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SHORT COMMUNICATION

Pathological Investigations in the First Reported Case of Rotaviral Enteritis in Racing Pigeons in Bulgaria

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ABSTRACT

The present report aims to present the pathomorphological characteristics in the organs and systems during rotavirus enteritis infection in young domestic pigeons, diagnosed for the first time in Bulgaria based upon macro- and microscopic changes in the organs in combination with the method of direct immunofluorescence. This concerns a private flock of domestic pigeons bred in Southern Bulgaria for sporting and racing purposes, the Pazardzhik High-Flying Pigeon breed. The total number of birds was 3,000, 800 having age from 3 weeks to 70 days. The described pathological changes in the various intestinal sections contribute to clarifying the diagnosis and differential diagnosis of rotavirus enteritis in pigeons from other diseases such as: *Coccidiosis, Salmonellosis, Trichomoniasis, Hexamitosis, Colibacteriosis,* and *Cryptosporidiosis* as well as other widespread intestinal diseases and viral infections in pigeon flocks.

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INTRODUCTION

Rotaviruses (RV), belonging to the *Reoviridae* family, are widely distributed in humans, animals, and birds (Jindal *et al.,* 2010 & 2014). They are considered as one of the most common enteric pathogens (Pantin-Jackwood *et al.,* 2008; Singh *et al.,* 2017). According to Koo *et al.* (2013), many bacteria and viruses cause diarrhoea in birds alone or in combination with other entero-pathogens. The pathogenesis of the *G18P [17]* genotype of rotavirus A (RVA) was first time evaluated by Rubbenstroth *et al.* (2020) in young pigeons. RVA has been demonstrated in domestic pigeons in many countries around the world, from different cases of clinically ill and asymptomatic pigeons (McCowan *et al.,* 2018). RVA is involved in a complex of diseases in young pigeons known as young pigeon disease syndrome (YPDS) (Dhama *et al.,* 2015). In most cases, RVA causes systemic infection in pigeons, and necrotic lesions have also been described in the livers of diseased birds. RVA infection spreads rapidly in a pigeon loft and usually causes high morbidity of up to 100%, and mortality ranging from over 50% (Blakey *et al.,* 2019). The course of the disease in affected flocks lasts about one week and it takes up to three weeks for the sick pigeons to recover (Rubbenstroth *et al.,* 2020). Clinical manifestations of RVA infection include apathy, anorexia, mucous or watery greenish diarrhoea, vomiting, stunted growth of young pigeons and subsequent death or illness. Neurological and respiratory signs have also been observed in 68-day-old pigeons with rotavirus infection (Rubbenstroth *et al.,* 2020; Meßmer *et al.,* 2022). Macroscopic changes observed in the organs are; a diffusely enlarged and spotted liver and a moderately enlarged pale spleen, enlarged and pale kidneys, and the intestinal tract with thin walls, along with watery & greenish intestinal contents (Schmidt *et al.,* 2021). After reviewing the literature, we found a lack of sufficient information regarding the pathological changes in the intestinal tract of domestic pigeons with rotavirus enteritis.

The present report aims to present the characteristic pathomorphological changes within the organs in rotavirus enteritis infection in young domestic pigeons bred for sports and competition purposes.

MATERIALS AND METHODS

Owner consent was obtained for the procedures undertaken and the use of the data for research purposes while this study was approved by the Ethics Committee at Trakia University (Decision No. 024/2025). This concerns a private flock of domestic pigeons bred in Southern Bulgaria for sporting and racing purposes, the Pazardzhik High-Flying pigeon breed. The total number of birds was 3,000, 800 ranging from 3 weeks to 70 days of age. All birds were raised in an aviary, with some compartments

being overcrowded. The rearing technology was to keep the breeding pairs separate from the young pigeons intended for racing flights. The birds were periodically vaccinated against salmonella, paramyxovirus, adenovirus, calicivirus and herpesvirus infection. The birds were fed with 20-component mixtures for racing pigeons in the amount of 22grams per pigeon while trace elements, vitamins and minerals were given *ad libitum*. Antiprotozoal preparations and antibiotics were administered preventively, and they were also dewormed against external and internal parasites. Clinical and epidemiological studies were conducted during the spring, summer and autumn seasons on the entire flock of 3,000 pigeons. Clinical signs of intestinal disease were evident in 70% of young and adolescent pigeons. Mortality in affected young pigeons was found to be between 30% and 57%. The observed signs were somnolence, anorexia, delayed growth and development, pale conjunctiva, ruffled feathers, vomiting of cloudy watery contents mixed with grains of food, a protruding sternum, yellow-greenish spots around the cloaca and tail feathers, pale greenish watery feces, weakness and immobility. In single birds, catarrhal purulent discharge from the nostrils and stridor were observed. The diseased young pigeons were treated with enrofloxacin 2ml/L of water, but no effects were observed.

