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Isolation and Identification of Pigeon Paramyxovirus-1 From kafrelsheikh Province, Egypt

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Abstract: The aim of the present study was the isolation and identification of Pigeon Paramyxovirus-1 (PPMV-1) viruses from Kafrelsheikh province, Egypt in the period between 2014 and 2017. Twenty five samples were collected and inoculated into the allantoic cavity of embryonated chicken eggs (ECE). Virus identification was performed by haemagglutinating (HA) test followed by haemagglutinating inhibition (HI) test. Further confirmation was done by examination of the infected allantoic fluid by electron microscope (EM).Results revealed 12 out of 25 samples were positive for PPMV-1.

Key words: Pigeon Paramyxovirus-1 · HA · EM · H1 · Egypt

INTRODUCTION

Pigeon Paramyxovirus 1 (PPMV-1) is a viral infection that is present worldwide causing high rates of pigeon illness and death. During 1981 to 1985, infections of racing and show pigeons with an APMV-1 became worldwide, causing a frequently fatal disease primarily associated with neurological signs. This virus was considered to be a variant from Newcastle Disease virus (NDV) of chickens and so termed Pigeon Paramyxovirus -1 [1]. It was suggested that PPMV-1 viruses havs been originated as an antigenic variant of NDV from the Middle-East (ME) in 1978 [2] while in Egypt it was first diagnosed in 1981[3].

The virus belongs to the genus Avulavirus within family Paramyxoviridae [4]. PPMV-1 is a non-segmented, single-stranded, negative-sense, enveloped RNA virus. Viral particles are observed by electron microscopy as pleomorphic, varying from spherical (150-300 nm in diameter) to filamentous (About 100 nm across and of variable length). Projections of approximately 8-12 nm are observed on the viral surface, corresponding to the fusion (F) and hemagglutinin-neuraminidase (HN) glycoprotein spikes. The "Herring bone" nucleocapsid (About 13-18 nm in diameter) might be seen either free or emerging from disrupted viral particles [5].

PPMV-1 attacks mostly pigeons and less frequently chickens, but there are cases of this virus being isolated from birds kept in captivity as well as from wild birds, including partridges, pheasants, swans, falcons, blackbirds, cockatoos and budgerigars [6]. Young pigeons were more susceptible to PPMV-1 infection than older ones. Mortality was 100% in young pigeons whereas adult had much lower mortality and morbidity rates [7].

During the course of infection of most birds with NDV, large amounts of virus are excreted in the feces. Ingestion of feces results in infection; this is likely to be the main method of bird-to bird spread for the pigeon variant virus which normally not produces respiratory signs in the infected birds [8].

Vertical transmission remains controversial where infected embryos have been reported during naturally occurring infections of laying hens with virulent virus but this generally results in the death of the infected embryo during incubation [9].

Laboratory diagnosis of PPMV-1 can be achieved by virus isolation in ECE or cell cultures such as Chicken embryo fibroblasts (CEF), Chicken embryo liver cells (CEL) and characterization of the isolated virus by serological methods as haemagglutination-inhibition and

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enzyme-linked immunosorbent assay (ELISA) tests [10]. In this study we report the isolation and identification of PPMV-1 strains isolated from pigeons in Egypt between 2014 and 2017.

MATERIALS AND METHODS

Field Samples: A total number of 25 freshly dead tissue samples (Brain, lung, trachea, liver, spleen and kidney) were collected from clinically diseased pigeons suffering from nervous manifestations and greenish diarrhea .These samples were collected during the period from August 2014 till January 2017. Collected organs were minced, homogenized and suspended in PBS to make 10% W/V suspension. Tissue supernatant was collected and stored at -80°C till being used in ECE inoculation.

Virus Isolation: Tissue supernatants were treated with antibiotic then inoculated via the allantoic cavity of 9-11-days-old specific pathogen free (SPF) ECE [11]. The eggs were incubated at 37°C and daily examined for embryonic death. Death recorded in the first 24 hr was recorded as non-specific death. The eggs were chilled at 4°C overnight and allantoic fluids were harvested to be used in HA test.

