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INFLUENCE OF PIGEON PARAMYXOVIRUS TYPE-1 ON CLINCOPATHOLOGICAL PROFILES IN RACING PIGEONS ASSOCIATED WITH RECENT OUTBREAKS IN IRAQ

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Abstract. The number of racing pigeons recently increased in Iraq accompanied by significant increases in the number of events similar to the neurotrophic velogenic group of ND viruses in different regions. Thus, this research was designed to study the influence of natural infection with PPMV-1 on clincopathological profiles in four PPMV-1-associated mortality events in racing pigeons in the southern part of Iraq. The result revealed that HI antibody titers above the positivity threshold of four events were seen in (9 out of 24) of tested pigeons in all events, and the predominant signs included polyuria followed by neural symptoms which appear in most of the affected pigeons in the flock. All events showed a high mortality rate (60% - 70%) and absence of gross abnormalities in necropsied pigeons. kidney showed widespread interstitial hemorrhage and intensive epithelial sloughing of renal tubular epithelium, marked white pulp hyperplasia and marked peri-arteriolar fibrosis with onion skin appearance in the spleen, and marked destruction of the superficial intestinal mucosa and intensive inflammation in the site of tissue destruction. This study demonstrates the role of PPMV-1 on clincopathological profiles in racing pigeons associated with recent outbreaks in Iraq, polyuria followed by neural symptoms represent the predominant symptoms in the affected pigeons.

Keywords: *Paramyxovirus-1, Pigeon, racing, Basra, Iraq.*

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1. Introduction

Pigeon paramyxovirus serotype 1 (PPMV-1) is a variant of the Newcastle disease virus (NDV) belonging to the class II genotype VI of the serogroup 1 of the genus Avulavirus, and family Paramyxoviridae (Alexander & Senne, 2008; Miller *et al*., 2010; Briand *et al*., 2012). Historically 3 panzootic of Newcastle Disease have been recorded in the last eighty years. The first panzootic was identified in Indonesia and England during the mid-1920s (Briand *et al*., 2012), while the second was recognized during the late 1960s in Europe but was thought to have originated in Asia. The third one involves a pigeon-adapted variant of avian (APMV- 1), which emerged first during the late

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1970s in the Middle East, and the virus was first isolated from pigeons in Iraq in 1978, simultaneously, the virus reached the eastern part of Europe and North Africa from the Middle East regions. During the period between 1981- 1985 infection of show and racing pigeons with (PPMV-1), became globally distributed causing a frequent disease primarily associated with neurological signs, and remains endemic in many countries (Alexander, 2001). It is thought that the rapid spread of PPMV-1 was related to trade, competition flights, and shows of carrier and ornamental pigeons (Aldous *et al*., 2012). Torticollis, a medical/veterinary term used for the wry neck, is a sign of abnormal positions of the head and neck often noticed in domestic pigeons and other species of bird, there are different reasons for this case which may include infectious, non-infectious, and nutritional causes. Is usually seen in ND (Ali *et al*., 2014). In 1978 a severe disease in pigeons in Baghdad, Iraq called 'Contagious paralysis' or 'Viral encephalomyelitis', was described by several authors, and the pigeon herpes encephalomyelitis virus (BVC 78 strain) was isolated. But based on these results of (Kaleta *et al*., 1985) the BVC 78 strain was permittivity classified as a pathotype variant of NDV, being more virulent for pigeons than for chickens, and the pigeon experimentally infected with this strain shows one-wing lameness, torticollis, and watery diarrhea, leading to progressive weakness.

The number of pigeon fanciers increased recently in Iraq accompanied by significant increases in the number of pigeons in different regions. It's well known that in regions with large populations of Columbiformes, the pigeon Newcastle disease is considered to be enzootic and causes neurological and viscerotropic signs, including high mortality (Aldous *et al*., 2004; Meulemans *et al*., 2002; Alexander, 2000). For this reason, many field outbreaks of (PPMV-1) have occurred and are still ongoing in different types of (racing and local) pigeons causing remarkable economic losses for fanciers. Thus, this study was designed to study the influence of natural infection with paramyxovirus type-1 on clincopathological profiles in racing pigeons associated with neurological signs.