Histopathological examination: The owner provided 32 corpses of dead pigeons for autopsy and subsequent diagnostic tests. After autopsy, tissue samples for histopathological examination measuring 1cmx1cm were obtained from the affected parts of the intestinal tract: duodenum with pancreas, jejunum and ileum as well as from spleen, kidney and liver. The materials for pathohistological examination were fixed in 10% neutral buffered formalin for 48-72 hours and embedded in paraffin and stained conventionally with haematoxylin-eosin (H/E) stain.

Immunofluorescence studies: Tissue samples from intestinal segments (duodenum, jejunum, ileum, cecum, rectum) were also obtained for immunofluorescence studies. Monoclonal conjugated antibody (anti Coronavirus and Rotavirus FITC conjugated 0.5ml (20X), BIO 023, (BIOX Diagnostics, Belgium) was used for this. After fixation with acetone, they were rinsed thoroughly with PBS and FITC conjugated monoclonal antibody was then added. The infiltrated lamellae and deparaffinated FITC orthotics were kept in a humid chamber at 37ºC. After 1 hour, FITC was removed by washing 3 times (10min) with PBS. They were then contrasted for 10 minutes with elongated blue at a concentration of 1:10,000 for historesis and 1:100,000 for cell culture lamellas. The final step was drying the lamellas and histosrets, fitting with PBS and glycerol in a ratio of 9:1 and observed under a fluorescence microscope. Parasitological studies were performed on diarrheal feces and intestinal contents using the Henriksen methods (Emmonya Biotech, Chelopech) – modified Ziehl-Neelsen staining and flotation studies using the Fulleborn's method for the detection of protozoan agents. Blood clots from the heart, liver, kidney, spleen and ligated small intestine were also used for bacteriological studies.

RESULTS AND DISCUSSION

External examination: After the pathological examination according to the standard autopsy protocol for bird, the following changes were noted: the bird carcasses were severely emaciated, the conjunctivae were pale, and eyeballs were sunken with a dried cornea. The beak was red, and catarrhal purulent discharge was observed from the nostrils of some birds. The tongue and throat were hyperaemic; there was a mucous discharge in the choana area. Hyperemia was observed on the soft palate. The plumage was bristling and moulting without shine of the feathers, there were stains in the cloaca area, the flight feathers and the feathers of the tail and under tail with watery greenish feces. Similar changes have been observed by a team of Western authors (Rubbenstroth *et al.,* 2020). The sternum was strongly convex with deformation in 4 of the autopsied birds. In a section of the skin, the pectoral muscles were poorly developed and had a pale colour. In a section of the pleuroperitoneal cavity in 13 pigeons, the liver was enlarged and occupied two-thirds of the volume of the cavity. It had rounded edges, a friable consistency, and numerous whitish spots on the surface with irregular shape. Other researchers also described the presence of necrosis in the liver in systemic rotavirus infection in pigeons (Blakey *et al.,* 2019) while Lublin *et al.* (2004) reported that the main pathoanatomical lesions of rotavirus enteritis in birds were: transparent intestinal walls, enlarged gallbladder, pancreatic atrophy, degeneration of the bursa of Fabricius, rickets and proventiculitis. In all 32 autopsied pigeons, the spleen was pale and reactive. The intestinal tract was diffusely hyperaemic, the intestinal wall was dilated, flaccid and severely thinned with visualization of the contents having a light green colour and a watery-mucous consistency (Fig. 1). On section of the intestine, the mucosa was severely thinned due to severe catarrhal desquamative processes. The macro-lesional changes in the liver, spleen, and intestines described in this study are similar to those described by Schmidt *et al.* (2021). The pancreas was locally hyperaemic, the kidneys were friable and slightly enlarged. The air sacs were turbid, and the pericardium was filled with clear fluid. In 4 pigeons, pneumonic areas of lobular nature were also detected in the lung. Bursa of Fabricius had reduced dimensions. In section of the cervical organs, the trachea had hyperaemic mucosa, the crop was empty, and its epithelium was peeled. Single punctate haemorrhages measuring about 1mm were observed bilaterally on the thymus. The infraorbital sinuses in 4 birds were filled with catarrhal purulent exudate.

