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STUDIES ON CURRENTLY CIRCULATING PARAMYXOVIRUSES IN PIGEONS

Reham A. Elbhnsawy, Abu El Azm K.I., Sanaa S. Awad and Eladl A.H.

Department of Poultry and Rabbit Diseases, Faculty of Veterinary Medicine, Mansoura University.

ABSTRACT

Twenty two pigeon paramyxovirus (PPMV) isolates were recovered from 50 tested diseased pigeons. Tested pigeons suffered from nervous manifestation, listlessness, inappetance, inability to fly and greenish diarrhea. Diseased pigeons were collected from Dakahlia governorate during 2014 and 2015.

Virus isolation was done in embryonated chicken eggs via allantoic sac inoculation. Isolates were identified as PMV by HA and RT-PCR tests.

Pathogenicity for five isolates was done via intracerebral inoculation of one day old 10 SPF-chicks for each isolate. Intracerebral pathogenicity index varied from 0.0 to 1.44. Although RT-PCR testing of liver, spleen, lung and brain collected from culled chicks on day 8 post inoculation for group with 0.0 pathogenicity index and negative control group were negative, yet testing of the same organs from dead birds of other challanged groups were positive for PMV.

INTRODUCTION

Pigeon variant Newcastle disease virus belongs to the genus *Avulavirus*, subfamily *Paramyxovirinae* and family *Paramyxoviridae*. The disease is caused by pigeon paramyxovirus type 1(PPMV-1) ***(Alexander and Senne, 2008; Miller et al., 2010; Briand et al. 2012; Terregino et al., 2013)***. It was first described in Middle East (Iraq) in 1978 ***(Kaleta et al., 1985)*** and spread rapidly to Europe ***(Biancifiori and Fioroni, 1983).***

Pigeon PMV-1 isolates produce neurological disease and mortality during ICPI measures in pigeons similar to those of moderately virulent NDV mesogens ***(Alexander, 1991).***

In Egypt, the disease was firstly diagnosed in pigeons as ND by ***El-Dahaby and Sokkar (1967).*** Similar cases were subsequently reported from pigeons with neurological signs ***(Ahmed and Sabri, 1969; Mohamed et al., 1980; Tantawi and Hassan, 1982; Shakal, 1989; Abou-Hashem, 1993; Abdel Motelib et al., 1994 and Awad, 1995)***.

Disease in pigeons characterize mainly by sudden onset of listlessness, in-appetence, nervous manifestations and inability to fly. Morbidity and mortality may reach 80-100% ***(Eisa and Omer, 1984).***

PM lesions of died pigeons were congestion of internal organs (lung, liver, heart

and proventriculus), enteritis, splenomegaly and hepatomegaly. Brain was congested and slightly swollen ***(Mushtaq et al., 2006 and El-Bagoury et al., 2014).Shaheen et al. (2005)*** did not find any obvious gross lesions in most cases of PPMV infection.

However, some PPMV-1 strains cause only minimal disease and show a low intracerebral pathogenicity index (ICPI) in chickens **(*Meulemans et al., 2002*);** nevertheless, they become potentially virulent as after serial passages in chickens **(*Alexander & Parsons, 1984; Collins et al., 1994; Kommers et al., 2001, 2003; Dortmans et al., 2011*)**.

Intracerebral inoculation of 1day old chicks with pigeon PMV-1 resulted in intracerebral pathogenicity indices of 1.0-1.8 and the virus is also pathogenic for chickens when inoculated intravenously ***(Alexander and Parsons, 1984)***.

A number of Newcastle disease outbreaks in poultry have been attributed to PPMV-1, which makes these pigeon-derived viruses a real and continuous threat to the poultry industry **(*Anonymous, 1984; Alexander et al., 1985b, 1997, 1998; Werner et al., 1999; Liu et al., 2006; Irvine et al., 2009*).**

Controversially, ***Al-mulhim et al. (2006)*** stated that "The pigeon owners agreed that most of the disease outbreaks occurred in summer. However, sporadic cases could also be seen throughout the year". they observed that a storm affection usually starts almost all the flock and morbidity reaching 100% and mortality may reach over 90%. They also found that all breeds and sexes were equally affected and all age groups were susceptible but young pigeons were affected mostly.