2. Materials and methods

Four PPMV-1-associated mortality events in racing pigeons in the southern part of Iraq (Basra province). The postmortem investigation of the 4 events was carried out at the Department of Veterinary Pathology and Poultry Diseases, University of Basra. PMV-1 infection in each event was confirmed according to the following: (1) isolation and identification of PMV-1(2) detection of PMV-1 antibodies of pigeon's serum samples by using hemagglutination inhibition test (3) presence of histopathological lesions compatible with PMV-1 infection in the internal organs (kidney, intestine, and spleen). Their detailed information on loft management, the date and age of each event, vaccination programs, symptoms, and mortality rate were recorded. The pigeons in all events were unvaccinated against ND.

2.1. PPMV-1 Isolation

Specimens of kidney, intestine, and spleen were collected from inspected birds from loft outbreaks. Normal saline was used for preparing homogenized tissues and the supernatants were inoculated in 9-11 days-old embryonated eggs via the allantois. Allantoic fluid of dead embryos was harvested and identified by standard

hemagglutination (HA) and standard specific antisera to the reference strains of paramyxovirus.

2.2 Serological examination

Conventional microtiter Hemagglutination inhibition (HI) test was performed to detect PPMV-1 antibodies in serum samples by using four Hemagglutination units (HA) of the LaSota strain as antigen. Results were recorded as log2 X and titers equal to or greater than 16 were considered positive for NDV (OIE 2004).

2.3. Histopathological study

The postmortem diagnostic technique was implemented based on standard procedure; tissues from affected pigeons were fixed in 10% neutral-buffered formalin, processed routinely, sectioned at 5 mm, and stained with hematoxylin and eosin.

3. Results

3.1. Clinical signs

Clinical signs observed in pigeons in these PPMV-1 outbreaks were similar. Predominant signs included polyuria followed by neural symptoms which appear in most of the affected pigeons in the loft. Torticollis, Shaking and nodding of the head, muscular tremors, wings and leg paralysis, are the most common neural signs. Swollen eyelids stuck together, and had a serous discharge, occasionally seen. Mortality started 5 days post-infection and birds died throughout the observation period (15 days). All events showed a high mortality rate (60% - 70%). Based on age, (65% - 75%) and (25% - 35%) mortality rate was reported in pigeons younger and older than 6 months, respectively (Table 1).

Table 1. Mortality rate throughout the observation period

Events	Number of pigeon	Mortality rate in age < 6 months	Mortality rate in age > 6 months	Total of mortality rate
1	75	75%	25%	60%
2	90	68%	32%	65%
3	60	73%	27%	70%
4	80	65%	35%	63%

3.2. Viral isolation Serological examination

PPMV-1 was isolated from most of the submitted samples. The HI antibodies titers above the positivity threshold of four were seen in (9 out of 24) of tested pigeons (Table 2).

Table 2. Positivity threshold of HI antibodies titers (Log2X) in tested pigeons

Events	Number of tested pigeon	Positive threshold antibodies titers	Negative threshold antibodies titers
1	6	2	4
2	6	3	3
3	6	2	4
4	6	2	4
Total	24	9	15

Positive threshold: titers (Log2X) equal to or greater than 4

3.3. Pathological and histological changes

The results revealed the absence of gross abnormalities in necropsied pigeons. Regarding histopathological analysis, the kidney section of the racing pigeon showed widespread interstitial hemorrhage and intensive epithelial sloughing of the renal tubular epithelium (Fig 1, 2, 3, and 4). Spleen shows marked white pulp hyperplasia and marked peri-arteriolar fibrosis with onion skin appearance (Fig 5 and 6). The intestine of racing pigeons shows marked destruction of the superficial intestinal mucosa and intensive inflammation in the site of tissue destruction (Fig 7, 8, and 9).