Histopathological changes: Pathomorphological changes observed in individual segments of the intestinal tract were expressed in desquamative and degenerative necrobiotic changes of the enterocytes. In the submucosa, the blood vessels were strongly dilated with congestion, and in the subserosa, edema was observed. In all examined intestinal sections, the histological structure of the villi was disturbed, as a result of desquamative and destructive processes (Fig. 2). Inflammatory cellular elements mixed with exudate, pronounced atrophy of the villi and their fusion were observed in the mucosa of the duodenum, jejunum and ileum. In contrast to our study McNulty (2003) reported desquamation and vacuolization of enterocytes along with inflammatory cell infiltration in the lamina propria. Other authors (Ciarlet *et al.,* 1998) described the presence of absorptive epithelial cells in the distal small intestine, inflammatory cell infiltration in the

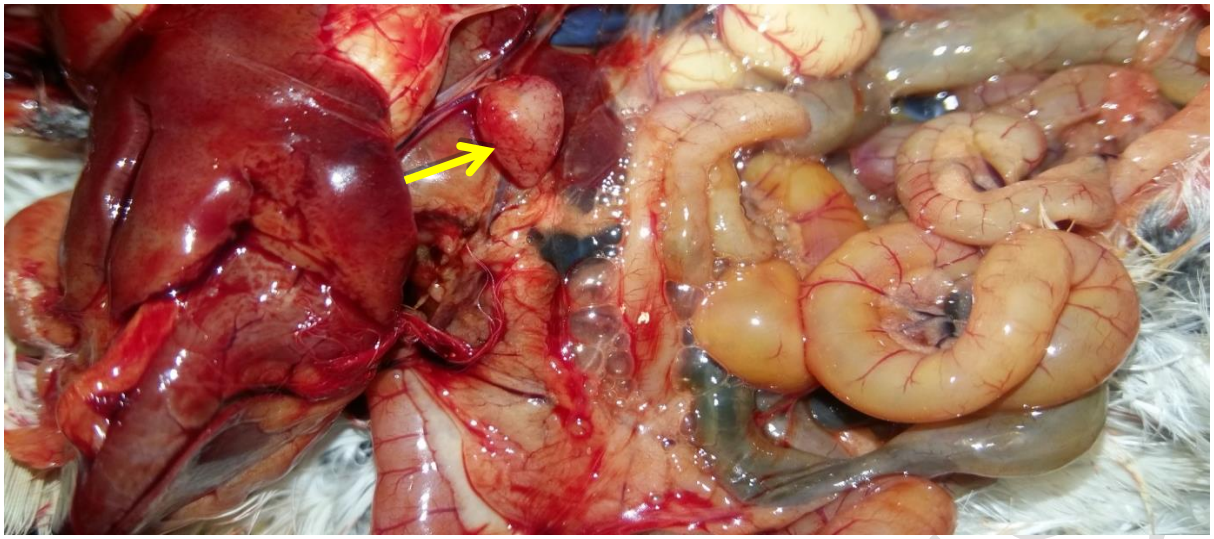

Fig. 1: Friable consistency of the liver a reacted spleen with a pale color and local hyperemia (arrow). Hyperemia of the intestinal tract, dilated, thinned and relaxed intestinal wall with visualization of the contents having a light green color and watery-mucous consistency a pigeon with rotaviral enteritis.

