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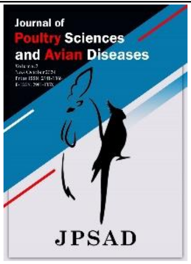

The role of migratory birds in the spread of coronaviruses

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ABSTRACT

Since the discovery of viruses, humans have always faced a great challenge in dealing with their subsequent diseases. Coronaviruses have created huge challenges in the world. Coronaviruses are RNA genome viruses from the *Coronaviridae* family that can cause different diseases in both poultry and humans, but no evidence has been provided for their zoonotic potential, which may be due to the different genera of the viruses that cause diseases in these hosts. There are different ways of transmission for the coronaviruses with airborne being the most common and important route. However, the role of migratory birds in their spread has been recently noticed. Due to the novelty and importance of this issue, we decided to conduct a review study on the role of migratory birds in the spread of coronaviruses. The results show that due to the high possibility of the virus mutations in the host's body, the interspecies barrier can be broken and we can see the direct transmission of the coronaviruses from birds to humans. However, due to the novelty of this topic, more studies are required to acquire a comprehensive knowledge on this matter.

Keywords: *Coronavirus, Migratory Birds, Pathogenesis, Poultry, Virus*

1 Introduction

Coronaviruses cause various diseases and have important effects on human and animal health. Acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome (MERS-CoV) as well as the recent global epidemic of the disease Covid-19 (SARS-Cov-2) have many pathologic effects, including mortality. In the countries where people have been infected by these viruses, it has had significant socio-economic impacts (1-3).

The *Coronaviredae* family is a large group of viruses that have an envelope, a single-stranded positive sense RNA

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genome. A virion consists of structural proteins including Spike (S), Envelope (E), Membrane (M), Nucleocapsid (N). The S protein has a prominent role in pathogenesis and also in immunogenesis (4).

This family consists of 2 sub-families named *Coronavirinae* and *Torovirinae*. There are 4 genera in the subfamily of *Coronavirinae*, which include *alphacoronavirus*, *betacoronavirus*, *deltacoronavirus*, and *gammacoronavirus*. *Alphacoronavirus* and *betacoronavirus* are mammalian viruses. *Deltacoronavirus* includes species of wild bird viruses, and *gammacoronavirus* includes infectious bronchitis virus (IBV), turkey coronavirus (TCoV), and coronaviruses isolated from pheasants, pigeons, ducks, and geese (4-6). Beluga whale coronavirus SW1 is also a gammacoronavirus (7). Some isolated coronaviruses, such as those isolated from leopards and Chinese mink, have not been assigned to any genus (8, 9).

Coronavirus infections in domestic animals, such as porcine epidemic diarrhea (PEDV) and recently porcine deltacoronavirus (PDCoV) as well as infectious bronchitis disease (IBV) and turkey coronavirus in birds, have certain effects on animal health and cause significant economic losses (10-13).

Breakdown of the interspecies barrier and transmission to new hosts occur frequently in coronaviruses, and the recent epidemics of SARS-CoV, MERS-CoV, and SARS-CoV-2 in human societies are among the most remarkable. There are cases of interspecies barrier failure and transmission to humans (14-16). Cattle coronavirus, dog respiratory coronavirus, camel coronavirus, and even human coronavirus OC43; All originated from a common ancestor, which shows the flexibility of the virus in choosing its host (17-19).

SARS-CoV disease originated from bats, while PDCoV disease interestingly seems to have originated from birds (12, 14, 15) and this issue has recently caused special attention to investigate and evaluate animals for focus on the identification of coronaviruses.

Wild birds are everywhere and very mobile. They are potential hosts with the ability to transmit viruses over long distances and beyond geographic and political boundaries. The role of wild birds in the spread of acute H5Nx avian influenza viruses has been proven (20) and migration patterns explain the phylogenetic patterns found in the matrix gene of LP avian influenza viruses (21).