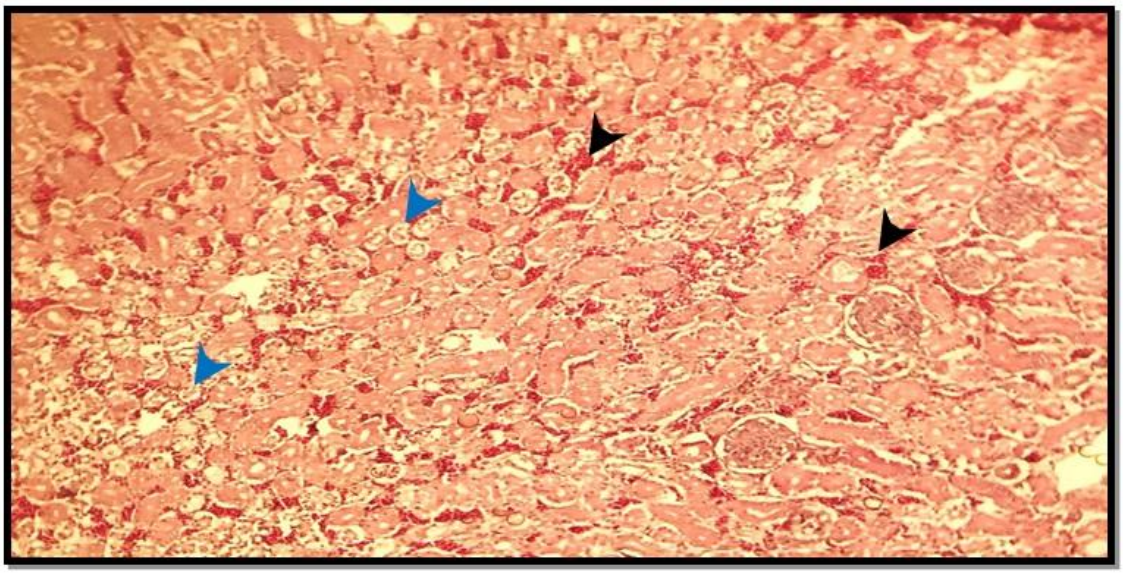

Figure 1. Kidney section of racing pigeon showing wide spread interstitial hemorrhage (black arrow) and intensive epithelial sloughing of renal tubular epithelium (blue arrow). H&E 4X

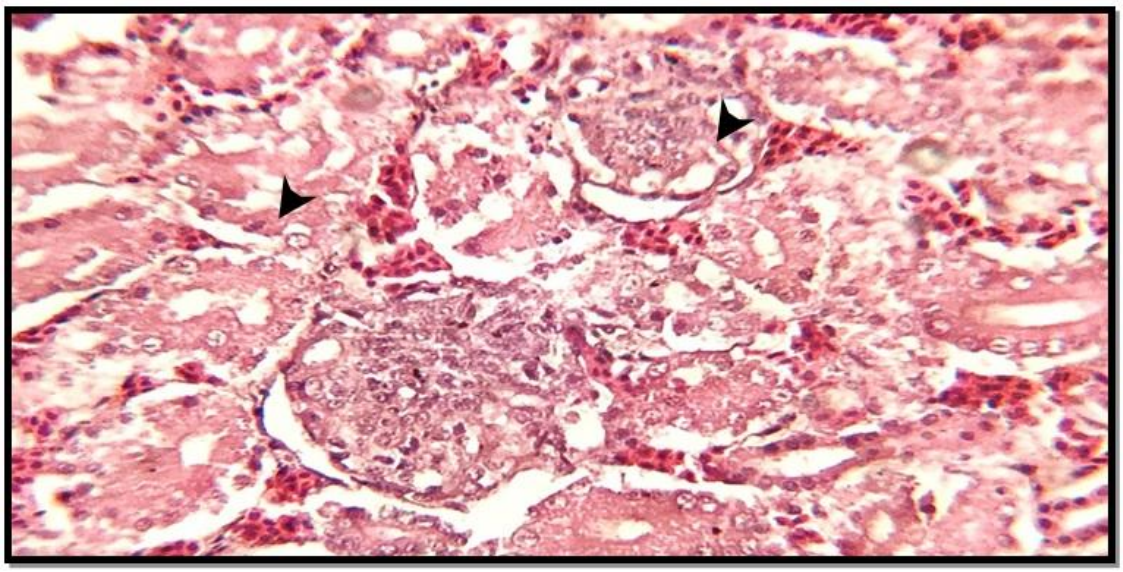

Figure 2. Kidney section of racing pigeon showing wide spread interstitial hemorrhage (black arrow). H&E 10X

