EVALUATION OF QUAIL SUSCEPTIBILITY AND ITS ROLE IN TRANSMISSION OF PIGEON PARAMYXOVIRUS TYPE 1.

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ABSTRACT

This paper aimed to study the susceptibility of quail to infection with pigeon paramyxovirus type-1 (PPMV-1) and their possible role for hazard spread of infection to pigeons. Quail showed high resistance to infection with PPMV-1 with mild clinical signs started by the 10th day post infection detected in 4 out of 20 qail and represented by dullness, ruffled feather, diarrhea and weakness but no nervous signs were observed. Recovery occurred to most of infected quail (19 out of 20) after 3 weeks post infection and one was died. In contact pigeons showed classical signs of PPMV-1 infection represented by greenish diarrhea, weakness and nervous signs. The morbidity and mortality rates were 20% and 5% respectively in quail and 25% and 20% respectively among in contact pigeons. PM lesion of died pigeons and quail showed congestion of internal organs with PPMV-1 was recovered from liver, spleen and kidneys of dead birds. Survived quail exhibited detectable HI and SNT antibody titers by the 1st week post infection. Vaccination of quail with the inactivated PPMV-1 vaccine resulted in good immune response of high antibody levels up to 10 weeks post vaccination although such levels were found to be lower than those induced by the same vaccine in pigeons.

Keywords: PPMV-1, Quail,Pigeons.

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1. INTRODUCTION

Pigeon paramyxovirus type 1 (PPMV-1) are variant strains of Newcastle Disease virus associated with infections of pigeons. They were classified in genus Avulavirus, sub-family Paramyxovirinae, family Paramyxoviridae (Lamb et al., 2005). The virus remained enzootic in racing pigeons in many countries, with regular spread to wild pigeons and doves and a continuing threat to poultry. Diarrhea and nervous signs were the main signs of PPMV-1 infections, preceded by marked drops in egg production in laying hens (Alexander and Parsons, 1984).

Although this virus is generally of no or low virulence in chickens, it may gain virulence during spread in flocks and considered as a hidden threat to poultry industry (Dortmans et al., 2010).

PPMV-1 was isolated and identified from outbreaks of nervous manifestations among pigeons in Egypt (Shakal, 1989; Moustafa et al., 2003; Ali, 2004; Gaber, 2004 and El-Morsi,, 2006). Despite the extensive and worldwide spread of quail farming, the epidemiology of PPMV-1 among these birds has been still questionable. The role of quail in transmission of PPMV-1 has not yet been

studied. So, the aim of the present work was directed to study the susceptibility of quail to infection with PPMV-1 and their possible role for hazard spread of infection to pigeons as well as evaluation of quail immune response to the inactivated pigeon paramyxovirus vaccine.

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1. Viruses:

2.1.1.1.Virulent pigeon paramyxovirus (PPMV-1):

A local isolate of PPMV-1 with a titer of 10^8 EID_{50}/ ml was supplied by Veterinary Serum and Vaccine Research Institutes, Abbasia, Cairo. This virus was used for experimental infection of quail.

2.1.1. 2.Cell culture adapted PPMV-1:

A cell culture adapted PPMV-1 with a titer of 10^8 $TCID_{50}$/ ml in African green monkey **(**VERO) cell line (Amer, 2008). It was used to monitor the levels of induced antibodies both in infected and vaccinated quail and pigeons using serum neutralization test (SNT).

2.1.2. Inactivated pigeon paramyxo vaccine:

A locally produced egg adapted inactivated pigeon paramyxo vaccine was supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. It was used for vaccination of experimental quail and pigeons at a recommended dose of 0.5 ml/bird by subcutaneous inoculation.

2.1.3.Embryonated Chicken Eggs (ECE):

Specific Pathogen Free (SPF) ECE (9 days old) were kindly supplied by Veterinary Serum and Vaccine Research Institutes, Abbasia, Cairo. These eggs were used in trials for virus recovery from experimentally infected birds.

2.1.4. Experimental birds:

2.1.4.1.Quail:

Fifty quail (30-45 days old) were supplied by a privet farm and reared under strict hygienic measure in an isolated and disinfected wire floored cages and checked serologically for freedom from pigeon paramyxovirus antibodies and used in the experiment.