RT-PCR used in diagnosis of PPMV-1 in South Africa from 2001 to 2006 ***(Abolink et al., 2008).***

The aim of this study was to investigate the incidence of PPMV-1 in sick pigeons in Dakahlia Governorate and the sensitivity of different primers used for diagnosis of PMV-1 in pigeons.

MATERIALS AND METHODS

Sample collection:

A total of 50 diseased pigeons were collected from different markets in Mansoura city, Dakahlia governorate, Egypt. Pigeons were sick and suffer from nervous signs, listlessness, inappetance, inability to fly and greenish diarrhea.

Sample preparation:

Samples were collected and pooled from brain, lung, kidney, spleen, liver and heart from each tested pigeon. Pooled organs were homogenized and saline with antibiotic and antifungal (gentamycin 50 mg and mycostatin 1000 units/ ml) were added (1:5). Mixtures were vortexed and centrifuged at1000 *xg* for 10 minutes. Supernatant were collected in sterile falcon tubes and preserved at -20^{0}C for further processing.

Virus isolation:

For each tested pigeon, 0.2 ml of tissue supernatant were inoculated in each of five 8 day old embryonated chicken eggs via allantoic route. Inoculated eggs were incubated at 37.5 ^{0}C and candelled daily for five days. Eggs with live embryos were chilled at 4-8^{0}C in refrigerator at fifth day post inoculation, eggs

with dead embryos were opened daily, and with live ones on fifth day post inoculation. Allantoic fluids were collected under sterile condition in falcon tubes and tested for HA activities by slide agglutination test. Collected allantoic fluids were reinoculated in allantoic cavity for two other passages.

Virus identification:

1-Rapid slide haemagglutination test:

It was done according to the standard method described by **Allan et al., (1978).**

2-Molecular identification

A-Total RNA Extraction:

Total RNA from allantoic fluids of tested samples was extracted with Thermo Scientific Gene Jet RNA purification Kit (#K0731, #K0732), Mammalian Tissue and Insect Total RNA purification protocol).

B- Reverse transcription-polymerase chain reaction and polymerase chain reaction:

Extracted RNA was tested by reverse transcription according to (Bioline, commercial kit United Kingdom). Reverse transcription was performed in a total volume of 50 µl containing 5 µl of the total RNA, 25µl of 2x My Taq One-Step Mix(1x), 1 µl of ribosafe RNAase inhibitor (10U/µl), 0.5 µl (10 µM) reverse transcriptase and 14.5 µl of DEPC-treated Water.

Amplification of 202 and 356 base pairs of the F gene were performed using 2µl of each primer (400nM) NDV/F and NDV/R in a total volume of 50 µl $MgCl_2$ in 1x reaction mixture (3mM).

Primer sequence of 356 base pair ***(Nanthkumar et al., 2000)*** was: Forward Primer 5- GCAGCTGCAGGGATTGTGGT-3 nucleotide position 158-177. Reverse Primer 5-TCTTTGAGCAGGAGGATGTTG-3, nucleotide position 513-493.

Primer Sequence of 202 base pair ***(Creelan et al., 2002)*** was: The Forward Primer 5-GGTGAGTCTATCCGGARGATACAA-3, Reverse primer 5- TCATTG GTT GCR GCA ATG CTC T-3

Thermocycler conditions were:

Cycles	Temperature	Time	Notes
1	45^0C	20min	Reverse transcriptase
1	95^0C	1 min	Polymerase activation
40	95^0C	10s	Denaturation
	60^0C	10s	Annealing
	72^0C	30s	Extension

C-Agarose Gel Electrophoresis for PCR products:

The RT-PCR products were subjected to electrophoresis in 1.5 % agarose gel using Tris/Borate/EDTA (TBE) (pH 8). Stock of agarose was made by adding 1.5 gm agarose to 100 ml 1X TBE, and heated in microwave until agarose was completely dissolved. 5µl of (10mg /ml) stock ethidium bromide was added to the gel. Gel was allowed to solidify at room temperature, comb was carefully removed. 1X TBE was added to gel tray to completely cover the gel and wells were filled by 5µl of marker and tested samples. Lid was placed on gel rig. Power source were turned on and gel was checked during the run till the dye front band has run to the bottom of the gel. Power source was turned off and gel image was obtained.