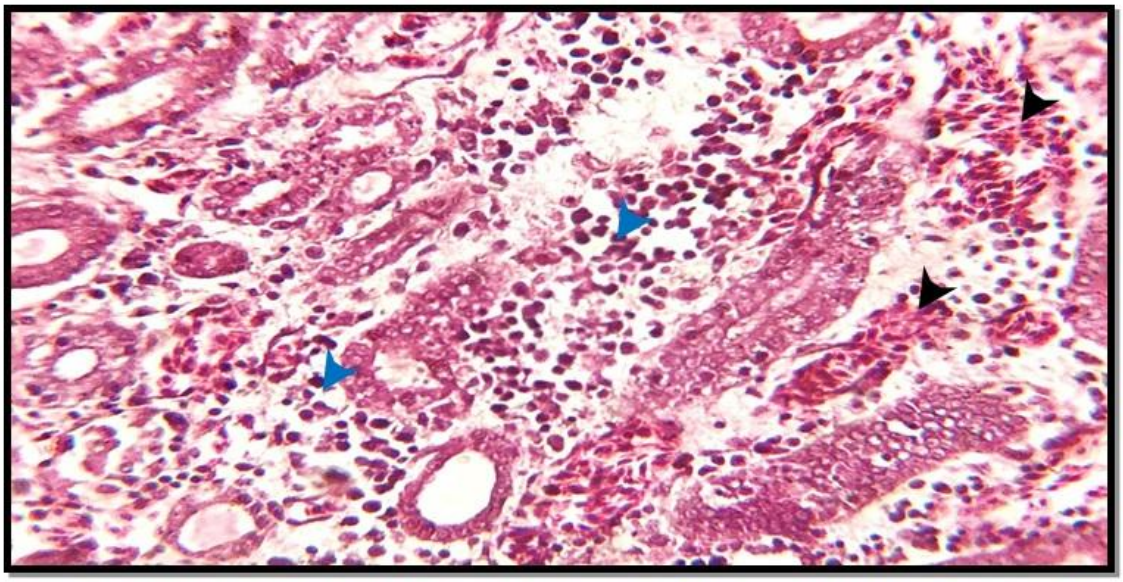

Figure 3. Kidney section of racing pigeon showing wide spread interstitial hemorrhage (black arrow) and intensive interstitial inflammation (blue arrow). . H&E 10X

