The Sudan J. Vet. Res. (1995-1996). 14. 15-21 *E.mail:cvrl@ sudanet.net*
With 1 table and 4 figures in the text.

Virus Distribution and Histopathological Changes in Chickens and Pigeons Experimentally infected With A Pigeon Paramyxovirus-1 (PMV-1)

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SUMMARY

The virus distribution and histopathological changes in organs of chickens and pigeons inoculated with PMV-1 pigeon strain were examined. Two groups of 4 weeks old chickens and adult pigeons were inoculated by mouth and intramuscularly respectively. Early distribution of the virus (2 days post infection) was demonstrated in various organs of both chicken and pigeon. At 4 days, the virus was recovered in all organs examined including the brain. The virus was recovered for up to 18 days post infection from various organs of infected pigeons and for up to 14 days from infected chickens. The shedding and distribution of the pigeon virus strain in organs of chicken were similar to those of the classical from of ND virus in chickens.

The neurological adaptation of the virus was shown by the virus recovery and the histopathological changes in the brain. Non-suppurative encephalitis, meningitis with mononuclear cell infiltration mainly of lymphocytes were recorded. In infected pigeons the hepatic cells were swollen, the sinusoids appeared narrow and lymphocytic infiltration were present in portal areas while in infected chickens, only centrolabular hepatocellular degeneration were seen.

INTRODUCTION

Several outbreaks of Newcastle disease (ND) in pigeons were reported (Vindevogel *et. al.*, 1982) and the role of pigeons as well as of other free flying birds as a source of virus spreading was discussed (Spalatin and Hanson, 1975). A pigeon virus strain has been described as being neurotropic for chickens (Alexander and Parsons, 1984), and the

clinical signs produced in pigeons were similar to those seen in chickens Lancaster and Alexander, 1975).

Using experimental infection, the pathogenicity of the pigeon strains for chickens was shown to be stronger than that of the lentogenic strains of chickens (Alexander and Parsons, 1984). Moreover, Shimizu *et. al.* (1966) showed that lentogenic strains of NDV isolated from chick ns could not be recovered from the brain of orally infected chickens.

The present study compares the virus distribution and histopathological changes in organs of pigeons with those of hickens which were both inoculated with a pigeon PMV-1(kh/94) solated from an outbreak in Sudan.

MATERIALS AND METHODS

Virus:

The virus used in this experiment was an avian paramyxovirus-1 strain isolated from an outbreak of a neurological disease that occurred in pigeons in Khartoum State in 1994. The main clinical signs were paresis, torticollis and incoordination. The morbidity and mortality rates were 36.3% and 15.3% respectively. The virus was isolated in embryonating chickens eggs from brain, bone marrow, spleen and trachea of diseased pigeons and designated as kh/94. It underwent 5 passages in embryonating chicken eggs before used (Ballal, 1995).

Inoculation of chickens:

Eighteen 4-weeks-old chickens that were free from NDV antibodies as indicated by Haemagglutination Inhibition (HI) test were inoculated per os with 10^5 EID_{50} of the virus. Starting from day 2 post inoculation, two chickens were sacrificed on alternate days and specimens were collected from trachea, brain, and spleen in addition to swabs for virus cloacal

Inoculation of pigeons

Twenty two adult pigeons that were free from NDV antibodies were inoculated intramuscularly with 10^5 EID_{50} of virus kh/94. On alternate days, two birds were killed and specimens were collected from the same organs as described for chickens and processed for re-isolation of the virus.

Histopathology:

Specimens were collected ten days post inoculation from infected chickens and pigeons. Pieces of liver, brain, kidney and proventriculus were preserved in 10% formalin, processed for histopathological examination and stained with Haematoxylin and Eosin stain.