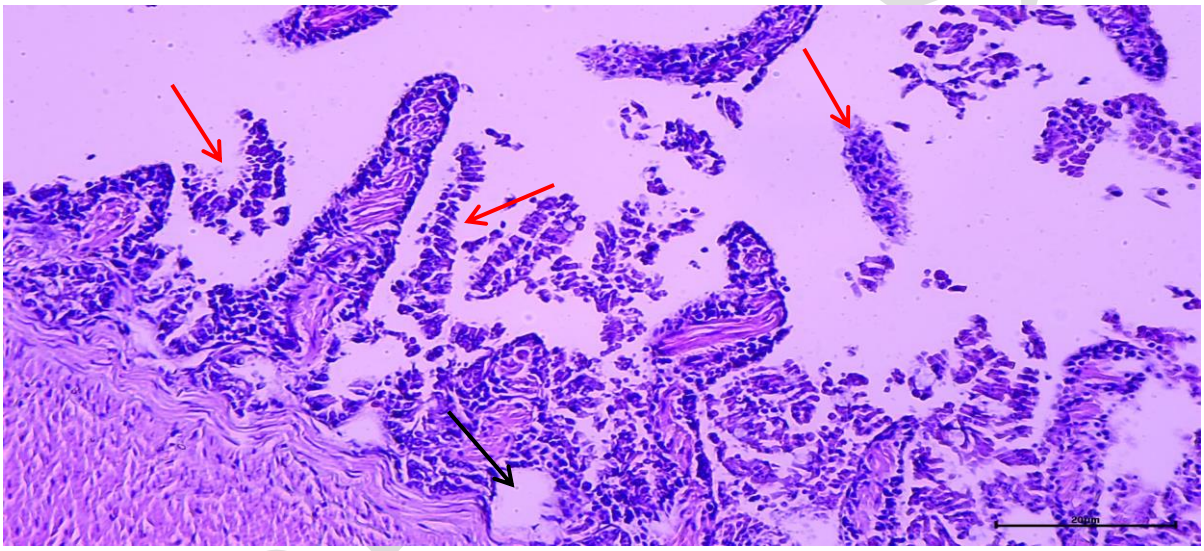

Fig. 2: Catarrhal-desquamative enteritis affecting all parts of the villi (red arrows). Exudate mixed with desquamated epithelial cells and inflammatory cellular elements, subserous edema and hyperemia of the vessels (black arrow) duodenum, pigeon with rotaviral enteritis, H/E, bar=10µm.

cecum, and marked villus atrophy in the duodenum and jejunum while Mori *et al.* (2002) reported the following microlesions in pigeons with *RV (PO-13)* infection: ballooning and degeneration of epithelial cells in the duodenum, jejunum, and ileum. There was lymphocyte infiltration in the crypts and lamina propria of the small intestine. However, Kim *et al.* (2020) observed lymphocyte infiltration in the lamina propria in rotaviral enteritis in birds.

Microscopic changes in the liver consisted of perivascular inflammatory infiltrates of lymphocytes and histiocytes, hyperemia of the sinusoidal capillaries, as well as degenerative and necrobiotic changes of hepatocytes. According to Blakey *et al.* (2019), hepatocyte necrosis and focal mononuclear cell infiltrates were common microlesions in rotavirus infection in pigeons while McCowan *et al.,* (2018) reported that in addition to necrosis in the liver, necrosis was also observed in the myocardium of the heart. The spleen showed edema, white pulp hyperplasia, and local haemorrhages. Desquamative and degenerative changes were observed in the epithelial cells covering the basal membrane of the renal tubules, cell casts, and vascular hyperemia. Similar pathomorphological changes to ours were found by Rubbenstroth *et al.* (2020) in experimental infection of pigeons with the *G18P[17]* genotype of rotavirus A (RVA). In all pigeons infected with *G18P[17]* they observed: multifocal, follicular hyperplasia of the white pulp of the spleen and multifocal, periportal, nodular lymphoid aggregates, siderocytes and degeneration and necrosis of the liver. However, in contrast to our results, Rubbenstroth *et al.* (2020) did not observe histological changes in the other organs and systems of the experimentally infected pigeons.

Immunofluorescence (IFA) studies: The direct immunofluorescence method used proved the presence of rotavirus antigens in tissue sections from the jejunum, no coronavirus antigens were detected in the intestinal segments examined (Fig. 3). Cryptosporidium oocysts and other protozoan agents were not detected in the studies