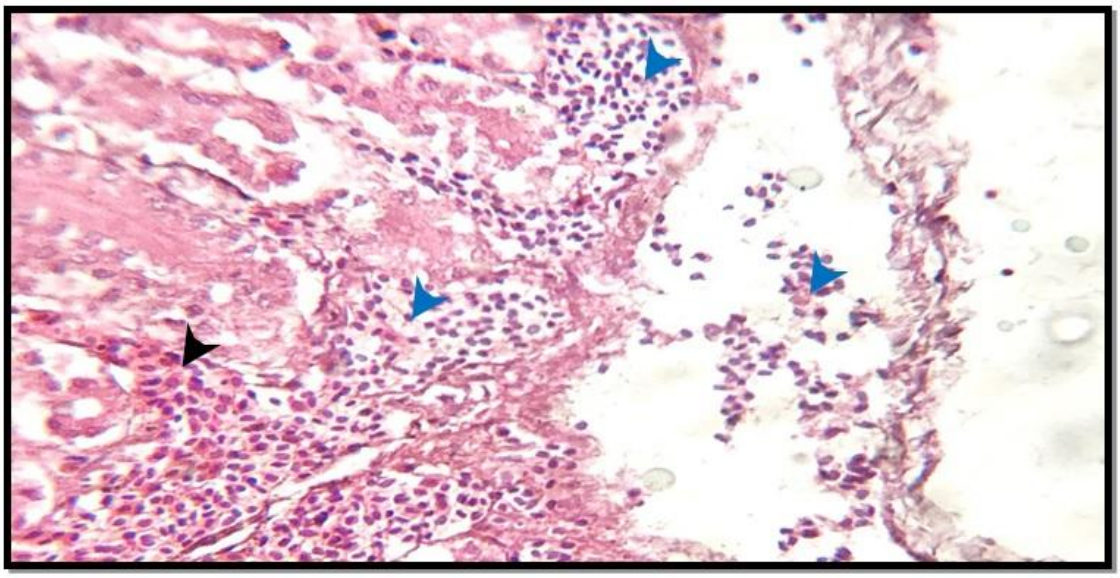

Figure 4. Kidney section of racing pigeon showing wide spread interstitial hemorrhage (black arrow) and intensive interstitial inflammation (blue arrow) in the sub-capsular region. H&E 10X

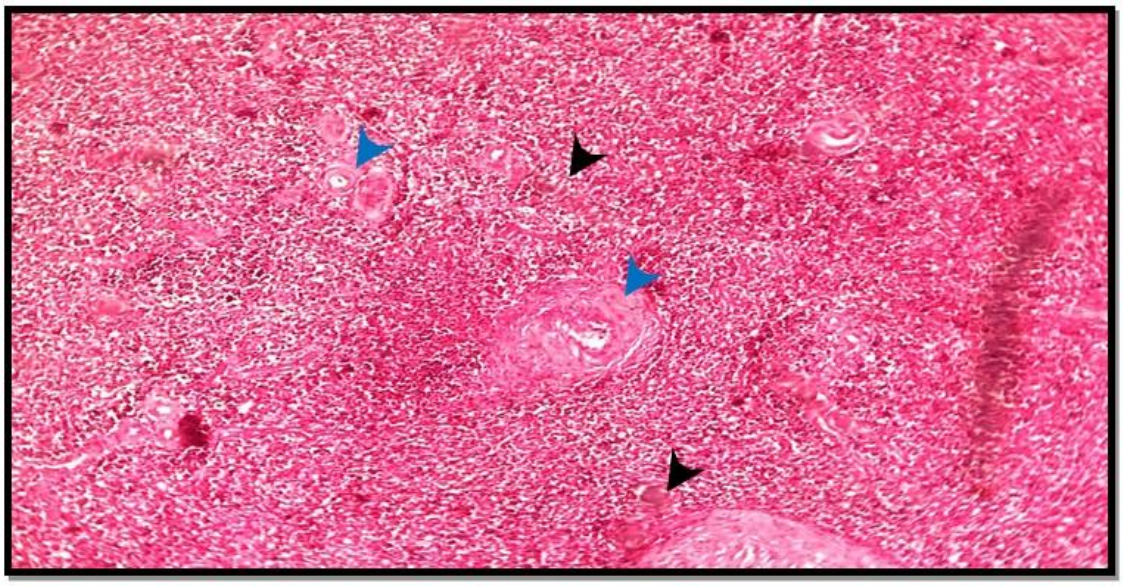

Figure 5. Splenic section of racing pigeon showing marked white pulp hyperplasia (black arrow) and marked peri-arteriolar fibrosis (onion skin lesion) (blue arrow). H&E 4XS