2.1.4.2.Pigeons:

Fifty native breed pigeons of native breed (3 weeks old) were purchased from local market and checked serologically for freedom from pigeon paramyxovirus antibodies and used in the experiment.

2.1.5. VERO cell line:

These cells were established by Yasumara and Kawatika (1963) and kindly obtained from the Department of pet Animal Vaccine Research, Abbasia Cairo. VERO cells were used in virus recovery and SNT.

2.1. 6.Chicken erythrocyte suspension:

Red blood cells from adult susceptible chicken were collected through the wing vein puncture on 4% sodium citrate as anticoagulant (1 ml of anticoagulant + 9 ml blood). An equal amount of physiological saline (0.85%), pH 7 was added to suspension and the cells were subjected to 3 cycles of washing and centrifugation in physiological saline at 800 rpm for 15 minutes. The packed red cells were then diluted in saline as 1% suspension for haemagglutination (HA) and haemagglutination inhibition (HI) tests in micro titer haemagglutination plates.

2.2. Methods:

2.2.1.Experimental design:

2.2.1.1. Experimental infection of quail with PPMV-1:

Fifty quail were divided into three groups and treated as follow:

Group (1): Twenty quail infected with virulent PPMV-1 strain using a dose of 0.5 ml/bird injected intramuscularly according to *Amer (2008*). These birds were

kept under observation for daily detection of clinical signs and mortality for 21 days.

Group (2): Twenty quail were subcutaneously vaccinated with inactivated PPMV-1 vaccine with 0.5 ml/bird inoculated backward in the dorsal aspect of the neck according to Amer (2008). The quail were weekly examined serologically up to 10 weeks post vaccination for antibody response. Ten quail of this group were challenged 3 weeks post vaccination against the virulent PPMV-1 while the other 10 quail were kept to follow up the level of induced antibodies.

Group (3): Ten quail were kept separately under hygienic measures without infection or vaccination as control.

2.2.1.2. Exposure of pigeons to experimental work:

The experimental pigeons were divided into three groups as follow:

Group (1): Twenty pigeons was housed in contact with the infected quail.

Group (2): Twenty pigeons was vaccinated with PPMV-1 vaccine with 0.5 ml/bird inoculated subcutaneously according to Amer (2008).

Group (3): Ten pigeons without vaccination and without infection was kept separately under hygienic measures as control.

2.2.2. Sampling:

2.2.2.1.Samples for virus recovery:

Specimens of lungs, trachea, liver, brain, and kidneys, were collected from freshly dead birds for virus recovery after application of the post mortem examination.

2.2.2.2.Serum samples:

Blood samples for serum collection were obtained from all bird groups one week intervals post application of the experimental work.

2.2.3.Virus recovery:

2.2.3.1.Egg inoculation:

The supernatant fluid obtained from the prepared specimens was inoculated at 0.2 ml/egg into 9 days old SPF embryonated chicken eggs through the allantoic sac as described by Anon (1971). Five eggs per sample were used. The eggs were sealed with liquid paraffin and incubated at 37.8 °C for 6 days with daily candling. Embryos died 24 hours post inoculation were considered as non specific deaths. Specificity of embryonic deaths was determined by detection of hemagglutinins in the allantoic fluid harvested from dead embryos using HA and HI tests.

2.2.3.2. Cell culture infection:

The obtained infected suspension of the prepared tissue specimens was inoculated in Vero cell culture three successive times where the obtained cytopathic effect was recorded and fully characterized through staining of infected cell culture.

2.2.4. Standard quantitative haemagglutination test:

This test was carried out for determination of the HA unit using 1% washed chicken erythrocyte suspension in saline. Two fold dilutions of the antigen in saline (1:2 to 1:2048) were prepared in U-form micro-plates and mixed with equal volumes (0.05 ml) of washed erythrocytes. End points were read after 30 minute at 4 °C according to Anon (1971).

2.2.5. Haemagglutination inhibition (HI) test:

It was done using the Beta procedure (constant virus plus diluted serum). The test was carried out according to Anon (1971) for titration of serum against PPMV-1.

2.2.6.Serum Neutralization Test (SNT):

SNT was performed in Vero cell culture using micro-technique according to Ferreira (1976) in flat bottom tissue culture micro titer plates for titration of serum against PPMV-1. The end point of neutralizing antibody titer was expressed as the reciprocal

of the final dilution of serum inhibiting the CPE.

