi, 2003) leading to the probability that there might be interference between them. Furthermore, Cook *et al.* (2001) who reported that IB virus vaccination interfered with the replication of aMPV resulted in a decrease in the antibody response but with no opposite effect on the induction of protective immunity. On the other hands, ducks inoculated with aMPV of turkey origin did not develop clinical symptoms of the disease, but they were assumed to play a role as non-clinical carriers of aMPV (Shin *et al*., 2001) and this may be the cause for the observed very low positive prevalence rate of aMPV in the analysed duck flocks.

Because the most of studied flocks were more than four weeks of age and non-vaccinated against aMPV, the results of this serological survey suggest field exposure of these flocks to aMPV and exclude the probability that were due to maternal antibodies. As well as, the all studied flocks did not suffer from any respiratory manifestation, but some of them have aMPV neutralizing antibodies that are consistent with (Pattison *et al*., 1989; Hafez and Lohren, 1990) who reported that chicken flocks did not have clinical symptoms may produce antibodies for aMPV. Additionally, the results showed that the net profits are significant between infected and non-infected aMPV flocks in different provinces according to the data of the body weights and feed conversion ratios collected from all tested flocks (data not shown). However, these differences in net profits between infected and non-infected may be due to other involving factors such as vaccination programmes, poultry house management and species of birds reared, etc.

CONCLUSION

Our results denote that the field strain of aMPV circulates among chicken and duck flocks, as well as, the low seroprevalence rate of aMPV may be enough to record that flocks are at the risk to aMPV infection. On the other hand, the results concluded that net profit as a measure of economic analysis showed that the flocks that have positive for aMPV showed decrease in net profit as a result of decrease body weight gain and egg production. Further research is recommended to isolate and characterize the currently circulating aMPV strains in Egyptian field with introducing routine diagnosis of aMPV in diagnostic laboratories as well.

AUTHOR DETAILS

[1]Department of Virology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt.
[2]Avian Diagnostic Laboratory, Abu-Kabir 44671, Ash Sharqiyah, Egypt.
[3]Dawagen Elkheir, Inc, Zagazig 44511, Ash Sharqiyah, Egypt.
[4]Department of Animal Wealth Development, Faculty of Veterinary Medicine, Zagazig University, Zagazig 4451, Egypt.

RECEIVED: May 2018; **ACCEPTED**: June 2018; **Published:** July 2018

REFERENCES

Abdel-Azeem, S.A., Giovanni, F., Antonella, D.Z., Michele, D., Elena, C., Caterina, L., Marco, M. and Mattia, C. (2014). First evidence of avian metapneumovirus subtype A infection in turkeys in Egypt. *Trop Anim Health Prod.* 46:1093–1097.

Banet-Noach, C., Simanov, L. and Perk, S. (2005). Characterization of Israeli avian metapneumovirus strains in turkeys and chickens. *Avian Pathol*. 34: 220-226.

Bennett, R.S., McComb, B., Shin, H.J., Njenga, M.K., Nagaraja, K.V. and Halvorson, D.A. (2002). Detection of avian pneumovirus in wild Canadian geese (Brantacanadensis) and blue winged teal (Anasdiscors). *Avian Dis.* 46:1025–1029.

Catelli, E., Cook, J.K.A., Chesher, J., Orbell, S.J., Woods, M.A., Baxendale, W. and Huggins, M.B. (1998). The use of virus isolation, histopathology and immunoperoxidase techniques to study the dissemination of a chicken isolate of avian pneumovirus in chickens. *Avian Pathol.* 27: 632–640.

Catelli, E., De Marco, M.A., Delugo, M., Terregino, C. and Guberi, V. (2001). Serological evidence of avian pneumovirus infection in reared and free-living pheasants. *Vet Rec*. 149: 56–58.

Cavanagh, D., Mawditt, K., Britton, P. and Naylor, C.J. (1999). Longitudinal field studies of infectious bronchitis virus and avian pneumovirus in broilers using type-specific polymerase chain reactions. *Avian Pathol*. 28: 593– 605.

Cavanagh, D and Naqi, S. A. (2003). Infectious bronchitis virus. In: Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R. and Swayne, D.E. (eds), *Diseases of Poultry*,pp.101-109. Ames: Iowa State Press.

Cook, J.K.A., Ellis, M. M. and Huggins, M.B. (1991). The pathogenesis of turkey rhinotracheitis virus in turkey poults inoculated with the virus alone or together with two strains of bacteria. *Avian Pathol*. 20:155–166.

Cook, J.K.A., Jones, B.V., Ellis, M.M., Jing, L. and Cavanagh, D. (1993a). Antigenic differentiation of strains of turkey rhinotracheitis virus using monoclonal antibodies. *Avian Pathol*. 22: 257-273.

Cook, J.K.A., Kinloch, S. and Ellis, M. M. (1993b). In vitro and in vivo studies in chickens and turkeys on strains of turkey rhinotracheitis virus isolated from the two species. *Avian Pathol*. 22:157-170.

Cook, J.K.A. (2000a). Avian rhinotracheitis. *Rev Sci Tech*. 19: 602–613.

