Pakistan J. Zool., pp 1-5, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230402210451

Short Communication

Occurrence of Virulent *Salmonella enterica* Among Migratory Birds: A Potential Zoonotic Risk

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ABSTRACT

Salmonella is considered as a foodborne pathogen causing many public health problems worldwide. However, salmonellosis is a zoonosis with wide range animal reservoirs; the role of migratory birds in the transmission of *Salmonella* spp. is not well known. This study aimed to underline the potential role of migratory birds in the transmission of virulent *Salmonella enterica* serovars and highlighting the possible zoonotic risk. Cloacal swabs were collected from 496 migratory birds (299 quails and 197 ducks) from Gamsa city, Egypt. The collected swabs were enriched and cultured for isolation of *Salmonella*. The isolates were identified using conventional methods including the colonial characters, Gram's staining and biochemical tests. Moreover, molecular detection of *invA*, *stn* and *spvC* genes was performed among the obtained isolates to identify the virulent strains. Of the examined birds, 3 yielded *Salmonella enterica* giving an overall occurrence rate 0.6% (3/496) whereas only migratory ducks were positive for *Salmonella* spp. with a prevalence 1.5% (3/197). Moreover, pintail is the only duck species that yielded positive results 4.3% (3/70). All *Salmonella* isolates are considered virulent strains as they carry *invA*, *stn* and *spvC* genes. The BLAST and phylogenetic analyses of the *spvC* gene sequences revealed that these sequences showed high genetic relatedness with those isolated from humans (Nigeria), cattle and quails (USA). This study sheds more light on the role of migratory birds in the epidemiology of exotic virulent *Salmonella enterica* strains with a possible zoonotic risk.

Article Information
Received 02 April 2023
Revised 05 December 2023
Accepted 17 December 2023
Available online 07 March 2024
(early access)

Authors' Contribution
AS collected samples, performed laboratory work, investigation, analysis of data and writing the manuscript. KAA-M and MAS contributed to conceptualization, investigation, analysis of data and writing the manuscript. All authors gave final approval and agreed the final version of the manuscript including its integrity and accuracy.

Key words
***Salmonella, spvC*, Migratory birds**

Climatic changes and food shortage force millions of birds to migrate every year between continents. During the migration journey of the birds, they may act as a biological vector and transmit many zoonotic pathogens like bacteria, viruses and parasites (Fuller *et al*., 2012; Korytár *et al*., 2020; Seleem *et al*., 2021). Zoonotic pathogens may find their way to humans either through direct contact with birds during hunting or through consumption of its meat (Konicek *et al*., 2016). The game bird meat includes wild turkeys, wild geese, wild ducks, grouse, quails and pheasant (Costa *et al*., 2016). Game meat consumption is dramatically increased throughout the world (Milner-Gulland and Bennett, 2003), as it is safe and free from hormones and antibiotic residues. Moreover, it is very low in microbial load compared with meat from farm animals (Costa *et al*., 2016; Hedman *et al*., 2020). On the other hand, migratory game birds may shed many zoonotic pathogens especially exotic ones in their droppings to contaminate their meat (Sauvala *et al*., 2021).

One of the most important bacterial enteropathogens that is transmitted by migratory birds is *Salmonella* species (Benskin *et al*., 2009; Giorgio *et al*., 2018; Malik *et al*., 2021). It is considered as a foodborne pathogen, which is usually transmitted through ingestion of contaminated food and water (Kim *et al*., 2013; Eng *et al*., 2015; Abdel-Kader *et al*., 2022). *Salmonella* serovars cause many diseases in humans like typhoid fever, gastroenteritis and diarrhea (Giannella, 1996; Kurtz *et al*., 2017). Wild birds constitute a potential reservoir to *Salmonella* species and thus, migratory birds may play a role in transmission of *Salmonella* during their journey (Ehuwa *et al*., 2021).

There are many virulence genes found in *Salmonella* such as *invA*, *stn* and *spv*C (Chaudhary *et al*., 2015), of which *invA* gene is found in *Salmonella* Pathogenicity Island 1 (SPI-1) and is considered as a marker for virulent strains of *Salmonella* spp. (Mthembu *et al*., 2019; das Neves *et al*., 2020). Also, *Salmonella* enterotoxin *stn* is considered as strong virulence factor and responsible for diarrhea (Nakano *et al*., 2012), while *spvC* gene is a determinant causing systemic *Salmonella* infection by inhibition of intestinal inflammatory response (Deguenon *et al*., 2019; Zuo *et al*., 2020). The current study has been conducted to underline the potential role of migratory birds

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0030-9923/2024/0001-0001 $ 9.00/0

in transmission of virulent *Salmonella enterica* serovars and highlighting the possible zoonotic risk.

