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Comparative Study on the Clinico-Pathological Response of the Collared Dove (*Streptopelia roseogrisea arabica*) and Pigeons (*Columba livia*) to Experimental Infection with the Pigeon Paramyxovirus-1

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Abstract: The present study constitutes the first record of susceptibility of the dove species *Streptopelia roseogrisea arabica* to the Pigeon Paramyxovirus-1 (PPMV-1). This dove is native to Africa and the Arabian Peninsula. So, it was novel to explore its susceptibility to the PPMV-1, as it may have an epidemiological significance for this disease in the region and internationally. The close comparative study, to examine the response of this dove in relation to that in pigeons to the PPMV-1, indicated that both species succumbed to the disease. Typical clinico-pathological picture was recorded in both species. However, variation was noted and discussed. The virus was reisolated from all the infected birds of the two species and they seroconverted. The possible role of this dove species, in the epidemiology of the PPMV-1 in the region was discussed.

Key words: Comparative study, collared doves, pigeons, pigeon paramyxovirus-1

INTRODUCTION

The collared dove (*Streptopelia roseogrisea arabica)*, (Fig. 1), is found in large numbers in date-palm plantations of most oases of the Kingdom of Saudi Arabia (KSA), where there is plenty of food and water. They are also found in other parts of the Kingdom.

The most deadly and widespread disease of pigeons in KSA, is the pigeon paramyxovirus-1 (PPMV-1)[1,2].

In spite of the close proximity of the doves with pigeons, in KSA, the epidemiological situation of the PPMV-1 infection in doves is not known in the country. However, a veterinarian mentioned that during 1995, he saw a dove (locally called *Bidy*), which entered the premises of pigeons that were suffering from PPMV-1 infection, showed typical clinical signs of PPMV-1 infection (torticollis, raffled feather, diarrhea, depression and shaking).

The present study was under taken to examine susceptibility of the collolared dove (*Streptopelia roseogrisea arabica*) to the PPMV-1 as compared to the disease in pigeons. The results are expected to give an idea about the possible role of this dove in the epidemiology of PPMV-1 in the region. This is particularly important, as this dove species is widely distributed in KSA and the Arabian peninsula and can come in close contact with pigeons and possibly other domestic or feral avian species.

Fig. 1: A healthy collared dove (*Streptopelia roseogrisae arabica*)

MATERIALS AND METHODS

Experimental pigeons: Apparently health locally-bred adult pigeons (*Columba livia*), were procured from

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apparently healthy non-vaccinated flock. The pigeons were seronegative when tested against the PPMV-1 isolate which we used in the present experiments, in the Haemagglutination Inhibition (HI) tests[4].

Experimental collared dove (*Streptopelia roseogrisea arabica*): These were adult doves, obtained from an apparently healthy flock. They were seronegative in the HI tests , when tested against the PPMV-1 virus strain, which we used in the present study,.

The Pigeon Paramyxovirus-1: This was isolated from a field outbreak in pigeons at Al-Ahsa oasis, Eastern Saudi Arabia. It was isolated and identified, locally, as an avian paramyxovirus-1, in the HI tests employing polyclonal hyperimmune serum against the La Sota Newcastle Disease (ND) vaccine virus. Then it was sent to the World Reference Laboratory for avian paramyxovirus-1, Weybridge, Surrey, UK., where it was classified as pigeon paramyxovirus-1 (PPMV-1), P-group[4].

Specific Pathogen Free eggs (SPF): These were provided by the Vaccine Production Centre, Ministry of Agriculture and Waters, Riyadh.

Chicken Red Blood Cells (RBSs): These were obtained from white leg horn chickens. The RBCs were prepared and used at 1% in normal saline as described by Cunningham[5].

Propagation of the PPMV-1 in SPF chicken embryo: The PPMV-1 was propagated and titrated in the SPF chicken embryos, by inoculation via the allantoic sac as described by Cunningham[5]. The egg lethal dose 50 (ELD_{50}) was calculated as described by Reed and Muench[6] and the virus was used at a titre of 10^7 ELD_{50} in the experimental infection studies. It was designated the inoculum in the subsequent experiments.

Experimental infection of pigeons: Fifteen adult pigeons were used in the experiment. They were divided into three groups (A,B and C). Each comprised 5 pigeons. Group 'A' were inoculated Intramuscularly (IM) with 0.2 mL each, of the inoculum; and each of group 'B', received 0.2 mL of the inoculum, orally. Group 'C' pigeons were given normal saline 0.2 mL each orally and kept as controls. Each group was kept in a separate confinement and were given food and water *ad libitum*.