3-Intracerebral pathogenicity index (ICPI):

One-day old SPF chicks purchased from Koom Oshiem Elfayoum, Egypt, were inoculated intracerebrally with 0.05 ml of 1:10 dilution of infected allantoic fluid. Chicks were

monitored for 8 days and scored daily as normal (score 0), sick (score1), dead (score 2). Total scores were registered and the mean daily scores were calculated to determine the ICPI ***(OIE, 2012)***.

4-RT-PCR retesting of organs collected from culled chicks on day 8 post inoculation.

RESULTS

Tissue samples were collected from 22 diseased pigeons that was obtained from different markets in Mansoura city, Dakahlia Governorate, Egypt. Pigeons suffered from nervous signs, greenish diarrhea, listlessness, inappetance and/or inability to fly in a correct way. Postmortem examination was almost negative except presence of greenish discoloration of proventriculus and gizzard contents. In one bird congestion of internal organs (lung, liver, and heart), enteritis, splenomegaly and hepatomegalywere found.

Tissue samples collected from 22 pigeons were processed for 3 cycles of egg inoculation. All collected allantoic fluid were positive for HA activity by the end of the 3rd cycle of egg inoculation.

RT-PCR test revealed positive results to the 22 processed pigeon samples with specific primers for F-gene of Newcastle disease designed by Nanthkumar et al., 2000 (365bp) and Creelan et al., 2002 (202bp) Fig.1.

Intracerebral pathogenicity index (Table 1) for 4 samples ranged from 1.31 to 1.44 and one sample had 0.00 ICPI.

Redetection of PMV-1from pooled grinding tissues of brain, liver, spleen, lung, kidney and heart collected from killed birds at day 8 post-intracerebral inoculation revealed negative results for both negative-control group and group with 0.00 ICPI while positive results obtained for other four tested samples with 1.31 to 1.44 ICPI. F- gene specific primer (Nanthkumar et al., 2000) were used in retesting of tissue samples (Fig.2).

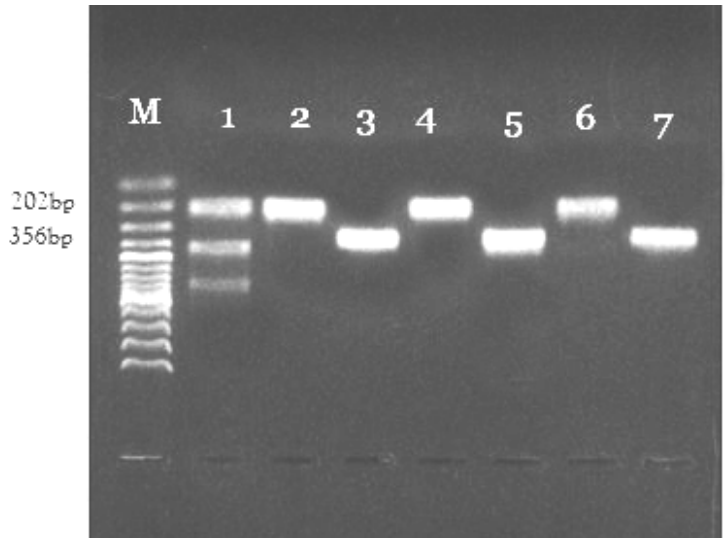

Fig. (1): F gene amplification by agarose gel electrophoresis of allantoic fluid of 4 samples. Respectively, each sample starting from lane 2 were tested by 202 bp primer ***(Creelan et al., 2002)*** and 356 bp primer ***(Nanthkumar et al., 2000)*** while lane 1 represent a sample tested by 202 bp, M= marker 100 to 1000bp.

Table 1: Intracerebral pathogenicity index (ICPI) of PPMV-1 isolates in day old SPF chicks.

