Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 3, 491-495

Investigation of four enteric viruses in diarrheic ducks in Egypt during 2021-2022

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ARTICLE INFO

Recieved: 17 November 2023

Accepted: 05 December 2023

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Keywords:

Ducks
PCR
Enteric viruses
Muscovy duck parvovirus
Avian rotavirus

ABSTRACT

The aim of this study was to examine viruses associated with gastrointestinal illnesses in ducks collected from four governorates in Egypt (Sharkia, Gharbia, Dakahlia, and Qaliobia) during 2021-2022. These ducks underwent comprehensive clinical examinations and post-mortem analyses. All the flocks exhibited various forms of diarrhea. Additionally, 59.5% of the duck flocks manifested respiratory symptoms, while 57% showed uneven growth, locomotory dysfunction (42.8%). Post-mortem findings consistently included enteritis in all examined flocks. To directly identify viruses associated with digestive illnesses, 42 aseptic intestine samples were obtained from recently deceased or sacrificed ducks. PCR analysis identified four positive samples out of the 42 (9.5%), with one containing Avian Rotavirus (AvRV) and three carrying Muscovy Duck Parvovirus (MDPV). Additionally, histopathological examination of the liver and intestine from PCR-positive flocks showed findings consistent with those typically observed in MDPV infections. This study concluded that the primary viruses associated with digestive illnesses in Egyptian ducks are MDPV and AvRV. Importantly, this research represents the first-ever detection of Rotavirus in ducks in Egypt.

Introduction

Diarrhea is a common sign of the most enteric diseases of ducks and chickens. Different viruses replicate at different parts of the gastrointestinal tract (GIT) tract and at different sites on the villi. Epidemiological investigations have revealed that the majority of these viruses do not establish long-lasting infections in avian hosts. Enteric viruses are commonly the cause of most of the primary abuses to the GIT of young poultry. This provides other agents, especially bacteria, to replicate, attach, and penetrate cells, leading to further damage. Injury of the GIT early in life could result in irreparable harm to the flock. Most of enteric viruses have been studied in birds include coronaviruses, rotaviruses, reoviruses, astroviruses, and enteroviruses (Saif, 2013).

In ducks, several different viruses have been identified as medically important causes of gastrointestinal tract infections especially Duck viral enteritis virus (DVEV), Muscovy duck parvovirus (MDPV), Avian rotavirus (AvRV) and Muscovy duck reovirus (MDRV) (Wu *et al*., 1994; Barnes, 1997; Shawky and Schat, 2002).

Duck virus enteritis (DVE) is a contagious virus infection that occurs naturally only in ducks, geese, and swans ranging in age from 7 days to adult breeders. In susceptible flocks, the common clinical signs include depression, loss of appetite, increased thirst, dehydration, weakness, ruffled feather, nasal discharge, ataxia, photophobia, tremor of head and neck, greenish and watery diarrhea, and soiled vent with a significant drop in egg production in laying flocks (Sandhu and Metwally, 2008). Petechial or ecchymotic hemorrhages on mycocardium, endocardium and free blood in the body cavity were seen in the experimental ducks. Hemorrhagic bands in the intestinal tract, raised plaque-like areas in the esophagus were visualized (Labib *et al*., 2014). The use of commercial live attenuated vaccine could ameliorate the clinical and pathological findings of the disease and considered as an effective tool to control DEV infection. (Abdullatif *et al*., 2020).

Waterfowl parvoviruses cause dreadful disasters in gosling and Muscovy ducklings (Barnes, 1997). Muscovy ducklings infected by MDPV were characterized by watery diarrhea, wheezing, and locomotor dysfunction. MDPV is mainly observed in Muscovy ducklings less than three-week-old, with the mortality rate reaching as high as 80% depending on age (Lin *et al*., 1991; Wang *et al*., 2017a). Although much lower mortality occurred after infection after three weeks of age, the ducks infected at an older age as well as most of the survived ducks in infection lost production performance because of abnormal feathering and mainly growth retardation (Glávits *et al*., 2005).

