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Short comminucation

Paratyphoid infection caused by *Salmonella* Typhimurium in a pigeon flock (*Columbia livia*) in Iran

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Summary

Salmonellosis is well known as a severe problem of all bird species, as well as humans, which is considered as one of the most common infectious diseases in pigeons. In the present study, a homing pigeon flock was referred with non-specific clinical signs such as diarrhea, progressive weight loss, weakness, drooping wings associated with a 21.05% mortality rate. At necropsy, severe necrotic hemorrhagic enteritis was found together with severe dehydration and pectoral muscle atrophy. In order to investigate the possibility of Salmonella infection, bacterial examinations were performed on Brilliant Green (BG) agar, Xylose-lysine deoxycholate (XLD) agar, and Salmonella-Shigella (SS) agar plates. Besides, biochemical tests were conducted to confirm Salmonella identification. Moreover, tissue samples were taken for histopathological examination. The results of bacteriological examinations showed the presence of *Salmonella* Typhimurium through *Salmonella* colonies on the applicable media, Gram stain smears, and biochemical analysis. At histopathological studies, which consistent with the bacteriological findings, severe enterocyte necrosis, heterophil infiltration, and hemorrhage were observed in the intestinal mucosa. Moreover, there was vascular congestion together with focal necrosis and inflammation in the liver, kidney, and spleen. In conclusion, it seems that infection by S. Typhimurium is frequently occurring in pigeons by diarrhea and enteritis. Thus, it should be considered in relation to public health.

***Keywords*:** Salmonella, pigeon, bacteriology, pathology, public health

Introduction

Pigeons are found in all regions of the world, which almost live near humans (as a pet bird) and other domestic animal species in nature. Besides, they are considered as a source of food (meat and egg) as a hobby, symbol, and for experimental purposes (Khordadmehr et al., 2018). Importantly, pigeons can play a role in spreading some zoonotic diseases to people

and other mammals and are a reservoir of some infectious diseases of poultry (Piasecki, 2006; Volkova et al., 2011; Greig and Ravel, 2009). Salmonellosis is well known as a severe problem in all bird species, which is one of the most common pigeon diseases, caused by *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) and *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis). Of note, it was also believed that Salmonellosis could lead to a high mortality rate among bacterial diseases in pigeons (Tanaka et al., 2005). Infection by this organism is frequently presented by enteritis, diarrhea, and septicemia in fetal cases (Farghaly and Heba, 2011). Currently, it is more likely to be found in birds from aviaries that have a notable rodent infestation.

The present study describes the clinical, pathological, and microbial finding of paratyphoid infection in a pigeon flock (with 57 pigeons), which was housed in Yazd Province; the central part of Iran.

Case presentation and methods

The owner of a pigeon flock (*Columbia livia*) consisting of 57 birds (in Yazd Province; central part of Iran with warm and dry climate) complained from a digestive disorder associated with closed eyes, diarrhea, progressive weight loss, weakness, depression, drooping wings, and 21.05% (12 birds) mortality, which occurred 3–5 days after clinical signs. In addition, there were mild conjunctivitis and fecal soiling of the feathers of the vent, a lesion consistent with diarrhea. At necropsy of the fresh pigeon carcasses, there was severe necrotic hemorrhagic enteritis together with severe dehydration and pectoral muscle atrophy (Fig. 1). Gas and fluid distended the intestine. Other visceral organs such as liver, kidney, spleen, and lung were enlarged and congested.

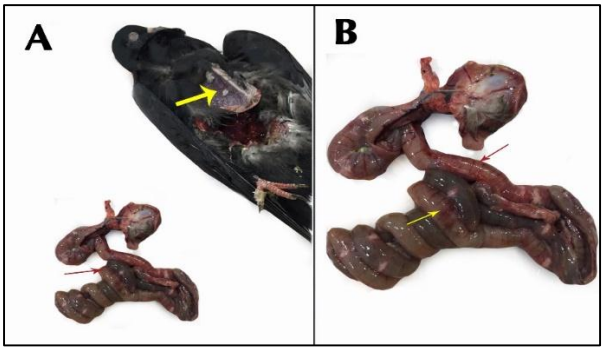

Fig. 1. Paratyphoid infection, pigeon. **A:** Severe hemorrhagic enteritis (red arrow) and atrophy of pectoral (yellow arrow) muscle were found at necropsy. **B:** Severe necrotic hemorrhagic enteritis (arrows) is the most frequent gross lesion of paratyphoid infection in pigeon.

Firstly, bacterial cultivation was performed. For this purpose, one gram of the intestinal content aseptically transferred into a sterile tube consisting of 10 ml of Tetrathionate Broth (Merck, Germany) and Selenite-F (Merck, Germany) broth medium. Then, the tubes were incubated at 37 °C for 24 h under aerobic conditions. Subsequently, a loopful of the media was streaked onto Brilliant Green agar

(BG, Merck, Germany), Xylose-lysine deoxycholate agar (XLD, Merck, Germany), and Salmonella-Shigella agar (Merck, Germany) plates which aerobically incubated at 37 °C for 24 h. Suspected colonies were taken and streaked onto Nutrient agar (Merck, Germany), which incubated at 37 °C for 24 h for purification. For morphological evaluation, the suspected colonies were stained by Gram stain and studied microscopically. Then, the colonies with morphological features of Salmonella were subjected to biochemical identification using the criteria as previously described (Ammar et al., 2014; Dutta et al., 3013).