3. RESULTS

3.1. Clinical examination of infected birds:

Experimentally infected quail showed clinical signs as weakness, diarrhea and ruffled feather as shown in photo (1). Morbidity and mortality rates were 20% and 5%, respectively. Quail could transmit infection to the pigeons in same cages in which pigeons showed typical signs of pigeon paramyxo viral disease as weakness, diarrhea, ruffled feather and nervous signs as shown in photo (2). Morbidity and mortality rates were 25% and 20%, respectively.

3.3. Examination of post-mortem lesions in dead birds:

PM lesion of died pigeon and quail showed congestion of internal organs (lung, liver, heart and proventriculus) as shown in photo (3).

3.4. Recovery of the virus from tissue samples of dead birds after isolation:

The virus was recovered from brain, liver and kidneys of died birds after serial passage in SPF embryonated chicken eggs and Vero cells that was detectable using HA test as shown in table (1).

3.5. Detection and titration of antibodies against PPMV-1 in survived quail and pigeons:

Survived quail and pigeons showed detectable HI and SNT antibody titers from the first week and up to 3 weeks post infection as shown in tables (2) and (3). Survived quail developed lower mean neutralizing antibody titers against PPMV-1 than that was developed in survived pigeons.

3.6. Evaluation of humoral immune response in quail and pigeons vaccinated with inactivated PPMV-1 vaccine:

Quail subjected to a trial of vaccination with inactivated PPMV-1 vaccine developed an increasing mean HI and serum neutralizing antibody titers (2 $\log_2$/ml and < 2, respectively) from the 1st week post vaccination, reached their peaks by the 6th week post vaccination (8 $\log_2$/ml and 32, respectively) and persisted up to 10 weeks post vaccination (the experimental period). However, such levels were found to be lower in quail than those induced by the same vaccine in pigeons as shown in tables (4) and (5).

Photo (1): Quail infected with PPMV-1 showing dullness and ruffle feather.

Photo (2): In contact pigeon to experimentally infected quails with PPMV-1 showing torticolis.

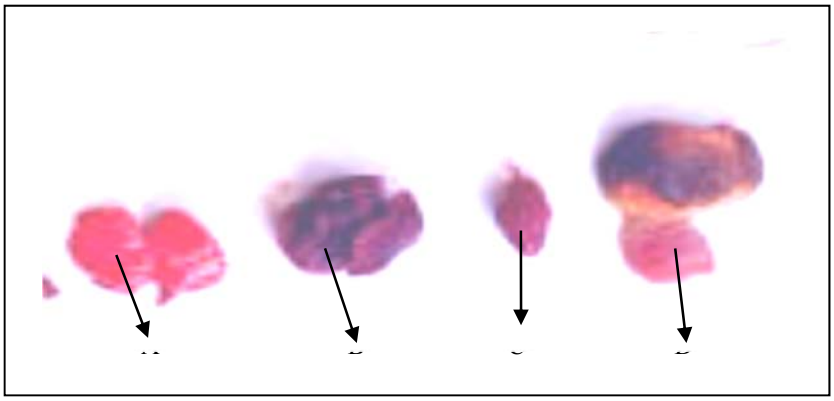

Photo (3): A- Lung B- liver C- heart D- proventriculus of Japanese quail infected with PPM-1virus showing congestion.

Table (1): Recovery of PPMV-1 from experimentally infected quail.

Tested Samples	Detection of the virus using HA test after serial passage					
	First passage		Second passage		Third passage	
	*ECE	**VERO	ECE	VERO	ECE	VERO
Liver	-	-	+	+	+	+
Spleen	+	+	+	+	+	+
Kidney	+	+	+	+	+	+

*ECE: Embryonated Chicken Eggs.
**VERO: VERO cell line.

Table (2): Serum HI antibody titers against PPMV-1 in survived birds.

Survived Bird	*Mean HI antibody titer against PPMV-1		
	1WPI**	2WPI	3WPI
Quail	2	4	5
Pigeons	3	4	5

*Mean HI antibody titer $\log_2$/ml = the reciprocal of the final serum dilution which inhibited agglutination of washed RBCs using 4HA unites of PPMV-1.
**WPI= week post infection.