Experimental infection of doves: Fifteen adult doves were used in the experiment. They were also divided into three groups (D, E and F). Groups D and E were inoculated IM and orally respectively as described for pigeons above, using the same inoculum and dose. Group F were left as control and were treated as group 'C' above. Each group was kept in a separate room and food and water were provided *ad lib*.

Each of the inoculated doves and pigeons was observed daily for any abnormality or death.

Cloacal swabs were taken from experimentally infected pigeons and doves on days 3, 7, 14, 21 and 28 post infection. Sera from surviving pigeons and doves infected I/M and per OS were collected on day 28 post infection and examined for presence of antibodies against the isolated PPMV-1, in the HI tests, as described below.

Pathological methods: Thorough post mortem examination was performed in dead or moribund pigeons and doves. All internal organs were inspected. Muscles, air sacs and joints were also examined. Whole brain and tissue samples were taken from heart, lungs, intestines, spleen, kidneys, liver, pancreas, pectoral muscles and eye. They were fixed in 10% aqueous formalin for histopathological examination. They were then trimmed and processed in paraffin using an automatic tissue processor. The aid of a rotary microtome and stained with haematoxylin and eosin (H&E)[7].

Portions of brain and spleen were collected in sterile containers and used for virus isolation.

Virus isolation from dead or moribund pigeons and doves: Brains and spleens from dead or moribund doves or pigeons were separately processed as 10% suspensions in Phosphate Buffered Saline (PBS) pH 7.4. Following clarification at 1500 r.p.m for 10 min, the supernatant was collected, antibiotics added (Penicillin G Sodium 10,000 mg mL) and Streptomycin sulphate (25 mg mL) and used to inoculate SPF eggs via the allantoic sac as described by Hanson[4] . The eggs were then incubated at 37°C and observed daily. Eggs dying within the first 24 hrs were discarded; those dying afterwards were collected and kept at 4°C for 24 hrs before the allantoic fluid was collected and stored at-86°C.

Each cloacal swabs was soaked in 1 mL of PBS pH 7.4- clarified at low centrifugation, processed as described above and inoculated into SPF via the allantoic sac. Subsequent processing of the eggs was as described above.

Virus identification: The HA and HI were performed as described by Hanson[4], using chicken RBCs, to identify the isolated virus employing polyclonal chicken serum against the La Sota ND vaccine strain.

Seroconversion: Convalescent sera from pigeons or doves (28 day post inoculation sera) were tested for presence of antibodies against the PPMV-1 strain, which was used in the experimental infection studies, using the HI as described by Hanson[4].

RESULTS

Experimental infection of pigeons

Clinical signs: The incubation period, morbidity and mortality rates are shown in Table 1. Following the incubation period, in both routes, the inoculated pigeons showed raffled feather and anorexia. This was followed by watery greenish diarrhea on day 6 Post Inoculation (P.I) From day 7 P.I. the nervous signs were apparent. These consisted of incoordination, dropping of wings, slight torticollis which became severe on the following days (Fig. 2). Tremor and leg paralysis were also seen. Pigeons of the IM route survived until they were killed on day 30 P.I. Two of the PO pigeons died between days 10 and 13 P.I.

Virus isolation and identification: A virus was isolated from the brains and spleens of the inoculated pigeons from both groups, A and B. Virus was also isolated from the cloacal swabs from the survived pigeon of both inoculated groups (A and B) up to day 28 P.I.

The isolated virus was identified as avian paramyxovirus-1, using the polyclonal anti La Sota ND vaccine strain in the HI tests.

Seroconversion: Antibodies against the PPMV-1 isolate were detected in the 28 P.I sera of the survived pigeons at a titre that ranged between 1/512 and 1/1024 .

Pathological investigations in pigeons

Gross description: Dead or killed pigeons from both groups (A and B) appeared dehydrated and the skin was not readily removed. Muscles, particularly the pectoralis muscle, were dark brown in colour. In some birds, the pectoralis muscle showed some haemorrhages.

The lungs showed congestion and patches of haemorrhages. The intestinal serosa exhibited yellowish mottling and occasional haemorrhages. The contents were watery. Liver was pale in colour and kidneys appeared oedematous. The brain showed marked congestion.

No salient gross lesions were observed in the other organs.