Sample Number	Month of isolation	Year of isolation	ICPI
Control	____	____	0.00
4	May	2015	0.00
7	April	2015	1.31
14	Janurary	2015	0.94
16	Janurary	2015	1.44
22	December	2014	1.36

Samples with ICPI range between 1.0-1.5 considered to be mesogenic strain ***(Alexander, 1998)***, and all our samples considered to be mesogenic. The one with zero ICPI considered to be inactive virus that proved by negative results in virus reisolation and RT-PCR test that may be inactivated during its storage in deep freezer (-20).

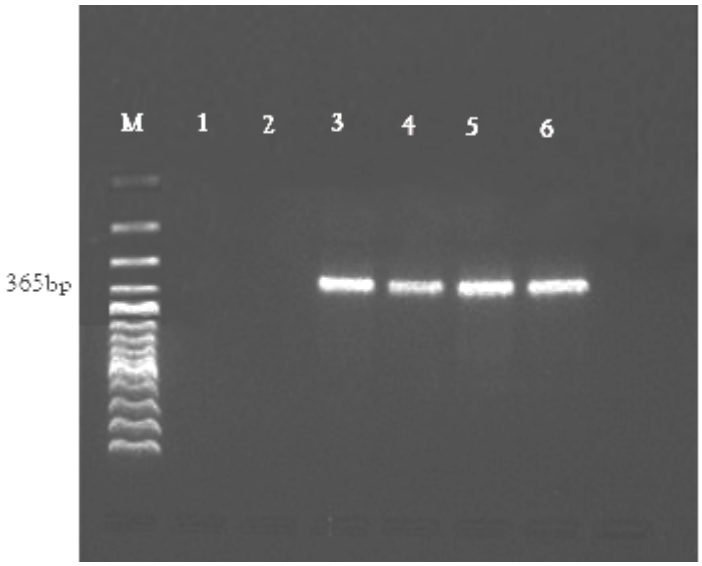

Fig. (2): F gene amplification by agar gel electrophoresis of tissue samples collected from different chicken groups post challenge and negative control group. Lane 1: -ve control, lane 2: group with 0.0 ICPI, lane 3: group with 1.31 ICPI, lane 4: group with 0.94 ICPI, lane 5: group with 1.44, lane 6 group with 1.36 ICPI, M = marker 100bp.

DISCUSSION

Pigeons are one of the few domesticated birds, which are kept by human for a variety of purposes such as food and hobby (racing). A variety of diseases seems may affect pigeons but viral diseases to be one of the most predominate one ***(Liu et al., 2003).***

Avian paramyxovirus type 1 (APMV-1) of pigeons (PPMV-1) is an antigenic and host variant of Newcastle disease virus (NDV) of chickens that is responsible for an autonomous Newcastle disease (ND)-like infectious disease of pigeons ***(Ujva´ri et al., 2003).***

As PPMV-1 is a common and serious problem nowadays for pigeon fancier in Egypt the objective of the current study is to isolate PPMV-1 in embryonated chicken eggs from naturally infected pigeons, identify the isolate by RT-PCR and measure their pathogenicity in one day old SPF chicks to determine their pathotypes.

In this study, RT-PCR used to identify our isolates by amplifying the fusion protein cleavage site (F protein) by two different primers designed by ***Nanthkumar et al., (2000)*** and ***Creelan et al., (2002)*** at 356 bp and 202 bp, respectively. Fusion protein gene corresponds to the cleavage site of the F0 precursor, which is a key determinant of NDV pathogenicity ***(Farkas et al., 2009a; Tan et al., 2004)***.

In our investigation, trials for isolation of PPMV-1 was done for twenty two prepared random samples collected from diseased and freshly dead pigeons that suffer from nervous manifestation, greenish diarrhea, listlessness, inappetance and inability to fly in a correct way. Tested samples were subjected to 3 successive passages in ECE. It was noticed that virus titer seems to increase due to increasing HA slide activity. No further passages were attempted, as we thought that the obtained third passages virus titers in some samples are satisfactory. Similar procedures were conducted by ***Alexander et al., (1985) and Abou Hashem (1993).***

All twenty two tested samples were positive by slide HA test and RT-PCR (100% of tested samples.