Table (3): Serum neutralizing antibody titers against PPMV-1 in survived birds.

Survived Bird	*Mean neutralizing antibody titer against PPMV-1		
	1WPI**	2WPI	3WPI
Quail	2	4	16
Pigeons	8	16	32

*Mean serum neutralizing antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of PPMV-1.
**WPI= week post infection.

Table (4): Humoral immune response in vaccinated quail and pigeons using HI test.

*WPV	*Mean HI antibody titer against PPMV-1										
	0	1	2	3	4	5	6	7	8	9	10
Quail	**0	2	4	5	6	7	8	8	8	8	8
Pigeons	0	2	3	4	8	9	9	9	9	9	9

*WPI= week post infection.
**Mean HI antibody titer $\log_2$/ml = the reciprocal of the final serum dilution which inhibited agglutination of washed RBCs using 4HA unites of PPMV-1.

Table (5): Humoral immune response in vaccinated quail and pigeons using SNT.

*WPV	*Mean neutralizing antibody titer against PPMV-1										
	0	1	2	3	4	5	6	7	8	9	10
Quail	**0	<2	2	4	8	16	32	32	32	32	32
Pigeons	0	4	8	16	32	64	64	64	64	64	64

*WPI= week post infection.
**Mean serum neutralizing antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of PPMV-1.

4. DISCUSSION

Despite of the still questionable epidemiology of pigeon paramyxovirus type 1 (PPMV-1) among quail, some papers showed high resistance of quail to infection with virulent PPMV-1 and could not transmit the infection to contact pigeons in the same cages (Tawfik et al., 2004). This could be attributed to the density of population, infective virus dose and virulence of the infecting virus as suggested by Alexander et al. (1985).

Our results revealed that quail showed susceptibility to PPMV-1 infection with low pathogenicity and mild clinical signs started by the 10th day post infection. These signs were detected in 4 out of 20 quail represented by dullness, diarrhea, ruffled feather and weakness with no nervous signs were recorded. These results agreed with those of Usman et al., (2008) who found that the major clinical signs in all affected quail were dullness and ruffled feathers .

Pigeons, in contact with infected quail, showed classical signs of PPMV-1 represented by greenish diarrhea, weakness and nervous signs in the form of torticollis and paralysis of the wings and legs. These results agreed with those of Biancifiori and Fioroni (1983).

Recovery occurred to most of quail (19 out of 20) after 3 weeks post infection and one was died. A total of 4 out of 20 in contact pigeons died. Monitoring of morbidity and mortality rates among experimentally infected quail and in contact affected pigeons revealed that the morbidity and mortality rates were 20% and 5% respectively in quail and 25 % and 20% among in contact pigeons. In this respect Biancifiori and Fioroni (1983) concluded that PPMV-1 showed high pathogenicity for pigeons (55% mortality) and low pathogenicity for quail.

Post mortem findings of dead quail revealed that lesions were detected in the lungs, spleen, proventriculus and intestine with the vent of such quail was solid with greenish feces. On the other hand PM lesions in pigeons kept in contact with infected quail showed congestion of internal organs “lung, heart and brain”. Similar findings were described by Alexander and Parsons (1984); Awad (1992); Abou Hashem (1993); Hassan (1997 and 2001) and Amer (2008) who reported that postmortem findings were represented by hemorrhages in the brain and congestion of most internal organs. Usman et al., (2008) found that post mortem lesions in affected quail included hemorrhagic lesions of the intestinal tracts and proventriculus and greenish diarrhea .

PPMV-1 was recovered from liver, spleen and kidneys of dead birds through the inoculation into allantoic cavity of embryonated chicken eggs and Vero cell culture for 3 successive passages. Standard quantitative haemagglutination test was carried out to confirm the virus incidence; on the recovered virus from collected allantoic fluid of inoculated embryonated chicken eggs and cell culture infected fluid. Methods used to recover PPMV-1 from specimens of dead affected quail and pigeons agree with Alexander and parsons (1984) and Alexander et al (1985).