During 1970s, Short beak and dwarfism syndrome (SBDS) was first reported in France in mule ducks, caused by a novel parvovirus-related virus (NGPV) (Palya *et al*., 2009). Since March 2015, there are numerous SBDS outbreaks in mule duck and Cherry Valley duck flocks in various regions of China (Chen *et al*., 2015; Li *et al*., 2017). The morbidity rate of NGPV was found to be 10–30% while mortality rate was about 2% (Fan *et al*., 2017). The ducks infected with NGPV, showed clinical symptoms like swollen tongue, shorter tibia and stunted growth (Palya *et al*., 2009). In this way, the disease causes huge economic losses to duck industry by reduction in weight and size of birds. However, the adult ducks were found resistant to SBDS disease (Palya *et al*., 2009). Pale thigh and heart muscles, serofibrinous perihepatitis, increased pericardial fluid, and ascites are instances of gross pathological lesions. In prolonged cases, the birds become stunted, with chronic liver congestion and ascites (Wool-

ISSN: 2090-6277/2090-6269/

cock *et al*., 2000; Glávits *et al*., 2005).

The infection of ducks with reovirus was first reported in South Africa in 1950 (Kaschula, 1950). The acute form of the disease is apparent in ducklings between 2 and 4 weeks of age, causing diarrhea and difficulty in movement, in which morbidity may reach from 10 to 30%, with mortality from 15% to 30% (Chen *et al*., 2012). Recently, the YB strain of MDRV causes extensive "white necrosis" in the liver and spleen of ducklings with high mortality (Wang *et al*., 2015 and Wang *et al*., 2017b).

Avian Rotavirus infection is often associated with outbreaks of diarrhea and general flock depression causing enteric syndrome in poultry (Barnes *et al*., 2000; Jindal *et al*., 2010; Otto *et al*., 2011). Naturally occurring rotavirus infection in poultry (turkeys, chickens, pheasants, partridges, and ducks) and the associated enteric disease signs generally occur in birds that are young, less than about 6 weeks old (Barnes *et al*. 2000; Barnes and Guy 2003; Jackwood *et al*, 2008). Diarrhea is the principal manifestation of disease, while in affected birds, decreased weight gain, dehydration and an increased mortality could also be observed (McNulty, 2003). The presence of large volumes of fluid and gas in the digestive tract and ceca is the most common observation at necropsy. Pallor of the intestinal tract with loss of tonicity is possible. Secondary observations may include dehydration, growth stunting, pasted and inflamed vents, anemia caused by vent pecking, litter in the ventriculus (gizzard), and inflammation and encrustation of plantar surfaces of the feet with droppings (Spackman *et al*., 2010). Therefore, the aim of this study was to identify the causes of viral duck enteritis and their prevalence among Egyptian flocks.

Materials and methods

Examined birds and clinical samples

One hundred and nine diseased ducks (n=109) were collected from 4 governorates (Sharkia, Gharbia, Dakahlia and Qaliobia) during 2021-2022 that were subjected to clinical and PM examinations. The diseased ducks were collected from different flocks and transferred to the laboratory at Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt. Forty-two (n=42) livers and intestine samples have been collected aseptically from freshly dead and/or sacrificed ducks in order to identify viruses directly using PCR tests. Additionally, liver and intestinal sections were kept in a 10% buffered formalin solution for histopathological evaluation.

Polymerase chain reaction (PCR)

DNA/RNA Extraction

It was performed using the WizprepTM Viral Mini Kit following the manufacturer instruction. Conventional PCR test has been applied for detection of incriminated four enteric viruses (Duck virus enteritis (DVE), Muscovy duck parvovirus (MDPV), Avian rotavirus (AvRV) and Muscovy duck reovirus (MDRV). The oligonucleotide primers (Metabion, Germany) (Table 1) were used.

cDNA synthesis for PCR detection of MDRV and AvRV

Five µl of template RNA was added to 2 µl Oligo (dt) 18 primer and completed to 13.5 µl nuclease free water. These reactions were incubated at 65°C for 5 min, chilled on ice then 4 µl 5X RT buffer, 0.5 µl H minus MMLV (200 unit/ µl) and 2 µl 10 mM dNTP mix were added. These reactions were incubated for 60 min at 42°C. The reaction was terminated by heating at 70°C for 5 min. The cDNA used immediately for PCR.

Conventional PCR reaction

The optimized components for each 20 µl reaction contained 10 µl PCR 2X master mix, 0.4 µl primer-pair (Forward and reverse primers, separately, 10 µM each), 5 µl DNA template, and 4.6 µl nuclease-free water. Tubes were transferred into a PCR instrument and run as using the thermal profile showed in Table 2. The PCR products were subjected to electrophoresis on 1.0 % agarose gel for analysis.