Although some researchers observed PM changes as represented by congestion of internal organs (lung, liver, heart and proventriculus), enteritis, splenomegaly , hepatomegaly, and brain congestion with slightly swollen in PPMV infection ***(Mushtaq et al.,2006 and El-Bagoury et al., 2014)***; yet we did not find such changes in diseased pigeons except in 1 pigeon that was confirmed to be positive for PPMV.

Due to pathotypic variation of Newcastle disease virus in pigeons, virus detection and identification must be followed by the assessment of virulence by conventional in vivo method (determination of ICPI) ***(Rott and Klenk, 1988)***.

In this study, the virulence of PPMV-1 isolates for chicks had a wide range. The ICPI of day old SPF chicks ranged from zero to 1.43 with mean 0.858, so the majority of isolates in this study were mesogenic isolates. While ***Werner et al. (1999)*** reported that the ICPI values of 149 isolates identified as PPMV-1 varied between 0.71 and 1.65, with a fairly even distribution between 0.8 and 1.5**.** Overall, the virulence of the PPMV-1 isolates was slightly lower than that described by ***Alexander et al. (1985a)***, but within the range of 0.56 to 1.85 reported in a study of 409 isolates from pigeons ***(Alexander et al., 1994)***.

Based on our findings, it appears necessary to include PPMV-1 as one of the most serious disease problem in Egypt and we

should recommend a PPMV-1 vaccine for pigeons in Egypt as well as careful attention for preventing the similarities of such virus to chicken farms.

We can conclude from our results that PPMV-1 is a predominant disease of Egyptian pigeons, all isolated PPMV-1 is a mesogenic strains, and RT-PCR using primers design to F gene is a good and fast tools of PPMV-1 identification and the infected pigeons with PPMV-1 represent a source of infection for susceptible chickens.

REFERENCES

Al-mulhim, A.; Abu-Elzein, E.M.E.; Gameel, A.A.; Manvell, R.; Alexander, D.and Al-Afaleq, A.I. (2006). Observations on the Pigeon Paramyxovirus-1 Infection in pigeons at Al-Ahsa Oasis of Eastern Saudi Arabia. Journal of Animal and Veterinary Advances 5(12): 1058-1061

Abd El-Motelib, T.Y.; El-Ballal, S.S. and Galal, B.E.(1994). Herpes virus encephalities in pigeons. Assuit Veterinary Medical. Journal, 30(60): 306-319.

Abolniki, C.; Gerdes1, G.H.; Kitching, J.; Swanepoel, S.; Romito, M.; and Bisschop, S.P.R. (2008). Characterization of pigeon paramyxoviruses (Newcastle disease virus) isolated in South Africa from 2001 to 2006. Onderstepoort Journal of Veterinary Research, 75:147–152.

Abou Hashem , N.A.(1993). Studies on some paramyxovirus infections in pigeons M.V.Sc. Thesis , poultry Diseases, Department of Poultry and Fish Diseases Faculty of Vetrinary Medicine, Zagazig University.

Ahmed, H.N. and Sabri, I. (1969). Isolation of Newcastle disease virus from pigeons infected with pigeon pox. Journal of Veterinary Science, United Arab Republic, 6: 47-53.

Alexander, D.J and parsons G. (1984). Avian paramyxovirus type-1 infections of racing pigeons: 2 Pathogenicity experiments in pigeons and chickens. Veterinary Record, 114: 466-469.

Alexander, D.J. and Senne, D.A. (2008). Newcastle diseases, other avian paramyxoviruses, and pneumovirus infection. In Saif, Y.M.; Fadly, A.M.; Glisson, J.R.; McDougald, L.R.; Nolan, L.K. and Swayne, D.E. (eds.), *Diseases of Poultry*. 12, Iowa-United State of America: Blackwell Publishing, Ames. Ch.2: 75-93.

Alexander, D.J.; (1998). Newcastle disease virus and other avian paramyxoviruses. In Swayne, D.E; Glisson, J.R.; Jackwood, M.W.; Pearson, J.E. and Reed, W.M. (eds.), *A laboratory Manual For The Isolation and Identification Of Avian Pathogens*. 4, Kennett Square, Pennsylvania New Bolten Center, United State of America: The American Association Of Avian Pathologists, .156-163.