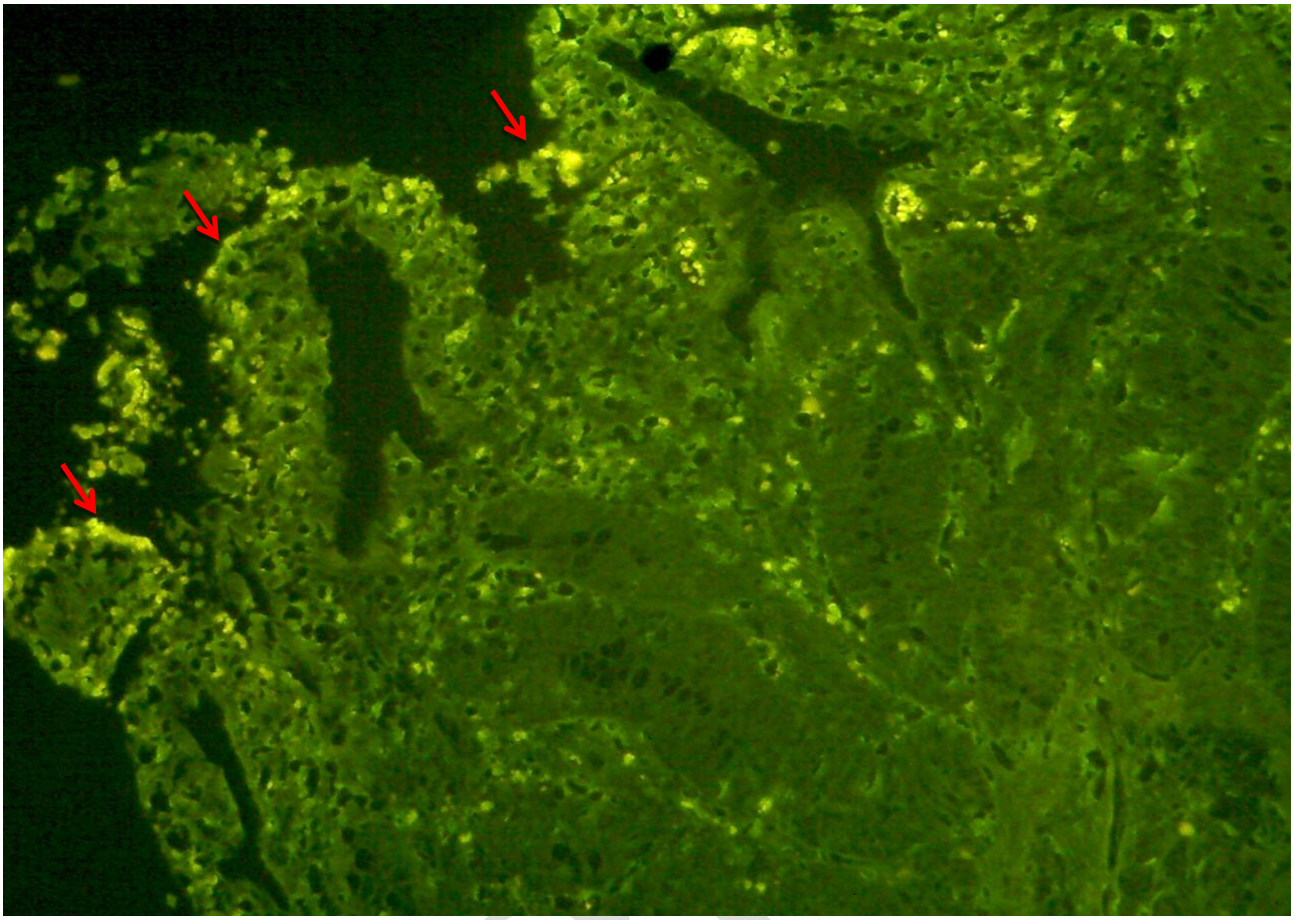

Fig. 3: Positive result for rotavirus antigen from immunofluorescence examination (arrows) of the jejunum of a pigeon with rotaviral enteritis.

conducted. No pathogenic microorganisms were isolated from the microbiological examination conducted for isolation and identification of aerobic and anaerobic microbial pathogens.

The presented report of rotavirus infection in domestic pigeons proves the spread of rotaviruses among flocks of domestic pigeons kept in Bulgaria, as well as their involvement in intestinal infections and in the complex of diseases known as young pigeon disease syndrome (YPDS). The described pathoanatomical and histopathological changes in the various intestinal sections contribute to clarifying the diagnosis and differential diagnosis of rotavirus enteritis in pigeons from other diseases such as: *Coccidiosis, Salmonellosis, Trichomoniasis, Hexamitosis, Colibacteriosis, Cryptosporidiosis* as well as other widespread intestinal diseases and viral infections in pigeon flocks.

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