Coronaviruses have been identified in a wide range of wild bird species on all continents, and it seems that they exist in wild birds of all continents. However, some studies failed to identify CoV. The main reason for this problem is probably that no single test method can detect all positive samples in terms of contamination with coronaviruses (22).

Since the diversity of CoVs in wild birds is unknown, different types of PCR are used to maximize the detected cases. These tests include:

- Pancoronavirus polymerase nested PCR
- Cov real-time PCR
- Modified Pancoronavirus polymerase real-time PCR

There are also other types of these tests. After this stage, positive samples for coronavirus are examined for genetic sequencing, and phylogenetic analysis is performed to compare the sequence of identified CoVs (23).

Among wild bird species from which coronaviruses have been successfully isolated, waterfowls such as Anseriformes (goose, duck, and swan) and Charadriiformes (seagulls, coastal migratory birds, and waders) had the largest share and most studies have focused on these two orders of birds (24-28).

Bird migration is one of the wonders of nature. Different birds make this risky journey; Rufous Hummingbird migrates in the north and south of the American continent, while the arctic tern migrates from pole to pole. About one-fifth of all bird species migrate. Of course, today, dangers such as illegal hunting, urban development, power transmission lines, and other human artifacts, along with climate change, destroy bird habitats and endanger their lives (29, 30).

To a geographical area that is occupied during the annual migration period of migratory birds present in that area, and includes specific lands and settlements for reproduction and wintering, as well as migration routes that connect these areas is called a migration flight path (30).

When traveling between breeding grounds and wintering grounds, birds do not randomly choose their flight paths. They pass through routes that have suitable settlements so that they can stop and rest and eat well. Many different species gather together to fly similar routes together. There are a total of 4 main migration routes on the planet (Figure 1) and some of them also sub-divided into more migration flying paths.

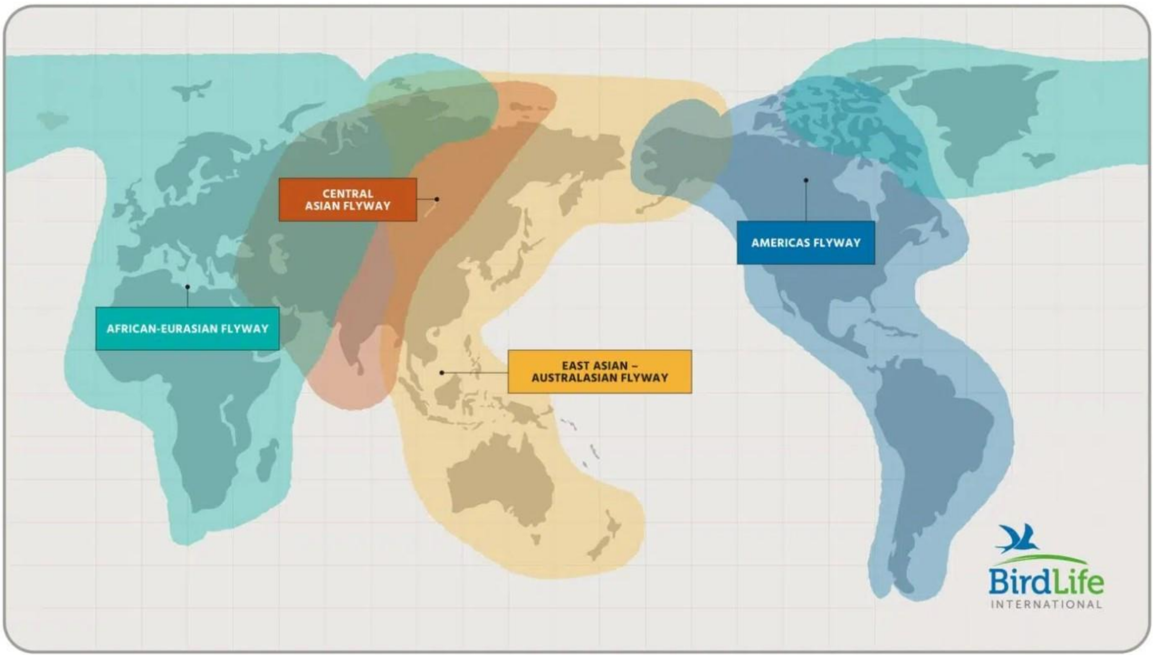

Figure 1. Main flight paths of migratory birds. This figure was taken from the original report by BirdLife International (31)