Alexander,D.J. (1991). Newcastle disease and other paramyxovirus infections. In Calenek,B.W; Barnes,H.J.; Beard,C.W; Reid,W.M. and Yoder,H.W,Jr.,(eds.), *diseases of poultry*. 9, Iowa-United State of America: Wolfe publications, Ch. : 496-519.

Alexander, D.J.; Parsons, G.; Manvell, R.J. and Sayers, A.R. (1994). Characterisation of avian paramyxovirus type 1 infections of racing pigeons in Great Britain during 1983 to 1990. *Proceedings of the*

European Commission meeting on Virus Diseases of Poultry-New and Evolving Pathogens, Brussels, 1992, 65-75.

Alexander, D.J.; Russell, P.H.; Parsons, G., Elzein, E.M.; Ballouh, A. and Cernik, K. (1985a). Antigenic and biological characterization of avian paramyxovirus type I isolates from pigeons: an international collaborative study. Avian Pathology, 14: 365-376.

Alexander, D.J.; Russell, P.H.; Parsons, G.; Abu Elzein, E.M.; Ballouh, A.; Cernik, K.; Engstrom, B.; Fevereiro, M.; Fleury, H.J.A.; Guittet, M.; Kaleta, E.F.; Kihm, U.; Kosters, J.; Lomniczi, B.; Meister, J.; Meulemans, G.; Nerome, K.; Petek, M.; Pokomunski, S.; Polten, B.; Prip, M.; Richter, R.; Saghy, E.; Samberg, Y.; Spanoghe, L. and Tumova, B. (1985). Antigenic and biological characterization of avian paramyxovirus type 1 isolates from pigeons—an international collaborative study. Avian Pathology, 14: 365–376.

Alexander, D.J.; Wilson, G.W.; Russell, P.H.; Lister, S.A. and Parsons, G. (1985b). Newcastle disease outbreaks in fowl in Great Britain during 1984. Veterinary Record, 117: 429-434.

Allan, W.H.; Lancaster, J.E.; and Toth, B. (1978). Newcastle disease vaccine their production and use. FAO animal Production and Health Series No.10 FAO, Rome, Italy.

Anonymous. (1984): Newcastle disease: outbreaks linked to pigeons. Veterinary Record, 114: 305-306.

Awad, A. M. (1995). Studies on viral infections in pigeonsM. V. Sc Thesis, Poultry Diseases, Faculty of Veterinary Medicine, Alexandria University.

Biancifiori, F.and Fioroni, A. (1983). An occurrence of Newcastle diseases in pigeon: Virological and serological studies on the isolates. Comparative Immunology, Microbiology and Infectious Diseases, 6: 247-252.

Briand, F.X.; Henry, A.; Massin, P.and Jestin, V. (2012). Complete genome sequence of a novel avian paramyxovirus. Journal of Virology, 86: 7710.

Collins, M.S.; Strong, I. and Alexander, D.J. (1994). Evaluation of the molecular basis of pathogenicity of the variant Newcastle disease viruses termed ‘‘pigeon PMV-1 viruses’’. Archives of Virology, 134: 403-411.

Creelan, J.L.; Graham, D.A. and McCullough, S.J. (2002): Detection and differentiation of pathogenicity of avian paramyxovirus serotype 1 from field cases using one-step reverse transcriptase-polymerase chain reaction. Avian Pathololgy, 31: 493-499.

Dortmans, J.C.; Rottier, P.J.; Koch, G. and Peeters, B.P. (2011). Passaging of a Newcastle disease virus pigeon variant in chickens results in selection of viruses with mutations in the polymerase complex enhancing virus replication and virulence. Journal of General Virology, 92: 336-345.

Eisa, M. and Omer, E.A. (1984). A natural outbreak of Newcastle disease in pigeons in Sudan. Veterinary Record, 114:297.

El-Bagoury, G.F.; El-Habbaa, A. S.; Ahmed, B.A. and Khodeir, M.H. (2014). Evaluation of quail susceptibility and

its role in transmission of pigeon paramyxovirus type 1. Benha Veterinary Medical Journal, 26 (2): 61-70.