Histopathological examination

Selected organs (livers and intestine) were fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin, paraffin sections were prepared and stained with hematoxylin and eosin then examined microscopically for histopathological findings (Suvarna *et al*., 2013).

Results

Clinical and Postmortem findings

The examined ducks of variable ages (20 Flocks during the first 3

Table 1. Primer sets used for PCR detection of the four enteric viruses in duck intestinal samples.

Virus	Amplified gene	5' -3' sequence of primers	Amplicon size	Reference
DVEV	DNA polymerase gene	F,5′GAAGGCGGGTATGTAATGTA-3′ R,5′CAAGGCTCTATTCGGTAATG -3′	446 base pairs (bp)	OIE (2012)
MDPV	Viral structural protein (VP 1) -encoding gene	F,5′CCTGGCTATAAGTATCTTGG-3′ R,5′GTAGATGTGGTTGTTGTAGC-3′	593 base pairs (bp)	Poonia *et al.* (2006)
MDRV	S3 segment encoding sigma B gene	F,5′GCTTTTTGAGTCCTCAGCGTG-3′ R,5′GATGAATAGGCGAGTCCCGC-3′	1202 bp base pairs (bp)	Li *et al.* (2018)
AvRV	Viral structural protein (VP 6) -encoding gene	F,5'CACCACGACTTATGCAGAGA-3' R,5'CTCCGAATGGATGCTACTGT-3'	493 base pairs (bp)	Otto *et al.* (2012)

Table 2. The optimized thermal conditions of PCR.

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	45 sec.	35
Anneal	55 (DVEV-MDPV) 57 (AvRV) 60 (MDRV)	45 sec.	
Extend	72	60 sec.	
Final Extension	72	5 min.	1

weeks of age, 18 flocks during the second 3 weeks of age and 4 breeder flocks) and different breeds (Pekin, Muscovy and Mallard) were suffered from general signs of illness expressed by off food, depression, ruffling feathers, weakness and different degrees and colors of diarrhea (100% of flocks), including watery, white, bloody, and greenish diarrhea. Also, respiratory signs as nasal and ocular discharge (59.5% of flocks), uneven growth (57% of flocks), locomotory dysfunction (42.8%), and short beak and swollen tongue (16.7% of flocks) were noticed. The examined ducks showed postmortem lesions as enteritis (100% of flocks), fibrinous pericarditis and perihepatitis (64% of flocks), necrosis on liver (28.5% of flocks), and haemorrhage on liver (9.5% of flocks). MDPV infected flocks showed clinical symptoms as dehydration, weakness, ruffled feathers, severe diarrhea, runting and stunting, swollen tongue and shortening of beak (Figure 1) while postmortem lesions showed pericarditis and increased pericardial fluid and perihepatitis. The main symptom of an AvRV-infected duck backyard flock was watery diarrhea, which was associated with reduced growth. Postmortem lesions revealed that the intestines contained abnormally high levels of fluid and gas, as well as ceca and enteritis. A comprehensive summary of the clinical symptoms and PM lesions of the affected duck flocks were summarized in Figure 2.

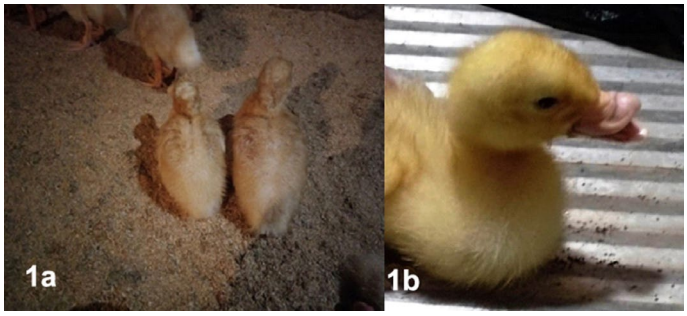

Fig. 1. MDPV infected flock showing (a) dehydration, weakness and ruffled feathers resulted from severe diarrhea, (b) swollen tongue and shortening of beak.