2 Migratory Paths

There are 3 immigration flight routes in America:

The Pacific Americas Flyway, the Central Americas Flyway, and the Atlantic Americas Flyway. These flyways connect the breeding areas of North America with the wintering areas in the Caribbean, Central and South America (32).

There are also 3 migration flight routes in the areas of Palaearctic–African flyways:

The East Atlantic Flyways, the Mediterranean/Black Sea Flyway, and the East Asia/East Africa Flyway constitute the largest migratory system of birds, and each year more than 2 billion birds of sparrows and other birds from breeding areas in Europe and Western and Central Asia migrate to wintering areas in tropical Africa (33).

Central Asian Flyway: It is considered the shortest migration flight route in the world and is located in the northern hemisphere. This route connects a large part of the northern belt and the lands around the North Pole to the Indian subcontinent. Limited species of migratory birds use this route; because the Tibetan plateau and the Himalayan Mountain range are a big barrier in this migration path (34).

East Asian-Australasian Flyway: It includes the northern and polar regions of Russia and North America to New Zealand and is used by more than 50 million migratory ducks every year (34).

According to the Environmental Department; Most of the migratory birds in the country are a group that spends autumn and winter and migrated to Iran from Western Siberia and Western Europe and are mostly seen in the northern provinces of the country. About 65% of the country's migratory birds are swans, geese, ducks, sandpipers, and gulls.

Shadgan Wetland is also considered one of the most important natural resources of Iran, which is located in the Khuzestan Plain and hosts a large number of unique native and migratory birds every year.

3 Evaluation of the role of Migratory Birds in Different Studies

The occurrence of porcine deltacoronavirus (PDCoV) in countries with the pig breeding industry has caused serious problems, and many countries, including the United States, have conducted extensive research on this problem. Part of this research was the investigation of the epidemiology of deltacoronaviruses in wild birds. In a study conducted by Paim et al. in 2019, the prevalence of delta- and gammacoronaviruses in land and water migratory birds in some American states was evaluated. A total of 1236 cloacal/fecal swabs were collected and tested using the genus-specific RT-PCR method. Sixty-one samples (4.99%) were positive for gammacoronaviruses and only 14 samples (1.13%) were positive for deltacoronaviruses. As a result, unlike the existing reports from Asia in this field; In America, gammacoronaviruses are more common than deltacoronaviruses, and this indicates that deltacoronaviruses probably spread less efficiently among birds. This problem can indicate the situation of deltacoronaviruses and the incomplete adaptation of these viruses to new host species, which limits their spread. The phylogenetic analysis of the N gene determined that the recently identified deltacoronavirus strains are closely related to the HKU20 strain isolated from the Wigeon duck (35).

In the study of Honkavouri et al. in 2014 on fecal samples collected from Semipalmated Sandpiper, sanderling, and ruddy turnstone in Delaware Bay, using the RT-PCR method, it was determined that out of the 8 samples, it was positive for contamination with coronavirus, and after additional tests and phylogenetic analysis, the virus isolated from the ruddy turnstone was classified as deltacoronaviruses. The results of this study, along with the results of other researchers' studies (24, 26), showed that the circulating coronaviruses in the population of migratory birds have a wide variety in addition to the high prevalence rate. Due to the high volume of birds that stop there during the annual migration, Delaware Bay is a suitable place for the emergence and spread of different variants of coronaviruses (36).