El-Dahaby, H. and Sokkar, I.M.H.(1967). Natural infection of Newcastle disease in Egyptian pigeon. Journal of Veterinary Science of United Arab Republic , 4 (11):55-62.

Farkas, T.; Szekely, E.; Belak, S. and Kiss, I. (2009a). Real-time PCR-based pathotyping of Newcastle disease virus by use of TaqMan minor groove binder probes. Journal of clinical microbiology 47: 2114- 2123.

Irvine, R.M.; Aldous, E.W.; Manvell, R.J.; Cox, W.J.; Ceeraz, V.; Fuller, C.M.; Wood, A.M.; Milne, J.C.; Wilson, M.; Hepple, R.G.; Hurst, A.; Sharpe, C.E.; Alexander, D.J.and Brown, I.H. (2009): Outbreak of Newcastle disease due to pigeon paramyxovirus type 1 in grey partridges (Perdix perdix) in Scotland in October 2006.Veterinary Record, 165: 531-535.

Kaleta, E.F.; Alexander, D.J. and Russel, P.H.(1985). The first isolation of avian virus responsible for the current panzootic in pigeons Avian Pathology,14: 553-557.

Kommers, G.D.; King, D.J.; Seal, B.S. and Brown, C.C., (2001). Virulence of pigeon-origin Newcastle disease virus isolates for domestic chickens. Avian Diseases, 45: 906-921.

***Kommers, G.D.; King, D.J.; Seal, B.S. and Brown, C.C. (2003*).** Virulence of six heterogeneous-origin Newcastle disease virus isolates before and after sequential passages in domestic chickens. Avian Pathology, 32: 81-93.

Liu, M.; Guan, Y.; Peiris, M.; He,S. R. J. Webby, D. Pervez and Webster, R.G. (2003). The quest of influenza A viruses for new hosts. *Avian Diseases,* 47: 849–856.

Liu, H.; Wang, Z.; Son, C.; Wang, Y.; Yu, B. and Zheng, D., et al. (2006). Characterization of pigeon-origin Newcastle disease virus isolated in China. Avian Diseases, 50: 636-640.

Meulemans, G.; Van den Berg, T.P.; Decaesstecker, M. and Boschmans, M., (2002). Evolution of pigeon Newcastle disease virus strains. Avian Pathology, 31:515- 519.

Miller P.J.; Afonso, C.L.; Spackman, E.; Scott, M.A.; Pedersen, J.C.; Senne, D.A.; Brown, J.D.; Fuller, C.M.; Uhart, M.M.; Karesh, W.B.; Brown, I.H.; Alexander, D.J. and Swayne, D.E. (2010). Evidence for a new avian paramyxovirus serotype 10 detected in rockhopper penguins from the Falkland Islands. Journal of Virology, 84: 11496-11504.

Mohamed, M.A.; Sokkar, S.M.; Yousif, Y.I.; Saad, F. and Bastami, M.(1980). Contagious paralysis of pigeons. Apreliminary investigation on the occurance of disease in Egypt. Journal of the Egyptian Veterinary Medical Association, 40 (1): 45-51.

Mushtaq, I.; Rizvi, F. and Ullah, M.S. (2006). Effect of pigeon origin Newcastle disease on various liver enzymes and associated pathological changes in experimentally infected pigeons. Pakistan Veterinary Journal, 26(4): 171-175.

Nanthakumar, T.; Kataria, R.S.; Tiwari, A.K.; Butchaiah,G. and Kataria, J.M.(2000). Pathotyping of Newcastle Disease Viruses by RT-PCR and

Restriction Enzyme Analysis. Veterinary Research Communications, 24: 275-286.

OIE (2012). Manual of diagnostic Tests and Vaccines for Terrestrial animals, birds and bees. Biological Standard Commission, Vol.1, Part 2, Chapter 2.3.14. Paris. 1-19.

Rott, R. and Klenk, H.D. (1988). Molecular basis of infectivity and pathogenicity of Newcastle disease virus. In Alexander, D.J. (Eds.), Newcastle disease. 8, Boston, Dordrecht, London: Kluwer Academic Publishers. Ch. 6: 98- 112.