Histopathological findings in pigeons: Both inoculated groups of pigeons showed similar lesions. Lung sections

Fig. 2: An experimentally infected pigeon holding its head under its body

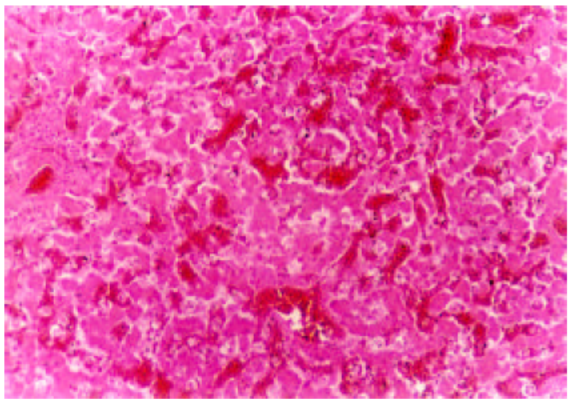

Fig. 3: Liver section showing marked sinusoidal congestion (HE X 160)

showed severe congestion and free erythrocytes. The primary bronchi showed active mucous glands. The secondary bronchi and parabronchi showed dilated lumina with flattening of the atria and occasionally thickening of atrial muscles. Peribronchial lymphoid hyperplasia was observed mainly around primary bronchi, usually in the form of lymphoid nodules.

The heart showed subepicardial lymphocyte infiltration.

Vacuolations were seen in muscular tunic of the duodenum with slight round-cell infiltration of the serosa. In some infected pigeons, the small intestine showed mononuclear cell infiltration in the tunica muscularis, specially inner muscles.

The caecum showed increased cellularity of lamina propria with distinct caecal tonsils. Muscular tunic was also infiltrated by mononuclear cells.

Congestion and parenchymal vacuolations were seen. Mural vacuolations were seen in large arteries.

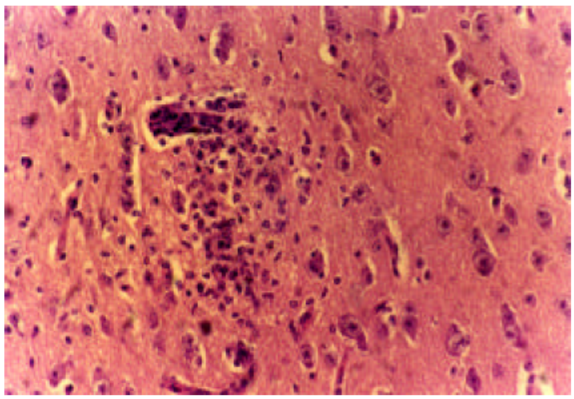

Fig. 4: Cereberum showing focal degeneration and glial cell proliferation (HE X 320)

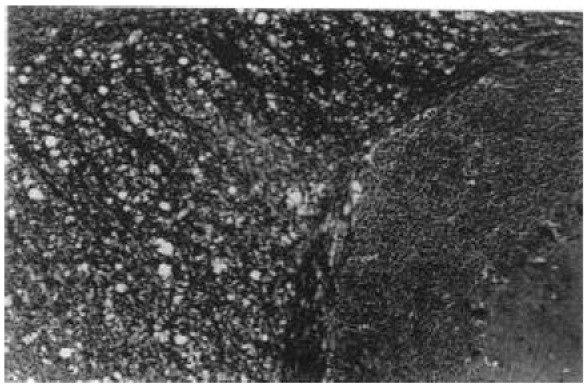

Fig. 5: Cerebellum exhibiting vacuolations in white matter. Note indistinct Purkinji cells (top left) (HE X 160)

Severe congestion and sinusoidal dilatation, hepatocytes swelling and vascuolar degeneration were observed in liver sections (Fig. 3). Focal areas of cellular necrosis with infiltration of lymphocytes and macrophages were also noticed. In many sections portal triads were enlarged, with mononuclear cell infiltration and proliferation of bile ducts; the latter were dilated and lined by more than one layer of epithelium.

Kidney sections showed multiple granulomas scattered throughout the renal parenchyma particularly the cortex. Diffuse interstitial infiltration of mononuclear cells was also noticed.

Slight interstitial cell proliferation was seen in most sections of the pectoral muscle. Two cases showed muscle degeneration and areas of haemorrhages.

All brain sections exhibited severe congestion and capillary proliferation.

In the cerebrum, some neurons showed evidence of degeneration characterized by central chromatolysis and

Fig. 6: An experimentally infected dove showing complete bending of the head to the back, depression, diarrhoea and raffled feather

Table 1: Morbidity, mortality and incubation period of the inoculated doves and pigeons

Species and route	I P	Morbidity rate	Mortality rate
Pigeons (I/M)	3 d	5*/5 (100%)	**0/5 (0%)
Doves (I/M)	1 d	5/5 (100%)	1/5 (20%)
Control Doves	-	0/5	0/5
Pigeons (P/O)	3 d	5/5 (100%)	2/5 (40%)
Doves (P/O)	2 d	5/5 (100%)	2/5 (40%)
Control Pigeons	-	0/5	0/5

IP =Incubation Period
d =day
I/M =Intramuscularly
P/O =Per Os
* =Ailing / No. inoculated
** =No. dead / No. inoculated

rounding of cells. This was associated with glial cell proliferation and satellitosis. proliferation of astocytes was also seen (Fig. 4).