In 2019, cloacal and pharyngeal swabs of 746 wild bird samples from different regions of Brazil were examined for the presence of coronaviruses using RT-PCR and Pancoronavirus-Nested PCR methods. Positive samples were sequenced for the presence of coronaviruses and phylogenetic analysis was performed to investigate the relationship between virus epidemiology and bird migration. Among the samples; 3 samples were positive for gammacoronaviruses, and 3 samples were positive for deltacoronaviruses. This study proved the presence of gamma- and deltacoronaviruses associated with birds in urban areas and also in the areas of industrial poultry farms in Brazil. It also showed that wild birds are potential carriers for CoVs and can pose a risk to public health as well as poultry farms (37).

In the study conducted by Chu et al. in 2011 in China, the prevalence of coronaviruses in wild aquatic birds was measured at 12.5%. The phylogenetic analysis of these viruses revealed that a wide range of delta and gammacoronaviruses are circulating in the bird population. Gammacoronaviruses were mostly found in goslings, while deltacoronaviruses can be found in Ciconiiformes, Pelecaniformes, and Anseriformes. In this study, it was observed that interspecies transmission of gammacoronaviruses occurs commonly among duck species. In return; Deltacoronaviruses probably have more host specificity. The investigations carried out in this study, including the analysis of the host's mitochondrial DNA sequence as well as the viral DNA sequence, determined that in some cases, coronaviruses coevolve with birds of the same order (24).

Previously in another study in China by Qian et al. in 2006, a new coronavirus was isolated from cases of urban pigeons with clinical respiratory disease and inflammation of the pancreas. The S protein sequence was very similar (about 78.5 to 96.6%) to the IB virus and to a lesser extent (37.6 to 37.8%) to turkey TCoV strains (38). Another strain of coronavirus that was isolated from peacocks by Sun et al. in 2007 was from the genus Gammacoronaviruses and had the most similarity with the Massachusetts strain of infectious bronchitis virus. In experimental infection, this virus had the ability to cause disease in chickens. These findings showed that peacocks and pigeons can also be natural reservoirs for coronaviruses (39).

A study in Australia in 2018, investigated the presence of coronaviruses in 918 samples taken from wild birds from different regions of Australia. Coronaviruses were detected in 141 samples (15.3%) of ducks, shorebirds, and herons. Examining the sequence of positive cases in terms of the presence of coronaviruses, showed that most of the detected cases are of gammacoronaviruses. Moreover, cases of deltacoronaviruses were also detected. Since no single test is able to identify all positive samples for coronaviruses, several different PCR methods were used to improve the detection rate of coronaviruses. Analysis of the genome sequence of the detected viruses showed that Australian duck gammacoronaviruses are similar to duck gammacoronaviruses in other parts of the world. Some of the gammacoronaviruses of shorebirds belonged to the lineage of Charadriiformes gammacoronaviruses, but other cases were more closely related to duck gammacoronaviruses (23).

Deltacoronaviruses of Australian ducks and herons belonged to the lineage of deltacoronaviruses of other ducks and herons, but in terms of nucleotide sequences, they differed by about 20% from the sequences of other deltacoronaviruses. The sequence of deltacoronaviruses isolated from shorebirds, together with the deltacoronavirus isolated from a ruddy turnstone in America, form a new lineage. In this study, it was found that Australian duck gammacoronaviruses are very similar to duck gammacoronaviruses that have been detected in other areas, and since Australian ducks are rarely in contact with migratory species in northern areas, it is assumed that Migratory shorebirds may be important carriers for the transmission of wild bird coronaviruses to/from Australia (23).

In the study of Hughes et al. in 2009, 441 samples of feces and pharyngeal swabs from 42 bird species were collected from northern England, including migratory and resident birds. In the investigations, it was found that ducks and waders are infected with coronaviruses. Phylogenetic analysis revealed that the sequences of the identified coronaviruses are genetically different. Most of the cases isolated from wild birds were very similar to gammacoronavirus causing infectious bronchitis, and these birds can be considered as a reservoir for this virus and also a potential risk for farmed poultry (27).