Shaheen, S; Anjum, A.D. and Rizv, F. (2005). Clinico-pathological observation of pigeons (Cloumba Livia) suffering from Newcastle Disease. Pakistan Veterinary Journal, 25(1): 5-8.

Shakal, M.A. (1989). Studies on paramyxovirus infection in pigeons in Egypt. Ph.D. Thesis , Poultry Diseases.Cairo University.

Tan, S. W.; Omar, A. R.; Aini, I.; Yusoff, K. & Tan, W. S. (2004). Detection of Newcastle disease virus using a SYBR Green I real time polymerase chain reaction. Acta Virologica, 48:23-28.

Tantawi, H.H. and Hassan, F.(1982). Pigeon herpes encephalomyelitis virus in egypt.Tropical Animal Health and Production, 14:20-22**.**

Terregino, C.; Aldous, E.W.; Heidari, A.; Fuller, C.M.; De Nardi, R.; Manvell, R.J.; Beato, M.S.; Shell, W.M.; Monne, I.; Brown, I.H.; Alexander, D.J.and Capua, I. (2013). Antigenic and genetic analyses of isolate APMV/wigeon/Italy/3920-1/2005 indicate that it represents a new avian paramyxovirus (APMV-12). Archive of Virology, 158: 2233-2243.

Ujva´ri, D.; Wehmann, E.; Kaleta,E.F.; Werner, O.; Savic´,V.; Nagy,E.; Czifra,G. Lomniczi, B.(2003). Phylogenetic analysis reveals extensive evolution of avian paramyxovirus type 1 strains of pigeons (Columba livia) and suggests multiple species transmission. Virus Research, 96: 63-73.

Werner, O.; Romer-Oberdorfer, A.; Kollner, B.; Manvell, R.J. and Alexander, D.J. (1999): Characterization of avian paramyxovirus type 1 strains isolated in Germany during 1992 to 1996. Avian Pathology, 28: 79-88.

الملخص العربى

دراسات على فيروسات الباراميكسو الجارى انتشارها فى الحمام

ريهام عبد المنعم البهنساوى، كامل ابراهيم ابو العزم، سناء سلامة عوض، عبد الفتاح حمدى العدل

قسم امراض الدواجن والارانب- كلية الطب البيطرى- جامعة المنصورة.

تمت هذه الدراسة على ٢٢ عينة من اجمالى ٥٠ عينة حمام أخذت هذه العينات من حمام يعانى من أعراض عصبية - الكسل - فقدان الشهية - عدم القدرة على الطيران - إسهال اخضر. تم تجميع الحمام المريض من محافظة الدقهلية خلال عامى ٢٠١٤ و٢٠١٥.

تم عزل فيروس الباراميكسو فيروس فى بيض دجاج مخصب خالى من المسببات المرضية بواسطة حقن كيس السقاء وتم التعرف على الفيروس بإستخدام إختبار التلازن الدموى وإختبار تفاعل إنزيم البلمرة المتسلسل العكسى كانت كل العينات الموجبة بإختبارالتلازن الدموى موجبة لفيروس الباراميكسو فى الحمام بإستخدام اختبار تفاعل انزيم البلمرة المتسلسل العكسى .

تم عمل إختبار الامراضية وذلك لخمس عينات بواسطة حقن الفيروس داخل المخ، تم إختبار ضراوة كل فيروس فى ١٠ كتاكيت خالية من المسببات المرضية . وتراوح مؤشر المرضية داخل المخ للخمسة عينات من ٠,٠ الى ١,٤٣. تم تجميع الأعضاء المختلفة (الكبد – الطحال – الرئة – المخ) من الكتاكيت التى أعدمت فى عمر ٨ أيام بعد الحقن للمجموعة التى أعطت ٠,٠ لمؤشر المرضية. بذلك تم التأكد من أنها سالبة لفيروس الباراميكسو، و بقياس مؤشر المرضية للأربعة مجموعات الأخرى على نفس الأعضاء أعطت نتيجة إيجابية لفيروس الباراميكسو فيروس وذلك باستخدام اختبارالبلمرة المتسلسل العكسى.