In the cerebellum, some Purkinje cells showed degenerative changes; nuclei and cell boundaries were blurred. The constant finding was vacuolation of the white matter, especially in between the folia. In vacuolated areas glial cells (oligodendrocytes) were observed to be sparse (Fig. 5).

Experimental infection in doves

Clinical signs: Table (1) shows the incubation period, morbidity and mortality rates.

The incubation period in doves was one day for the IM route and two days for the PO route.

The first obvious sign, in the group of doves inoculated IM, was diarrhea on day 2 PI. This was accompanied by depression and raffled feather (Fig. 6).

From day five PI nervous signs were evident; these included nodding, wing shaking and slight torticollis.

The first signs to be seen on doves, inoculated orally, were nodding of head, raffled feather and severe

depression. Diarrhoea was detected on day 5 PI. In the progress of the disease slight nervous signs were seen. These were wing shaking and slight torticollis (Fig. 7).

Virus reisolation and identification: Virus was isolated from rectal swabs of both groups of doves up to day 28 PI. The isolated virus was identified as avian paramyxvirus-1 using polyclonal serum against the La Sota ND vaccine virus in the HI test described by Hanson[4].

Seroconversion: Antibodies against the PPMV-1 virus isolate were detected in the 28 PI sera of the survived doves at a titre ranging between 1/512 and 1/1024.

Pathological investigations in doves

Gross pathological lesions in doves: The gross pathological lesions seen in both groups of the experimentally infected doves, were similar to those seen in pigeons.

Histopathological findings in doves: Doves infected I/M and P/O showed similar lesions, but of a lesser grade, to those in experimental pigeons in the liver, kidney and lungs.

The kidney sections showed congestion and few aggregates of lymphocytes in the cortex. Lymphoid nodules were also seen in the lung sections. The liver showed slight cell lymphocyte degeneration with mild portal infiltration of round cells.

The muscles showed variable degrees of interstitial cell proliferation associated with myodengeneration at some places.

Brain sections showed congestion, mild glial cell proliferation in cerebrum and slight vacuolations in white matter in cerebellum.

No lesions could be seen in the intestines.

DISCUSSION

The collared dove (*Strrptopelia roseogrisea arabica*) is widely distributed in the Arabian Peninsula and Africa. They often come in contact with domestic poultry such as backyard chickens and domestic pigeons (*Columba livia*). They can also visit large poultry farms, seeking food.

At Al-Ahsa oasis, these doves are found in large numbers especially in date-palm plantations. They come in-contact with domestic pigeons. In spite of this and although PPMV-1 infection is widespread in pigeons in the area, no outbreaks of the disease were reported in doves. However, the disease could have been overlooked in them.

Accordingly and since the PPMV-1 situation in doves in KSA is not known and that the international literature does not mention any record of the natural nor experimental susceptibility of this dove species (*Streptopelia roseogrisea arabica*) to PPMV-1, we thought of conducting the present study.

This study confirmed and for the first time, susceptibility of the dove *Streptopelia roseogrisea arabica* to PPMV-1 infection.

This information is not only significant to the local epidemiology of PPMV-1 in the Arabian peninsula, but also to the international epidemiology of the disease.

The clinical signs, explicited by the experimentally infected doves, were similar to those seen in the experimentally and naturally infected pigeons. However, the incubation period was shorter in doves (one day for the IM group and 2 days for the PO group).

The clinical signs seen, in both the PO and the IM routes in both species, were depression, raffled feather, nervous signs and diarrhea. Although these signs were seen in the two species still some variation was evident. The doves showed marked raffled feather and depression than the pigeons. The most prominent nervous sign in doves was nodding. Torticollis in doves was slight (Fig. 7), while in pigeons it was so pronounced (Fig. 2).

Both pigeons and doves excreated virus up to day 28 PI. This aids in contamination of the environment and spread of the disease.

In the two species, the morbidity rate was 100%, for both routes. The mortality rate was similar in both species (40%) when received virus PO. However, one dove (20%) died of those inoculated IM, while all pigeons, which received the virus I M, survived.

All the survived pigeons and doves showed nervous sequelae that was characterized by various degrees of torticollis, imbalance and ataxia.

From the foregoing, it could be concluded that the dove, *Streptopelia roseogrisea arabica*, which is native to the Arabian Peninsula and Africa, can highly likely play a role in the epidemiology of the PPMV-1 infection in the region.

Further studies on the exact role of this dove in the epidemiology of PPMV-1 in KSA must be fully understood.

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