In a study conducted by Hepojoki et al. in 2017 in Finland, 939 samples from 61 different bird species were tested by RT-PCR to identify the RNA of coronaviruses. Of these samples, 51 samples (5.4%) of 8 bird species were positive for contamination with coronaviruses. Then the positive samples were subjected to phylogenetic evaluation. The viruses identified from wild ducks were 99% similar to other viruses of the gammacoronavirus genus. In terms of phylogenetic relationship, these viruses were most related to duck virus isolates from America, Russia, China, Hong Kong, and South Korea.

Gammacoronaviruses identified from seagulls in this study were 99% sequence similar to virus isolates from seagulls in Siberia and Alaska. Viruses isolated from pigeons and an isolate from long-tailed duck were about 89% similar to duck gammacoronaviruses. Two isolates of lesser black-backed and black-headed gulls in terms of nucleotide sequence were 83 and 85% similar to deltacoronaviruses of birds and mammals, respectively. These viruses are classified in the clade of deltacoronaviruses and were most closely related to the White eye coronavirus isolated in Hong Kong. None of the coronaviruses isolated from wild birds in this study were closely related to the infectious bronchitis virus strains circulating in the Finnish poultry population (40).

In the study of Rohiam in Egypt in 2019, cloacal/feces samples from 557 wild birds from four different species were tested for the presence of coronaviruses by RT-PCR method. A total of 14 samples (2.5%) were considered positive and subjected to phylogenetic analysis. Also, the sequence of the protein S gene was determined in these samples. Phylogenetic analysis confirmed that all the viruses identified in this study belong to gammacoronaviruses (41). The results of these studies are summarized in Table 1.

Table 1. The summery of studies to investigate the presence of coronaviruses in wild birds worldwide

Investigated species	Location	Results	Reference
Land and water migratory birds	USA	4.99% Gammacoronaviruses 1.13% Deltacoronaviruses	(35)
-Semi-palmated sandpiper -Sanderling -Rudy turnstone	Delaware bay (USA)	12.5% Deltacoronaviruses	(36)
Wild birds	Brazil	0.4% Deltacoronaviruses 0.4% Gammacoronaviruses	(37)
Wild aquatic birds	China	12.5% (Both gamma and deltacoronaviruses)	(24)
Urban pigeon	China	A novel coronavirus 78.5 to 96.6% similar to IBV and 37.6 to 37.8% similar to TcoVs	(38)
Peacock	China	A novel gammacoronavirus with the most similarity to Mass strains of IBV	(39)
Wild birds	Australia	15.3% (Mostly gammacoronaviruses)	(27)
42 bird species	Northern England	Estimated prevalence of 1.6% (mostly similar to H120 strain of IBV)	(27)
61 bird species	Finland	5.4% (Mostly gammacoronaviruses)	(40)
4 species of wild birds	Egypt	2.5% (Gammacoronaviruses)	(41)

4 Conclusion

According to the results obtained from studies in this field from all over the world, migratory birds can be infected with a wide range of coronaviruses and they are potentially a source of risk for the spread and transmission of coronaviruses to other animals, especially domestic animals and poultry farms. On the other hand, the pattern of infections in migratory birds - despite the high diversity, which was observed in the identified viruses – aligns with the main migration pathways due to the nature of coronaviruses, in which mutations and breaking the interspecies barrier usually occur. To achieve a better understanding on this issue, it seems necessary to conduct more research in this field. It is worth mentioning, in order to facilitate the comparison of results from epidemiological investigations; it would be better to use similar laboratory methods all over the world. Additionally, it is very important to conduct more extensive studies in the field of pathogenicity and identification of isolated viruses.

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Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

All the authors contributed to all parts of this research.

Data Availability Statement

Data are available from the first author upon reasonable request.

Ethical Considerations

There is no ethical consideration.

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