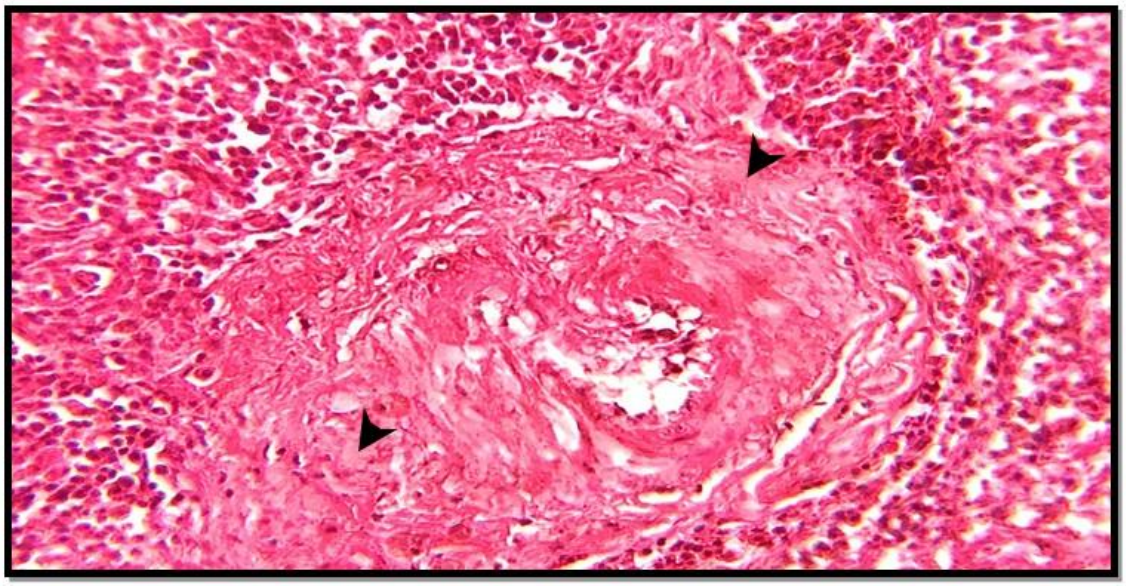

Figure 6. Splenic section of racing pigeon showing marled peri-arteriolar fibrosis (onion skin lesion) (black arrow). H&E 40X

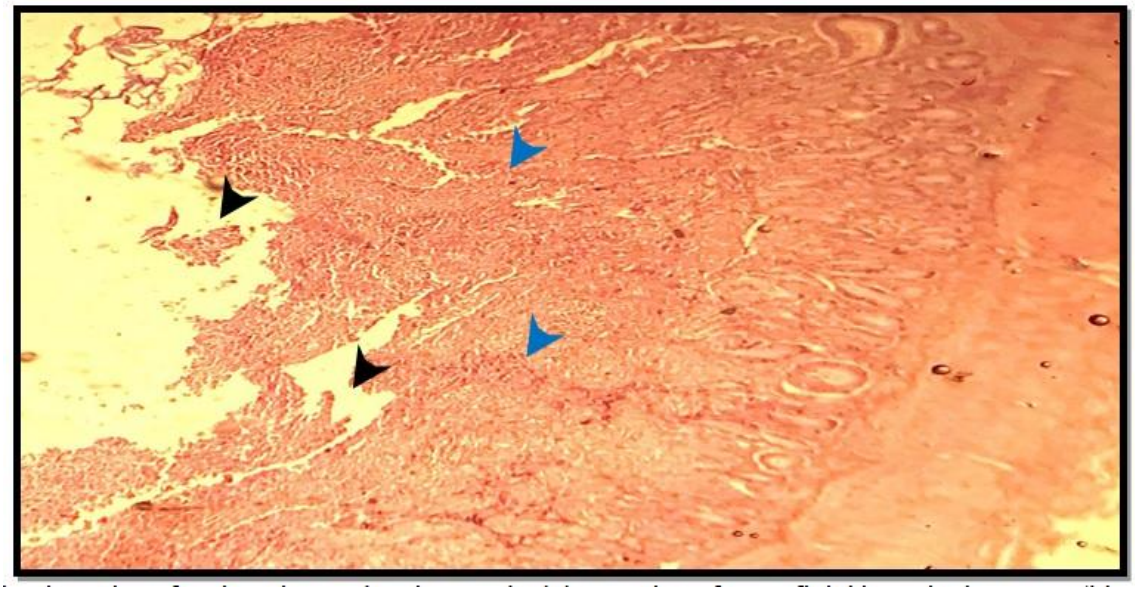

Figure 7. Section intestine of racing pigeon showing marked destruction of superficial intestinal mucosa (black arrow) and intensive inflammation in the site of tissue destruction (blue arrow). H&E 4X