It was found that survived quail exhibited detectable antibody titers of 2, 4 and 5 $\log_2$/ml using HI and 2, 4 and 16 using SNT by the 1st, 2nd and 3rd weeks post infection, respectively. Parallel to these findings, in contact survived pigeons exhibited detectable antibody titers of 3, 4 and 5 $\log_2$/ml using HI and 8, 16 and 32 using SNT by the 1st, 2nd and 3rd weeks post infection, respectively. These results could be attributed to the natural resistance of quail to PPMV-1 infection reacting against it by induction of antibodies although with low

titer and come in agreements with findings of Dortmans et al., (2011).

Vaccinated quails showed lower immune response to PPMV-1 vaccine than pigeons where they exhibited detectable antibody titers of 2 log_2/ml and <2 using HI and SNT, respectively on the 1st week post vaccination and 8 log_2/ml and 32 using HI and SNT, respectively on the 6th week post vaccination that remaining constant up to 10 weeks post vaccination. Vaccinated pigeons exhibited detectable antibody titers of 2 log_2/ml and 4 using HI and SNT, respectively on the 1st week post vaccination and 9 log_2/ml and 64 using HI and SNT, respectively on the 6th week post vaccination that remaining constant up to 10 weeks post vaccination. The lower obtained levels of PPMV-1 antibodies in vaccinated quails than in vaccinated pigeons could be attributed to the species susceptibility. The recorded results come in agreement with Knoll et al (1985) who concluded that pigeons immunized with homologous oil emulsion vaccine against PPMV-1 infection, and Hassan (1997 and 2001) and Amer (2008) who monitored the induced immunity in vaccinated pigeons with the inactivated cell culture PPMV-1 vaccine; it was found that the vaccine was able to induce detectable levels of specific PPMV-1 antibodies by the first week post vaccination (titer of 4 by SNT and 8 by HI). These antibodies were increased to reach a titer of 32 by SNT and 64 by HI on the third week post vaccination then decreased to 16 and 32 respectively after application of the challenge test.

It could be concluded that quail could be infected with PPMV-1 to less extent than pigeons and could transmit the infection to pigeons. Quail respond to PPMV-1 vaccine in a level lower than that in pigeons. It could be suggested that on the long term the virus may circulate in quail and increase in virulence resulting in disease outbreaks and according to these findings, it could be recommended to vaccinate quail against PPMV-1.

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تقييم قابلية السمان للاصابة ودوره في نقل عدوى فيروس باراميكسو الحمام (1)

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الملخص العربي

تهدف هذه الدراسة الى معرفة مدى استجابة السمان للإصابة بمرض فيروس باراميكسو الحمام (1) ودور السمان فى إمكانية نقل العدوى للحمام المخالط له. وقد أظهر السمان قابلية للإصابة بهذا المرض حيث ظهرت عليه أعراض بسيطة فى اليوم العاشر بعد الحقن فى 4 من 20 من السمان المحقون بالفيروس تمثلت فى الخمول وانتفاش الريش والاسهال والضعف والهزال دون ظهور اى اعراض عصبية. وقد زالت الاعراض عن معظم السمان (19 من 20) بعد ثلاثة أسابيع من العدوى بينما نفقت سمانة واحدة. وقد استطاع السمان المصاب نقل العدوى إلى الحمام المخالط والذى ظهرت عليه العلامات التقليدية للإصابة بمرض فيروس باراميكسو الحمام نوع 1 والمتمثلة فى الاسهال الاخضر والضعف والأعراض العصبية. وقد كانت نسب الاصابة والنفوق 20% و 5% على الترتيب فى السمان و 25% و 20% على الترتيب فى الحمام المخالط. بعمل الصفة التشريحية للسمان والحمام النافق وجد احتقان في الاعضاء الداخلية وقد أمكن عزل الفيروس من كبد وطحال وكلى الطيور النافقة .وقد أظهرت طيور السمان /الحمام الحية نتائج ايجابية لعيارية الاجسام المضادة لفيروس بارامكسو الحمام بإستخدام إختبارى تثبيط التلزن الدموى والمصل التعادلى من الاسبوع الاول بعد العدوى. وعند تحصين السمان بلقاح باراميكسو الحمام المثبط اظهرت النتائج استجابة سيرولوجية عالية إستمرت لمدة عشرة أسابيع بعد التحصين ولكن اقل من مثيلتها فى الحمام المحصن.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(2):61- 70, يونيو 2014)