Virus isolation:

Ten percent homogenates (w/v) from collected specimens were prepared in sterile normal saline as described by Cunningham (1966). Antibiotics were added at a final concentration of 10^3 I.U Penicillin, 10^3ug Streptomycin and 25 I.U Fungizone per ml of the suspension. Samples were then inoculated into the allantoic cavity of 10 day-old embryonating chicken eggs. Five eggs were used for each sample and each one received 0.2ml of inoculum. Inoculated eggs were incubated at 37°C in an egg incubator and candled daily for 5 days. Allantoic fluids were harvested from dead embryos and from surviving embryos at the end of the period and tested for the presence of haemagglutination activity using chicken red blood cells (Burnet, 1942).

RESULTS

Reisolation of virus:

Table 1. shows the results of reisolation of virus from inoculated chickens and pigeons. Early distribution of virus was demonstrated in trachea, cloaca and spleen of both chickens and pigeons. While no virus was recovered from all chicken tissue samples collected at 15 and 18 days post inoculation, virus shedding was demonstrated in pigeons up to 18 days post inoculation from trachea and cloaca.

No gross lesions were seen on post mortem examination of all infected chickens and pigeons.

Table (1): Shedding and distribution of the Pigeon virus kh/94 in 4- week-old chickens and adult pigeons.

Species	Tissue	Days post inoculation								
		2	4	6	8	10	12	14	16	18
Chickens	Trachea	+	+	+	+	+	+	+	-	-
	Cloaca	+	+	+	+	+	+	+	-	-
	Spleen	+	+	+	+	+	+	-	-	-
	Brain	-	+	+	+	+	+	+	-	-
Pigeon	Trachea	+	+	+	+	+	+	+	+	+
	Cloaca	+	+	+	+	+	+	+	+	+
	Spleen	+	+	+	+	+	+	+	-	-
	Brain	-	+	+	+	+	+	+	-	-

+ : Virus isolated
- : No virus isolation.

Histopathology:

Examination of the brains showed nonsuppurative encephalitis, meningitis with mononuclear cell infiltration mainly of lymphocytes and neuron degeneration was evidenced in brains of some pigeons by chromatolysis, shrunken irregular outline, basophilic cytoplasm and karyolysis (Fig.1). Kidneys showed interstitial nephritis with lymphocytic infiltration (Fig.2). Neutrophilic infiltration was also recorded in kidneys of some pigeons. Examination of the proventriculus revealed lymphocytic infiltration of the lamina propria and between the acini of the compound gland. In chickens, livers showed only centro-lobular hepatocellular degeneration (Fig. 3) while in the case of pigeons. the hepatic cells were swollen, the sinusoids appeared narrow and focal areas of lymphocytic infiltration were present in the portal areas.

DISCUSSION

Hofstad (1951) stated that the study of virus distribution and histopathologic changes in organs of chickens inoculated with NDV is one way to reveal their pathogenicity, but the pathogenicity of NDV strains greatly varies with the host infected (Higgins, 1971). The pathogenicity and the biological characters of the pigeons NDV were generally similar to the neurotropic form of NDV in chickens (Alexander

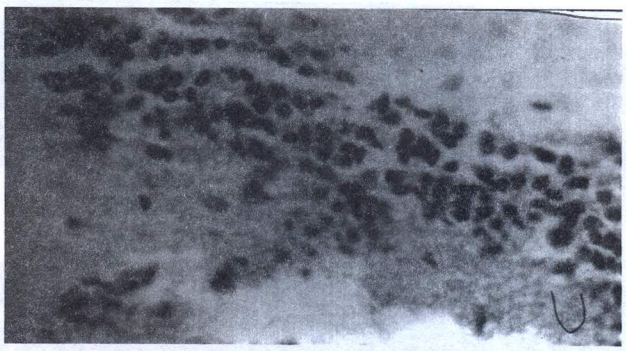

Fig. 1-A: Brain, meningitis with mononuclear cell infiltration mainly lymphocytes.