Haemagglutination and Hamagglutination Inhibition Tests: The collected allantoic fluids were subjected to a rapid slide haemagglutination (RHA) test followed by microtitre plate HA and HI tests according to OIE Terrestrial Manual [11]. Hyperimmune anti-PPMV-1 serum, prepared in rabbits by repeated injection with a PPMV-1 inactivated vaccine (Veterinary Serum and Vaccine Research Institute, Abassia, Cairo) was used in HI test. Commercially available Hitchner and Lasota vaccines were also used in HI test for compatibility evaluation and comparison.

Preparation of PPMV-1 Polyclonal Antiserum: Two female rabbits of 3 months age and about 2.5 kg body weight were used for antiserum preparation. They were injected through s/c route, monitored daily and examined for specific side effects. Locally produced PPMV-1 inactivated vaccine (Veterinary Serum and Vaccine Research Institute, Abassia, Cairo) was used for antiserum preparation.

Transmission Electron Microscope: For specimen processing, infected allantoic fluid was centrifuged at 2000g for 10 minutes to remove large particles (e.g., bacteria or cell debris). For negative staining, a drop of the supernatant was placed directly onto a formvar-coated grid and leaved for one minute. The excess liquid is removed from the grid by touching its border with a cut piece of filter paper. The grid is immediately floated in a drop of 1% phosphotungistic acid (PTA). After staining for one minute, the excess stain is removed with filter paper and the grid left to dry for a few minutes, before insertion into the microscope column. Examination of specimen by TEM (JEM-2100, Jeol, Japan) was performed in TEM unit in the National Research Center (Giza, Egypt).

RESULTS

Clinical Signs and Gross Pathology: The infected pigeons showed anorexia, nervous manifestations and greenish white diarrhea. The nervous symptoms include head deviation (Torticollis) and opisthotonus position (Fig. 1a, 1b). Mortality rates were up to 50% in some pigeon lofts. Post mortem examination of infected pigeons revealed enteritis along the intestinal tract with congested pectoral muscles, lung, liver and splenomegally.

Virus Isolation and Identification: The tissue homogenate of the field samples were inoculated via the allantoic route and examined daily by candling. Death recorded within the first 24 hr. was excluded as nonspecific death. No embryo mortalities were detected during the first 4 days of inoculation in the first egg passage. No gross changes were detected in the dead embryos. Congestion of the chorioallantoic membranes (CAM) were observed in some ECE. Harvested allantoic fluids from dead eggs were harvested for HA and HI tests.

Haemagglutination and Haemagglutiination Inhibition Tests: Rapid slide HA test was performed for all harvested allantoic fluids and revealed that 12 out of 25 samples were positive for HA (Fig. 2a-2b).

Microtiter plate HA test revealed12 positive HA samples with titer ranged from 2^6 to 2^{11}, followed by HI test using specific hyperimmune serum. HI titer of hyperimmune serum was variable for different samples and ranged from 2^8 to more than 2^{12}. HI titers of the antiserum were 2^{12} for Hitchner and 29 for Lasota vaccines.

Transmission Electron Microscope: Direct observation of negative contrast TEM images revealed presence of roughly spherical to pleomorphic shaped particles of varying sizes and diameters (Fig 3; a, b, c and d). These particles were enveloped and covered by evenly distributed external spikes.

Fig. 1: Clinically affected pigeon showing nervous manifestations due to infection with PPMV-1. (a) Torticollis, (b) Opisthotonus position

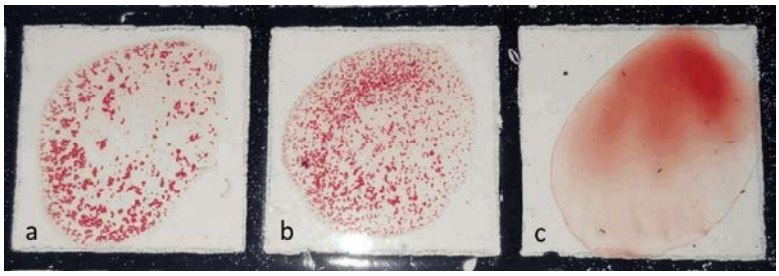

Fig. 2: Rapid slide haemagglutination test. (a) and (b) show positive HA while (c) shows negative HA activity