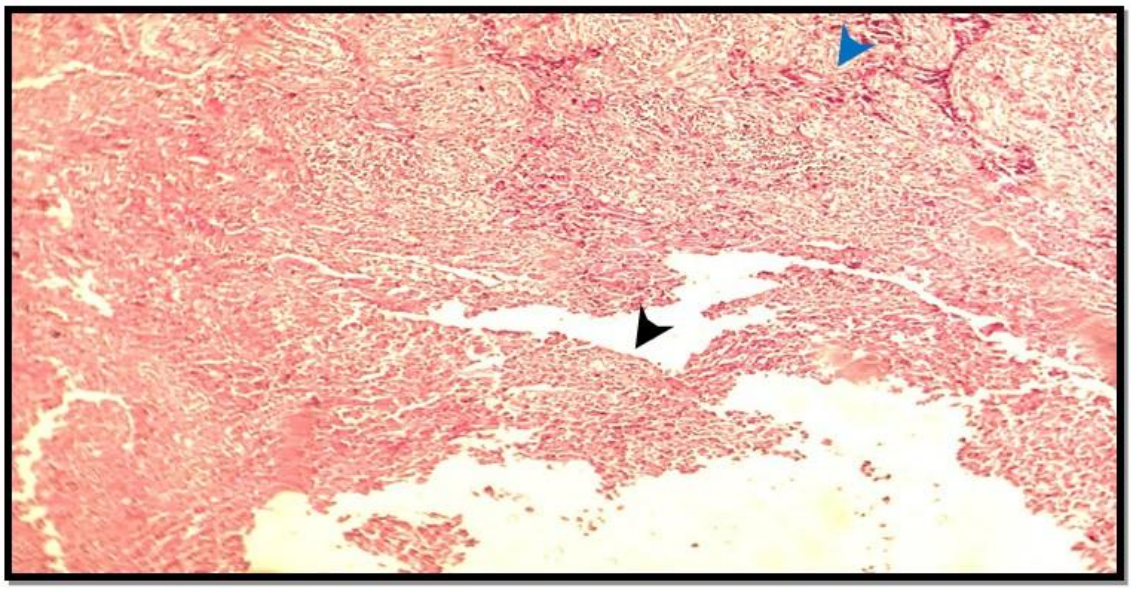

Figure 8. Section intestine of racing pigeon showing marked destruction of superficial intestinal mucosa (black arrow) and intensive inflammation in the site of tissue destruction (blue arrow). H&E 10X

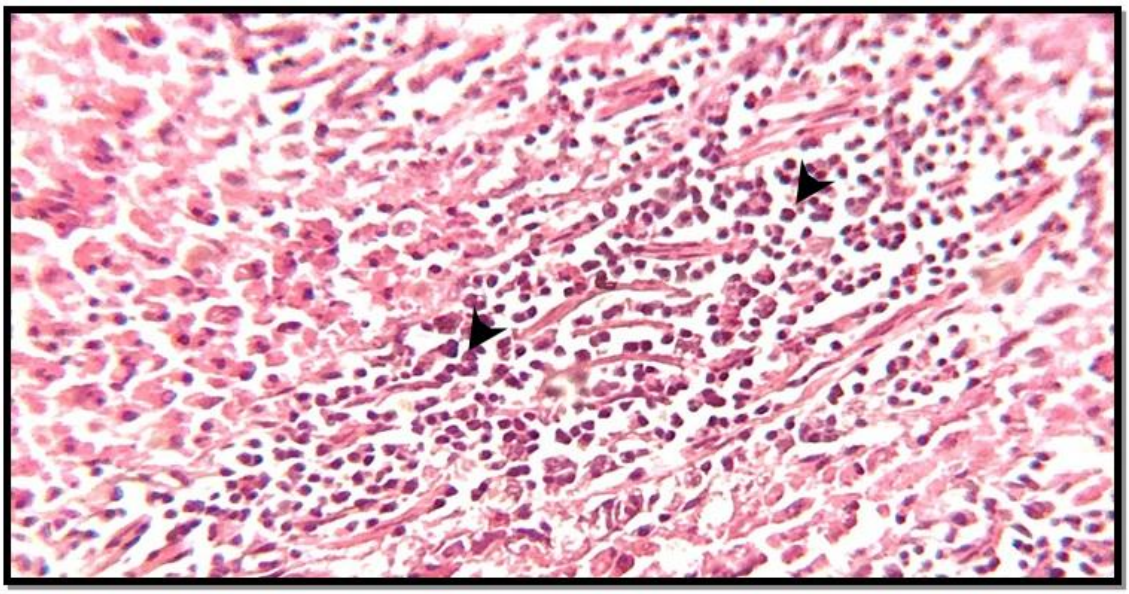

Figure 9. Section intestine of racing pigeon showing intensive inflammation in the site of tissue destruction (black arrow). H&E 40X