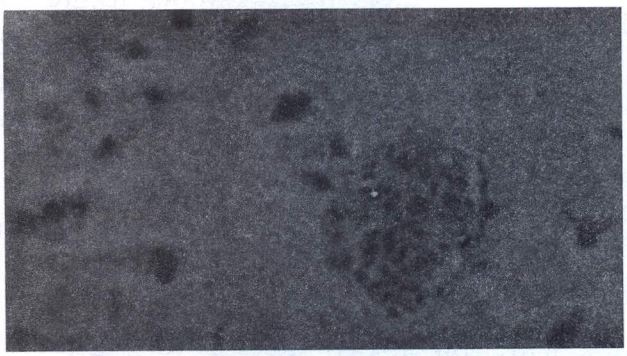

Fig. 1-B: Neuron degeneration.

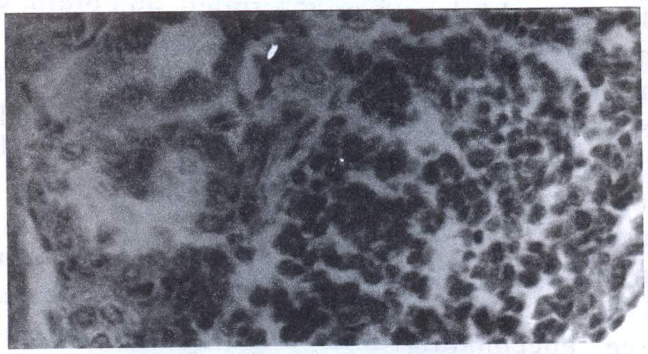

Fig. 2: Kidney, portion of kidney showing multifocal interstitial nephritis with lymphocytes.

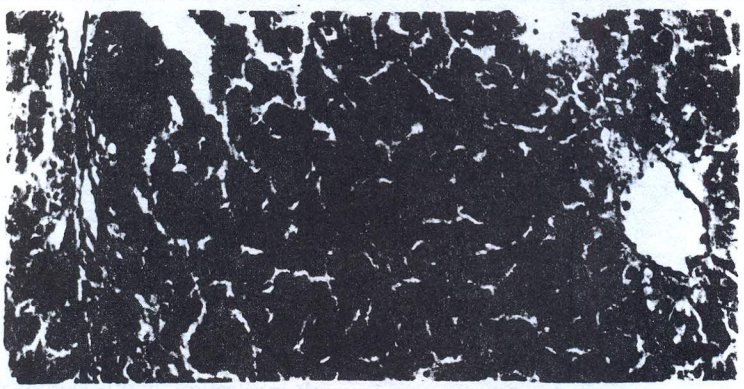

Fig. 3: Liver, centrolobular hepatocellular degeneration in liver.

et. al., 1984). In the present study, the neurotropic nature of the pigeon virus kh/4 for chickens was shown by the virus recovery from, and the histopathological changes observed in, the brains of experimentally infected chickens. These changes were characterized by non-suppurative-encephalitis, neuron degeneration and meningitis with mononuclear cell infiltration mainly of lymphocytes. These findings are similar to those reported by Maeda *et al.* (1987).

In this work the distribution of the pigeon virus kh/94 in organs of infected chickens showed the persistence of the virus up to 14 days post inoculation in the trachea, brain and cloaca and up to 12 days in the spleen. In the infected pigeons the virus was recovered up to 14 days from the spllen, and brain, and up to 18 days from the trachea and cloaca of one pigeon. These results were similar to the findings shown by Pearson *et al.* (1987). In another similar study, Shirai *et al.*(1988)isolated a pigeon virus from experimentally infected chickens up to 10 days post inoculation from spleen and brain and up to 8 days from faeces and trachea.

On histopathological examination, the predominant lesions observed in the organs of chickens and pigeons were non-suppurative encephalitis and degeneration of the liver and kidneys. The results also indicated similarity in virus distribution and histopathological changes for pigeon virus kh/94 in organs of chickens and pigeons. Fr experimental reproduction of the clinical disease in pigeons and chickens the route of infection

appeared to be very important (Alexander and Parsons, 1984). For this reason, the intramuscular route was used for infection of pigeons instead of the oral one because the latter route of infection failed to show a marked evidence of the disease in infected pigeons (Ballal, 1995).

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