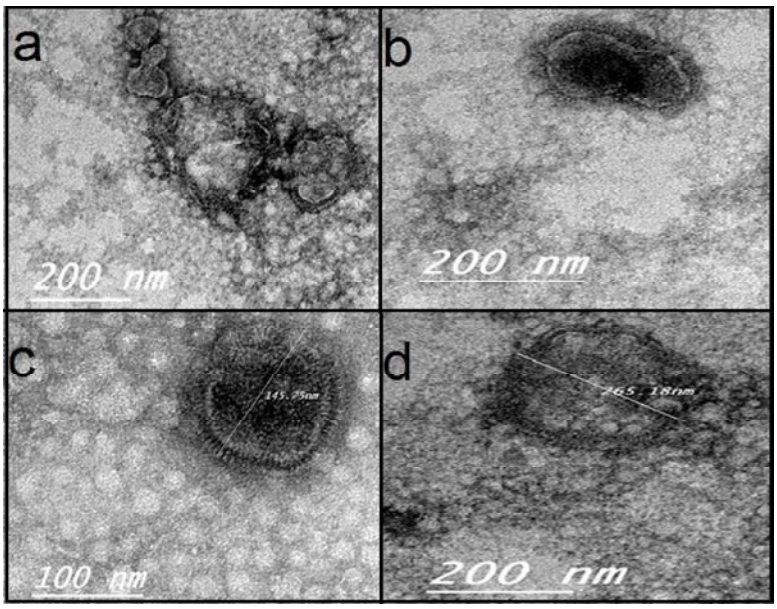

Fig. 3: TEM negative staining of allantoic fluids showing APMV particles, (a) and (b) show pleomorphic while (c) and (d) show roughly spherical shapes

DISCUSSION

Pigeon paramyxovirus type 1 is a contagious viral disease affecting pigeons since the late 1970s. The disease was initially reported in the Middle East and was responsible for the panzootic during the 1980s. Outbreaks of ND in pigeons are still being reported across the world, including Egypt leading to a degree of economic loss [12, 13]. In this study, 25 tissue samples were collected from field cases of pigeons for isolation and identification of PPMV-1. The examined pigeons were suffering from the typical signs of PPMV-1 infection including neurologic signs and diarrhea. Virus isolation was performed via allantoic route of 10-days-old ECE [11]. Subsequently, HA activity of the harvested allantoic fluids was tested by Rapid slide agglutination test and microtitration HA test. This biological property of the virus and its blocking by specific antisera have been used as useful tools in the diagnosis of the disease [14]. HA test revealed that 12 out of 25 samples were positive for HA. Several passages were performed for HA negative samples where OIE [11] reported that at least one further passage through ECE are required for negative samples before being discarded.

Haemagglutination inhibition test with polyclonal antisera of rabbits immunized with PPMV-1 was applied to the HA positive allantoic fluids [15] which revealed that all tested allantoic fluids were HI positive .

HI titers of polyclonal antiserum were variable with the tested samples and ranged from 2^8 to 2^{12} which indicated that the samples with higher titers have more compatibility with the vaccinal PPMV from which antiserum had been prepared. In this study, we found that the HI titre using Lasota and Hitchner as antigens was higher than when using PPMV-1 isolates where the variations in HI antibody titers rely on the virus strain used as an antigen in the HI assay [16]. The same result was reported by Alexander [7]. However other authors as [17] reported that the commercially usable ND vaccines as Lasota and Hitchner may not provide effective protection for PPMV-1 infection in pigeons.

One HI positive sample was selected for Transmission Electron Microscope (TEM) identification. Direct observation of negative contrast TEM images revealed presence of roughly spherical to pleomorphic shaped enveloped viral particles of varying sizes and diameters which revealed presence of PPMV 1 particles with their morphological characters which were identical to APMV morphology and this confirmed the results of HI. These results come on accordance with Murphy *et al*. [18] who reported that early virus classification depends heavily on the viral morphology as shown by EM.

In conclusion, there are different PPMV-1 circulating in the Egyptian fields which are not closely related to the commercially available vaccines and therefore it is strongly recommended to develop and apply advanced diagnostic and control measures including effective vaccination programs using vaccines based on VIb viruses in Egypt to safeguard pigeon's health to reduce the economic losses.

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