4. Discussion

Pigeon racing is enjoyed by more than two million fanciers throughout the world. In Iraq, hundreds of dedicated fanciers compete in races of distances ranging from 60 to 1000 kilometers every year. Pigeon racing is unlike other races. In pigeon racing, all the pigeons begin from the transport truck, whereas the finish line is each pigeon's home loft. In Iraq, field outbreaks of pigeon Newcastle disease recently increased in racing pigeons and these results might be attributed to the presence of healthy carrier pigeons in the same transport truck in addition to that in races some of these PPMV-1carriers lost their way in faraway towns and cities. Racing pigeons sport allow the spreading of PPMV-1 across vast distances and has been linked as a method of transmission to new premises (Hines & Miller, 2012). The experimental study by Qiu et al., (2017) indicated that infected pigeons with PPMV-1 continued to shed the virus, even at 21 days post-infection, they suggested that healthy pigeons may be vial carriers. According to epidemiological studies, genotype VIb was detected in healthy-appearance pigeons and doves (Wang *et al*., 2015; Kim *et al*., 2008).

Clinical Signs reported in the current study are commonly like to the signs caused by the neurotropic velogenic group of ND viruses. Polyuria followed by neural symptoms represent the predominant symptoms in most of the affected pigeons in all events, this result is in accordance with results of earlier studies by (El Mubarak *et al*., 1990; Isidoro *et al*., 2017). Some reports state that pigeons infected with viscerotropic strains sometimes exhibit polyuria prior to neural symptoms (Pestka *et al*., 2014). However, the present study revealed that polyuria was reported in most affected birds.
Estimated mortalities in 305 birds were reported in 4 events (60% - 70%). Based on the age, a high mortality rate (65% - 75%) was recorded in pigeons younger than 6 months. In recent years, high mortality from 40% to 80% or 100% in some cases has been observed in ND-infected pigeons in China(Guo *et al*., 2014; Zhao *et al*., 2010; Wang et

al., 2015) and a 92% mortality rate has been reported in Eurasian Collared-Doves and Rock pigeons in the United States(Isidoro *et al*., 2017).

The current study revealed that grossly pathological changes were often unremarkable or non-characteristics in necropsied pigeons. This result agrees with an earlier study which has been reported in Columbiformes, birds infected with neurotropic vNDV strains remain alert prior to developing neurological signs such as torticollis, ataxia, or a wing or leg paralysis, and all tissues including the brain, may be grossly normal (Cattoli *et al*., 2011; Barton *et al*., 1992 ; Johnston & Key1992). Regarding histopathological analysis, the kidney section of a racing pigeon in a current study shows widespread interstitial hemorrhage and intensive epithelial sloughing of renal tubular epithelium, marked white pulp hyperplasia and marked peri-arteriolar fibrosis with onion skin appearance in spleen, and marked destruction of superficial intestinal mucosa and intensive inflammation in the site of tissue destruction. These results are in line with previous studies (Isidoro *et al*., 2017; Yuzbasioglu & Gurel, 2022).

Although the HI antibodies titers above the positivity threshold of four events were not seen in most of the tested pigeons. However, positive antibody titer for some pigeons (9 out of 24) was detected. This is agreed with the results of (Barbezange & Jestin, 2003) who detected positive antibody titer in (6 out of 13) pigeons experimentally infected with PPMV-1. These results could be steam from using the LaSota strain as an antigen for the HI test in the present study, and higher titers might be detected with the homologous strain (Stone, 1989).

5. Conclusion

This study demonstrates the role of PPMV-1 in association with clinical signs and histological lesions in the racing pigeon in Iraq polyuria followed by neural symptoms represent the predominant symptoms in most affected pigeons in all events. Consequently, fanciers should be properly educated to improve biosecurity with appropriate disinfectants. Vaccination is still important in effectively controlling the disease to minimize virus transmission in homing pigeon lofts.

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