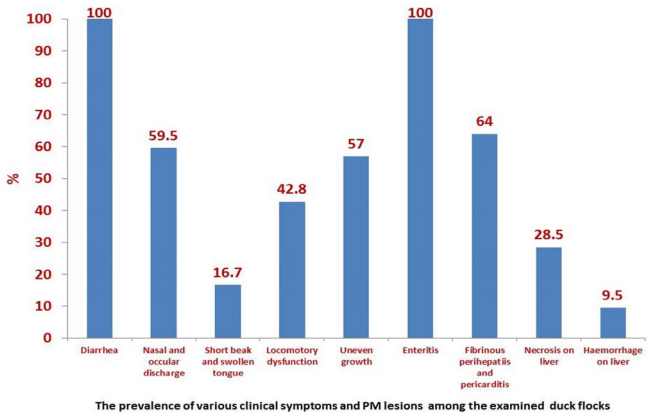

Fig. 2. Clinical signs observed by the farmers, categorized by organ system, for the highly pathogenic avian influenza virus infected. chicken (left) and duck farms (right) and exceedance of daily mortality (>0.5%) and mortality ratio (MR) thresholds.

Screening for enteric viruses using the polymerase chain reaction (PCR)

Affected intestine of examined flocks were subjected to four monoplex conventional PCR including two RT–PCR based tests (against AvRV and MDRV) and two PCR based tests (against MDPV and DVE). Only 4 samples out of 42 were positive, 1 sample was positive for AvRV from backyard duck flock (Figure 3), and 3 samples were positive for MDPV (Figure 4).

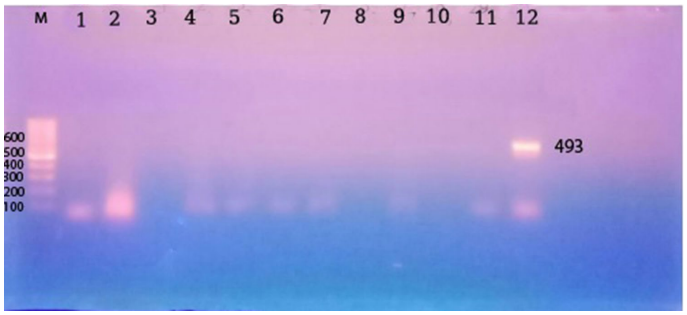

Fig. 3. Agarose gel electrophoresis of the RT-PCR products of AvRV. Lane M: Ladder (marker). Lane 12: AvRV PCR product of 493 bp.

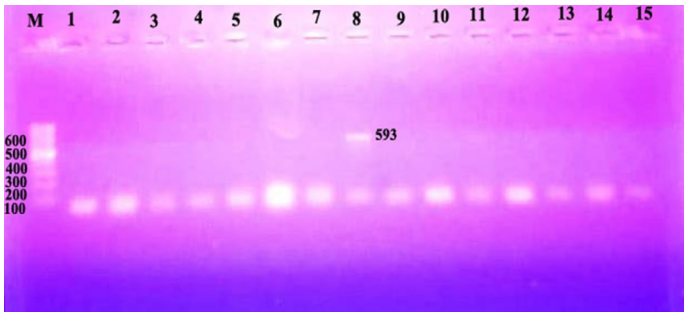

Fig. 4. Agarose gel electrophoresis of the RT-PCR products of MDPV. Lane M: Ladder (marker). Lane 8: MDPV PCR product of 593 bp.

Histopathology

Ducks from flocks infected with MDPV had common hepatocyte vacuolar degeneration and single cell necrosis in their livers. A small number of mononuclear cell infiltrations, hypertrophy of the blood vessel endothelium, and blood vessel congestion were visible in the portal area. Additionally, there were instances of severe blood vessels congestion and fatty changes in the liver cells. Infiltrations of mucosal inflammatory cells were visible in the intestine of the MDPV-infected flocks (Figure 5).