Cook, J. K. A., Huggins, M. B., Orbell, S. J., Mawditt, K. and Cavanagh, D. (2001). Infectious bronchitis virus vaccine interferes with the replication of avian pnemovirus vaccine in domestic fowl. *Avian Pathol*. 30: 233–243.

Cook, J.K.A. and Cavanagh, D. (2002). Detection and differentiation of avian pneumoviruses (metapneumoviruses). *Avian Pathol*. 31:117-132.

Fardos, A.H. (2009). Effect of some feed additives on economic and productive efficiency of broilers. M.V.Sc. Thesis, University of Zagazig, Egypt.

Gharaibeh, S.M., and Algharaibeh, G.R. (2007). Serological and molecular detection of avian pneumovirus in chickens with respiratory disease in Jordan. *Poult Sci*.86: 1677–1681.

Giovanardi, D., Lupini, C., Pesente, P., Rossi, G., Ortali, G. and Catelli, E. (2014). Longitudinal field studies of Avian Metapneumovirus and Turkey Hemorrhagic Enteritis Virus in turkeys suffering from colibacillosis associated mortality. *Vet res commun*. 38:129–137.

Gough, R.E., Collins, M.S., Cox, W.J. and Chettle, N.J. (1988). Experimental infection of turkeys, chickens, ducks, geese, guinea fowl, pheasants, and pigeons with turkey rhinotracheitis virus. *Vet Rec*. 123: 58–59.

Gough, R.E., Manvell, R.J., Drury, S.E.N. and Pearson, D.B. (1994) Isolation of avian pneumovirus from broiler chickens. *Vet Rec*. 134: 353–354.

Gough, R.E. (2003). Avian pneumoviruses. In: Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., McDougald, L.R. and Swayne, D.E. (eds), *Diseases of Poultry*, pp.93-99. Ames: Iowa State Press.

Hafez, H.M. and Lohren, U. (1990). Swollen Head Syndrome: Clinical Observations and Serological Examinations in West Germany. Dtsch Tierarztl Wochenschr. 97: 322-324.

Lamb, R.A. and Kolakofsky, D. (2001). Paramyxoviridae: the viruses and their replication. In: Knipe, D.M. and Howley, P.M. (eds), *Fields Virology*, pp. 1305 -1340. Philadelphia, PA, USA.

Mahmoud, A.H., Fahmy, H.A., Gafer, J.A.M. and Arafa, A. (2008). Investigation on Turkey Rhinotracheitis in Commercial Turkeys in Egypt. In: Hafez, H.M. (eds), *7th edn International Symposium on Turkey Diseases*, pp. 186 -196. Berlin, Germany.

Naylor, C.J. and Jones, R.C. (1993). Turkey rhinotracheitis: a review. *Vet Bull*. 63: 439–449.

O'BRIEN, J.D.P. (1985). Swollen head syndrome in broiler breeders. *Vet Rec*. 117: 619-620.

Omar, M A. E. (2003). Economic and productive efficiency of poultry farms in relation to veterinary inputs. M.V.Sc. Thesis, University of Zagazig, Egypt.

Pattison, M., Chettle, N., Randall, C.J. and Wyeth, P.J. (1989). Observations on swollen head syndrome in broiler and broiler breeder chickens. *Vet Rec*. 125: 229–231.

Picault, J.P., Giraud, P., Drouin, P., Guittet, M., Bennejean, G., Lamande, J., Toquin, D., and Gueguen, C. (1987). Isolation of a TRTV like virus from chickens with swollen-head syndrome. *Vet Rec*. **121**: 135.

Pringle, C.R. (1998). Virus taxonomy—San Diego 1998. *Arch Virol*.143:1449–1459.

Shin, H.J., Njenga, M. K., McComb, B., Halvorson, D.A. and Nagaraja, K.V. (2000). Avian pneumovirus RNA from wild and sentinel birds in the US has genetic homology with APV isolates from domestic turkeys. J Clin Microbiol. 38: 4282–4284.

Shin, H.J., Njenga, M.K., Halvorson, D.A., Shaw, D.P. and Nagaraja, K.V. (2001). Susceptibility of ducks to avian pneumovirus of turkey origin. *Am J Vet Res*. 62:991–994.

Stuart, J.C. (1989). Rhinotracheitis: turkey rhinotracheitis (TRT) in Great Britain. In: Nixey, C. and Grey, T.C. (eds), Recent Advances in Turkey Science. *Poultry Science Symposium*, pp.217-224. Butterworth, London.

Sugiyama, M., Koimaru, H., Shiba, M., Ono, E., Nagata, T. and Ito, T. (2006). Drop in egg production in chickens by experimental infection with avian metapneumovirus strain PLE8T1 derived from swollen head syndrome and the application to evaluate vaccine. *J Vet Med Sci*. 68:783-787.

Toquin, D., Bayon-Auboyer, M.H., Eteradossi, N. and Jestin, V. (1999). Isolation of a pneumovirus from a Muscovy duck. *Vet Rec*.145: 680.

Cite this article as:
Nagy ***et al.,*** **(2018)**: Seroprevelance of Avian Metapneumovirus in Egyptian Chicken and Duck Flocks with a Reference on Economic Impact. *J. of Virol. Sci.,* Vol. 4: 8-14, 2018.