Materials and methods

Cloacal swabs collected from 496 migratory birds (299 quails and 197 ducks) after catching them directly at Gamasa city, Egypt. The birds were captured live by expert hunters using the nets. The collected swabs were inserted were inserted in sterile tubes containing buffer peptone water Van der Zee (2003) and transported to the laboratory in the icebox (García *et al*., 2011). The birds were identified according to Carboneras (1992).

The collected swabs in buffer peptone water tubes were incubated at 37 °C for 16-18 hours, then 1ml from each tube was added to 10 ml Rappaport-Vassiliadis (RV) broth (OXOID, England) and incubated at 42 °C for 24 h. Loop-fulls of RV broth were streaked on xylose lysine deoxycholate (X.L.D) medium at 37 °C for 24 h. The suspected *Salmonella* colonies were identified by Gram's staining and conventional biochemical tests according to OIE (2018). Then, confirmed by RapID ONE System used for identification of *Enterobacteriaceae* (Oxoid, UK).

For molecular identification of virulent *Salmonella enterica* PCR reactions were performed to detect *invA*, *stn* and *spvC* genes among the obtained isolates. DNA was extracted from *Salmonella* isolates using boiling method then the extracted DNA was stored at -20°C for further investigation (Soumet *et al*., 1994). Uniplex PCR was carried out, targeting *invA*, *stn* and *spvC* genes using specific primer sets. *invA* gene primers F (5′-GTGAAATTATCGCCACGTTCGGGCAA-3′) and R (5′-TCATCGCACCGTCAAAGGAACC-3′) amplify 284 bp according to Oliveira *et al*. (2003), while *stn* gene primers F (5′-TTGTGTCGCTATCACTGGCAACC-3′) and R (5′-ATTCGTAACCCGCTCTCGTCC-3′) target 617 bp according to Murugkar *et al*. (2003). *spvC* gene primers F (5′-ACCAGAGACATTGCCTTCC-3′) and R (5′-TTC TGATCGCCGCTATTCG-3′) amplify 467 bp according to Huehn *et al*. (2010). The amplification conditions of *invA*, *stn* and *spvC* genes were initial denaturation at 94°C for 5 min, denaturation 94°C for 30 sec, annealing at 55°C for 30 sec, 59°C for 40 sec and 58°C for 40 sec for *stn*, *invA* and *spvC* genes, respectively, extension at 72°C for 40 sec and final extension at 72°C for 7 min for 35 cycles. A T3 Thermal cycler (Biometra, Germany) PCR system was used for PCR reactions. The PCR products (10 µl) was run on 1.5% agarose. Amplicons of the three *spvC* positive isolates were purified using QIAquick PCR product extraction kit (Qiagen Inc. Valencia, CA) and the sequencing was conducted by using Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). The obtained *spvC* gene sequences were deposited in the GenBank under the following accession numbers: MW701427 - MW 701428 - MW701429.

spvC gene sequences were analyzed using nucleotide BLAST on the NCBI website (www.ncbi.nlm.nih.gov/BLAST) to identify the most similar sequences available in the GenBank. Afterwards, *spvC* gene sequences were aligned against some selected similar sequences from different sources retrieved from the GenBank. The alignment of the sequences was done using Clustalw Multiple alignment (BioEdit 7.0.9) and a phylogenetic tree has been constructed using the neighbor-joining method based on *spvC* partial gene sequences with MEGA 7 software (version 7.0.26).

Results and discussion

Out of 496 examined migratory ducks and quails, 3 yielded *Salmonella enterica* giving an overall occurrence rate 0.6%. All isolates were obtained from migratory ducks with a prevalence rate 1.5%, while none of the examined quails yielded positive results. Moreover, only pintail ducks (*Anas acuta*) were positive for *Salmonella enterica* 4.3% (Table I). All 3 *Salmonella enterica* isolates were found to be virulent strains as they possess *invA*, *stn* and *spvC* genes.

Table I. Occurrence of *Salmonella enterica* among migratory birds.

Species	Numbers examined	Numbers positive (%)	*InvA* gene	*Stn* gene	*Spvc* gene
Coturnix coturnix	299	0	-	-	-
Anas acuta (Pintail)	70	3(4.3%)	+ve	+ve	+ve
Anas clypeata	8	0	-	-	-
Anas crecca	109	0	-	-	-
Anas platyrhynchos	5	0	-	-	-
Fulica atra	5	0	-	-	-
Total of the examined ducks	197	3(1.5%)	+ve	+ve	+ve
Total	496	3(0.6%)			

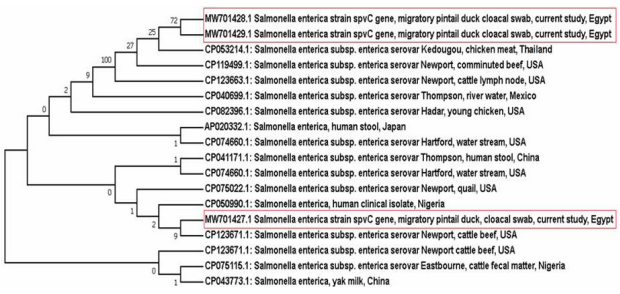

Fig. 1. Neighbor-joining phylogenetic bootstrap consensus tree shows the evolutionary history and genetic relatedness of the obtained *spvC* gene sequences of *Salmonella enterica* recovered from migratory ducks and selected similar sequences retrieved from the GenBank records.