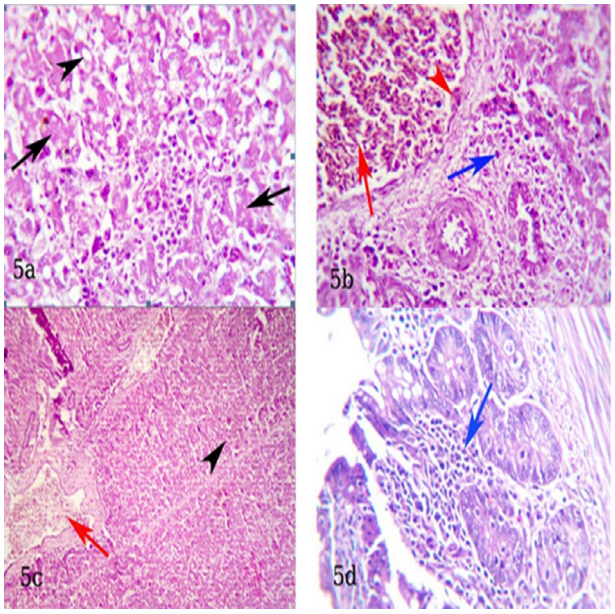

Fig. 5. Liver of MDPV infected flock showing (a) diffuse vacuolar degeneration of hepatocytes (black arrowhead) with coagulative necrosis (black arrows) due to infection with MDPV, (b) portal area showed congestion of blood vessels (red arrow), hypertrophy of endothelium of blood vessels (red arrowhead) and few mononuclear cell infiltrations (blue arrow), (c) fatty changes in hepatic cells (black arrowhead) with severe congestion of blood vessels (red arrow), and (d) the intestine showed mucosal inflammatory cell infiltrations (blue arrow).

Discussion

In order to investigate the causes of viral enteric illnesses in duck flocks from various locations in the four Egyptian governorates (Sharkia,

Gharbiya, Dakahlia, and Qalyubia), this study examined 42 flocks of ducks using clinical, molecular and histological methods. According to history and clinical investigation, the diseased flocks were suspected to have viral infections.

The most often detected clinical signs among the investigated flocks were various degrees and colors of diarrhea (100% of flocks). Also, approximately more than half of the examined flocks suffered uneven growth (57%) and a short beak and swollen tongue in 16.7%. The most prominent clinical sign in in rotavirus infected flock was watery diarrhea, similar results were recorded by Barnes (1997) who noticed a more severe enteric manifestation in 12 to 21-day-old birds, characterized by unrest, litter eating, watery feces, wet litter, and severe diarrhea. Also, Otto *et al*. (2012) reported that group A and D rotavirus dominated in chickens and turkeys causing diarrhea, growth retardation and/or runting and stunting syndrome (RSS).

MDPV infected flocks suffered from swollen tongue, shorter tibia and stunted growth. The obtained results were similar to that previously recorded by Palya *et al*. (2009) who detected short beak and dwarfism syndrome (SBDS) in studied ducks between 2002 and 2006 in Budapest, Hungary, after infecting day one to day 21 of age mule ducks orally with three different parvovirus strains. Saleh and Khodier (2020) observed the same SBDS symptoms in Mule, Muscovy, and Pekin duck flocks aged from 2 days to 2 weeks in several duck-producing areas in Egypt starting in 2015. These symptoms included significant growth retardation, tongue protrusion accompanied with a reduced beak, enteritis, and paralysis. However, the infection had a significant impact on the development of the tarsus and beak. MDPV did not produce serious lesions in the internal organs. The shortening of the beak is suggested as a consequence of virus effect on beak morphogenesis. Numerous facial prominences combine to form the beak. These prominences work together proportionately during development to form a unique beak. There are two proliferative zones in the frontonasal mass of the beak in ducks. These growth zones are associated with bone morphogenetic protein 4 (BMP4) activities that might be affected during parvovirus infection which in turn can result in the modulation of the beak shape (Wu *et al*., 2004).

Although DEV is the most predominant enteric virus in Egypt (Abd El-Ghany, 2021), no sample tested positive for DEV. This might be because most of the examined duck flocks were previously vaccinated with the DEV vaccine, however MDRV may not have been recognized since Egyptian duck flocks were not known to harbor it.

Our observations of respiratory symptoms could be the result of either managemental faults or bacterial causes such as Pasteurella species that were previously identified by Awad-Alla and Mahmoud (2011) in 100 ducks which had a history of respiratory problems and mortality in several private duck farms in the Sharkia Governorate of Egypt. The locomotive disorders in our study may be associated with several bacterial pathogens, including Staphylococcus aureus (S. aureus), Salmonella species and Escherichia coli, which have direct associations with leg pathologies in poultry (Abd El-Hamid *et al*., 2019).