Besides, the tissue samples (intestine, liver, kidney, and lung) were taken for histopathological examination, which were placed in a 10% buffered formalin solution. The samples were processed routinely, sectioned 4-5 µm, and stained by hematoxylin and eosin (H&E). Finally, the prepared tissue sections were studied by a light microscope (Olympus, Japan).

Results

Salmonella colonies presented pink, red with a black center, and pale with a black center on BG agar, XLD agar, and SS agar, respectively. Gram-negative rod-shaped Bacillus was detected on Gram's stain smears of suspected colonies. The results of biochemical tests were shown in Table 1.

At the histopathological examination, there were severe enterocyte necrosis, ulceration, and hemorrhage in the intestinal mucosa associated with fibrin deposition and heterophil infiltration (Fig. 2). The lesions extended into the submucosa muscularis, which accompanied with crypt dilatation and abscess formation. Moreover, vascular congestion together with focal necrosis and inflammation were observed in the liver, kidney, and spleen. The lung was significantly congested.

Table 1. The results (positive +/negative -) of biochemical test for *S. typhimuruim*

Biochemical test	Result
Catalase	+
Oxidase	-
Citrate	+
Gas	+
Gram Staining	-
H2S	+
Indole	-
Motility	+
MR*	+
VP*	-
TSI*	K/A gas
Urease	-
Glucose fermentation	+ (gas)
Lactose fermentation	-
Mannitol fermentation	+
Sucrose fermentation	-
Lysine decarboxylase	+
ONPG*	-
Ornithine decarboxylase	+

MR: Methyl Red; TSI: Triple Sugar Iron; VP: Voges Proskauer; ONPG: β-galactosidase

Discussion

In the present study, the results of biochemical tests were in agreement with the previous study for the identification of *S.* Typhimurium in the pigeon (Ammar et al., 2014; Malorny et al., 2011). Cultivation of Salmonella in simple media associated with traditional serotype designations (*S. Enteritidis, S. Typhimurium*) is still frequently used for brief diagnostic and epidemiologic studies (Gast, 2013; Voogt et al., 2001), which were subjected in the present study. Incidences of infection can vary substantially across national borders, even within the same geographic region (Pieskus et al., 2008), and national *Salmonella* isolation rates can sometimes alter notably from year to year (Eyigor et al., 2005). It seems that among Salmonella species, the occurrence of *S.* Typhimurium was demonstrated to be dominant among the detected Salmonellae (Takana et al., 2005). A previous study reported infection rates of 40% and 20% in pigeons (squabs) in Egypt by *S.* Typhimurium and *S.* Enteritidis, respectively (Ammar et al., 2014).

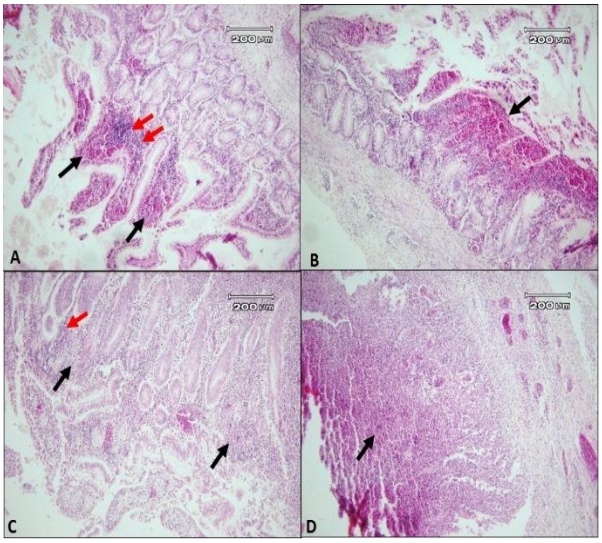

Fig. 2. Paratyphoid infection, pigeon. A and B: Small intestine showed severe hemorrhagic enteritis (black arrows) associated with heterophil infiltration (red arrows) in the intestinal mucosa. C and D: there were enterocyte necrosis (black arrow), ulceration, and heterophil infiltration (red arrow) in the intestinal mucosa. H&E.

Salmonella sp. generally are invasive, leading to significant lesions outside of the intestinal tract. It was clearly investigated that *S.* Typhimurium and *S.* Enteritidis, which are the main cause of bacterial infection in pigeons occasionally result a typhoid condition characterized by gastroenteritis, oophoritis/orchitis, arthritis, conjunctivitis, and high mortality (Dutta et al., 2013). In this regard, it is believed that homing pigeons that are housed in groups in pigeon lofts can facilitate long-term maintenance of a *Salmonella* infection (Ammar et al., 2014), which should be considered for public health. Similar histopathological lesions were previously presented in pigeons (Phangcho

2001, Dutta et al., 2013) such as severe necrosis in the intestinal mucosa, together with mucosal sloughing, vascular congestion, heterophilic and mononuclear infiltrations (Dutta et al., 2013). It was obviously represented that flagella, fimbria, and LPS play important roles in gastrointestinal attachment by Salmonella (Carroll et al., 2004, Garber et al., 2003; Dibb-Fuller and Woodward, 2000).

Conclusion

Regarding many of the serotypes that are most prevalent in humans (notably *S.* Enteritidis and *S.* Typhimurium) are similarly common in poultry and other birds like pigeon (Gast, 2013), prevention, diagnosis, and treatment of the affected birds are necessary for public health. Of note, homing pigeons occasionally are as pet birds and have close contact with the human. Thus, the health condition of these birds should be given special attention.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Not applicable

Ethics approval

Not applicable

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