The identity percentages of the obtained *spvC* gene sequences based on BLAST analysis are shown in Table II. The phylogenetic bootstrap consensus trees of *spvC* gene sequences showed high genetic relatedness with those of both humans and animals in USA, Nigeria and Thailand (Fig. 1).

Table II. The identity of the *spvC* gene sequences of *Anas acuta* after BLAST analysis on GenBank.

Source	Country	Identity %	Accession number	Study accession number
Human stool	Japan	97.95	AP020332	MW701427
Human stool	Nigeria	97.95	CP050990	
Chicken meat	Thailand	97.95	CP053214	
Cattle-lymph node	USA	99.76	CP 123663	MW701428
Beef	USA	99.76	CP123671	
Sheep (cecal)	USA	99.76	CP123669	
Comminuted beef	USA	99.76	CP119507	MW701429

The movement of the migratory birds across countries and continents allows them to act as potential spreaders of many pathogens, especially zoonotic ones (Altizer *et al*., 2011; Contreras *et al*., 2016). There is a scarcity of data about the role of migratory birds in the transmission of *Salmonella* spp. which may be owed to the difficulties in collection of samples from migratory birds. Majority of previous researches focused on the role of migratory Northern pintail as a reservoir for avian influenza virus (Jahangir *et al*., 2009; Wei *et al*., 2020).

The current study revealed that the overall occurrence of *Salmonella enterica* among the examined migratory birds was 0.6%. Only ducks yielded positive results with a prevalence rate 1.5%. Interestingly, all *Salmonella* isolates were recovered from pintail ducks giving a prevalence rate 4.3%. Such result is higher than that detected by Wei *et al*. (2020), who recorded that the overall occurrence of *Salmonella* among pintail ducks was 0.93% and higher than that detected by Grigar *et al*. (2017) who did not isolate any *Salmonella* spp. from pintail ducks in their study.

On the other side, none of the examined quails yielded *Salmonella* spp., such result which is matched with that obtained by Dipineto *et al*. (2014) who found that all examined common quails were negative for *Salmonella* spp., Also, Musa *et al*. (2023) who did not isolate *Salmonella* spp. from migratory birds including quails.

Since the *spvC* gene is important in *Salmonella* pathogenesis, it was selected for gene sequencing. The results of the BLAST analysis of the obtained sequences revealed that *spvC* gene sequences of *Salmonella enterica* isolated from migratory ducks (pintail) showed high identity (97.95%) with those of *Salmonella enterica* isolated from human stool in Nigeria and Japan also showed high identity with those of cattle lymph node and sheep in USA. Moreover, it showed high identity percentages with those isolated from chicken meat (Thailand) and comminuted beef (USA).

Furthermore, the phylogenetic tree of *spvC* gene sequences demonstrated that one of these sequences is found in the same clade with *Salmonella enterica* isolated from a quail in the USA and a human from Nigeria, whereas, the other two sequences were placed in the same clade with *Salmonella enterica* from both chicken meat in Thailand and comminuted beef in the USA. Strikingly, the results of the phylogenetic analysis is strongly augmented by the migration pathway of the birds according to Birdlife International Fact Sheet (2023) which determined the northern pintail distribution countries to include Egypt, USA, Mexico, Japan, China and Thailand. Moreover, Miller *et al*. (2005) recorded that the northern pintail may migrate to North America while Jahangir *et al*. (2009) and Wei *et al*. (2020) pointed out that northern pintail migrates to South Korea, Japan and China. Accordingly, pintail may catch such *Salmonella* strains which circulated in one country and subsequently distribute such exotic strains along the migration journey. A matter which explains the high genetic relatedness between the obtained isolates and those circulated among different hosts in the countries along the migration pathway to draw a conclusion about the role of migratory pintail in the epidemiology of such virulent *Salmonella enterica* strains and underscore the potential zoonotic risk through handling of such birds or consuming their contaminated meat by migratory game bird meat lovers.

Conclusion

The role of migratory birds especially pintail ducks as a transmitter of virulent *Salmonella enterica* strains during their migration journey with a potential zoonotic risk cannot be ruled out.

Ethics statement

The protocol of this study was approved by the institutional animal care and use committee (IACUC), faculty of veterinary medicine, Cairo University. Approval number: Vet Cu 28/04/2021/298

Statement of conflict of interest

The authors declare no conflict of interest.

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