The necropsy revealed enteritis in the ducks under investigation. This conclusion was supported by Otto *et al*., (2006) who recorded rotavirus-infection in 5-to-14-day-old broiler chicks from eight flocks suffering from runting and stunting syndrome (RSS) with intestinal lesions in Northern Germany. The rotavirus-infected flock's intestines' postmortem lesions showed that it had abnormally high levels of gas and fluid as well as ceca and enteritis. Spackman *et al*. (2010) observed the same postmortem picture in the intestines of turkey poults infected by avian rotavirus aged from 3 to 14 days in 2009 and noted this lesion.

Additionally, in this study, pericarditis, increased pericardial fluid and serofibrinous perihepatitis were noted as necropsy findings in MDPV infected flocks and these results were agreed with Mahardika *et al*. (2016) who studied 230 Muscovy ducks infected by MDPV of various ages in Bali, Indonesia, and reported severe enteritis, hepatitis, and fibrinous pericarditis. Hemorrhages and necrosis were observed on the liver of affected duck flocks (9.5% -28.5 %) respectively, which may be attributed to the high incidence of mycotoxicosis in ration in Egypt (Azzam and Gabal 1998; Grozeva *et al*., 2012). Many studies have been conducted on the effects of mycotoxins on poultry, as well as acceptable threshold limits. However, it has been established that ducks are more susceptible to mycotoxins than chickens or turkeys (Scott and Dean, 1991).

For the direct detection of four suspected enteric viruses (DVE, MDRV, MDPV, and AvRV) from intestinal tissue, a conventional PCR test has been utilized. According to Poonia *et al*. (2006), a portion of the MDPV (Viral structural protein (VP 1) -encoding gene) was amplified using PCR, and according to Otto *et al*. (2012), a portion of the AvRV (VP 6) gene was amplified using RT-PCR and specific primers. Only four positive samples out of 42 were reported, with one flock testing positive for AvRV (493 base pairs) and three flocks testing positive for MDPV (593 base pairs).

Only four samples confirmed viral infections; the remainder could be attributable to bacterial infections or managemental disorders. Many bacterial agents, such as Enterobacteriaceae and Pasteurella, have been implicated with duck enteric disease. Ibrahim (2003) observed whitish and greenish diarrhea in ducks infected with E. coli O86:K61, whereas Barrow *et al*. (1999) isolated and identified S. typhimurium from ducklings during their first two weeks of life in enteritis affected ducklings. Megahed *et al*. (2023) observed white fluid feces and stunting in 2-4 weeks old ducklings in Egyptian farms infected with Pasteurella multocida and Riemerella anatipestifer.

The lack of rotavirus vaccinations and the possibility of horizontal transmission from rearing different bird species in backyards may be the reasons behind our study's discovery of AvRV in Egyptian ducks kept in backyard. The primers used to identify rotavirus are not limited to ducks only. They are used to detect rotavirus in chickens and turkeys, indicating the probability of infection from other birds. Furthermore, the finding of rotavirus in a backyard flock highlighted the possibility of inter species transmission and justifying the need for additional research to determine the sequence of rotavirus infections in Egyptian duck farms. According to Otto *et al*. (2012), group A and D rotaviruses were predominant in chickens and turkeys, producing diarrhea, growth retardation, and/or runting and stunting syndrome. Similarly, RVs are one of the reasons of watery diarrhea, gut dilatation, and impaired food absorption in domestic birds such as pigeons and parakeets which are less common in pet birds like parrots than in pigeons (Lublin *et al*., 2004).

Histopathological examination of affected livers with MDPV showed extensive hepatocyte necrosis (apoptosis), hydropic degeneration with varying degrees of fatty changes, Inflammation in the portal tract and in between the liver cells, marked dilatation and congestion of central veins and dilated sinusoids with hemolyzed RBCs. The intestine of MDPV-infected flocks showed mucosal inflammatory cell infiltrations. These findings were consistent with those reported by Mahardika *et al*. (2016) who noticed diffuse hepatitis, sever enteritis and epicarditis in Muscovy ducklings aged 2-3 weeks in Bali, Indonesia, and it was due to Muscovy Duck Parvovirus Infection.

Conclusion

Muscovy duck parvovirus (MDPV) and avian rotavirus (AvRV) are implicated in the digestive illnesses of Egyptian ducks. Moreover, detection of Rotavirus in ducks from Egypt in this work is considered the first record which is crucial, as it may present a major challenge to the duck industry and offers insights for future disease management strategies and the overall well-being of duck populations in Egypt.

Conflict of interest

The authors declare that they have no conflict of interest.

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