Dispensable role of wild rodents in avian influenza A virus transmission in Gyeonggi province, Korea

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Abstract

Avian influenza A viruses (IAVs) present significant threats to both animal and human health through their potential for cross-species transmission and global spread. Clade 2.3.4.4 H5Nx highly pathogenic avian IAVs initially emerged in East Asia between 2013 and 2014. Since then, they have spread to Europe, Africa, and America via migratory bird flyways. However, beyond viral transmission primarily facilitated by migratory birds, the potential involvement of other intermediate factors for virus transmission remains poorly investigated. This study aimed to investigate the role of wild rodents as intermediary hosts in the ecology of avian IAVs in Gyeonggi province, South Korea. By capturing and analyzing 189 wild rodents near poultry farms and migratory bird habitats in 2013 and 2014 and employing serological assays and virus isolation techniques, we found no evidence of IAV infection among these populations. Our results suggest that wild rodents may not significantly contribute to the transmission dynamics of IAVs within these regions.

Keywords: avian influenza; rodents; transmission; surveillance

Introduction

Wild waterfowl serve as the natural reservoir for avian influenza A viruses (IAVs) and harbor a diverse subtype of avian IAVs. Avian IAV infections in wild waterfowl typically manifest as mild or asymptomatic. However, these birds can carry the virus over long distances, contributing to its global spread. This dissemination of avian influenza viruses by wild waterfowl heightens the risk of cross-species transmission to other animals, including domestic poultry, pigs, humans, and various mammalian species [1,2].

The transmission of avian IAVs to domestic poultry poses significant challenges for the poultry industry, leading to reduced meat and egg production and substantial economic losses. Furthermore, the transmission of avian IAVs to farmed animals increases the likelihood of spillover events to humans, raising public health concerns. Various IAV subtypes, including H1, H3, H5, H6, H7, H9, and H10 have been reported to affect humans, and poultry-to-human transmission is close-
ly associated with these events [1,3]. Highly pathogenic avian influenza (HPAI) viruses such as H5N1, H5N6, H7N7, and H7N9 have also been occasionally transmitted to humans in close contact with infected poultry, resulting in fatal outcomes [3,4]. Therefore, vigilant monitoring of IAV transmission from wild waterfowl to poultry is essential not only for the poultry industry but also for preventing zoonotic spread to humans.

Direct or indirect contact is recognized as the main source of avian IAV transmission to domestic poultry [5]. In open-door areas on a free-range poultry farm, wild waterfowl can access feed on the ground, facilitating direct contact with domestic poultry. Risk factors for indirect contact include wind-borne spread, food and water contamination, movement of vehicles and people, and virus-contaminated fomites [5–7]. In addition, other intermediate species, including rodents, may play a role in virus spread to domestic poultry [8]. Rodents are known to be susceptible to several IAV subtypes and are used as disease models [9]. Moreover, they are abundant around poultry farms and share their habitat with wild waterfowl, suggesting the potential role of rodents as intermediate species for IAV spread.

During the initial outbreak of H5N1 HPAI virus in Hong Kong in 1997, dogs, cats, rats, and mice residing near poultry markets underwent infection screening [10]. Although the virus was not directly isolated from these animals, some rat sera showed evidence of hemagglutination (HA) inhibiting activity. A similar study was conducted on poultry farms several weeks after the H5N8 HPAI virus outbreaks in Canada, but researchers found no evidence of infection in blood samples and respiratory tract tissue from trapped mice [11].

Materials and Methods

Capture of wild rodents

In this study, we captured wild rodents, including striped field mice, house mice, Eurasian harvest mice, and lesser shrews, twice in the spring of 2013 and 2014 in Gyeonggi province using Sherman traps. The traps were installed within a 100-meter radius of poultry farms (Hwaseong, Anseong, Paju, Yeoncheon, and Pyeongtaek) and migratory bird habitats (Sihwa Lake). The captured wild rodents were anesthetized using diethyl ether, followed by the collection of blood samples and euthanasia via cervical dislocation. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals [19]. The periods of wild rodent captures were selected based on the duration of antibody responses to avian IAV infection and the increased survival rate of trapped wild rodents.

ELISA to detect influenza-specific antibodies

For serological examinations to detect IAV antibodies, serum samples from the wild rodents were mixed with receptor-destruction enzyme (Denka Seiken, Japan) at a 1-to-3 volume ratio and then incubated at 37°C for 16 hours. After incubation, the serum samples were inactivated at 56°C for 30 minutes and then serially diluted 10-fold with phosphate-buffered saline (PBS). The presence of antibodies against the nucleocapsid protein (NP) of IAVs was measured using a competitive ELISA kit (BioNote Inc., Korea). Controls and serum samples were added to an NP-coated 96-well plate, followed by the addition of NP antibody-horseradish peroxidase. After adding substrates and stopping solutions, the optical density (OD) at 450 nm was measured with a reference wavelength at 620 nm. To assess the presence of IAV NP antibody, PI values were calculated as follows: \[1 - (OD_{sample}/mean\ OD_{negative\ control})\times 100.\] A PI value above 50 was considered positive. The serum samples were stored at −80°C, and serological examinations were conducted twice, once in 2013 and again in 2014, after all samples collected in each respective year.

Virus isolation

The lungs of the wild mice were excised and lysed with TissueLyzer 2 (Qiagen, USA), centrifuged at 3,000 rpm for 10 min, and then diluted 10-fold with PBS-containing antibiotics. Each 200-µl portion of the lung sample supernatant was inoculated into 10-day-old specific pathogen-free embryonated chicken eggs (ECEs; Charles River Laboratories, USA) via the allantoic cavity. After 3 days of incubation, the allantoic fluid was harvested and checked for the presence of the virus using the HA
test, following the World Health Organization Manual on Animal Influenza Diagnosis and Surveillance [20]. The supernatants of the lung samples were stored at −80°C, and egg inoculation were conducted twice, once in 2013 and again in 2014, after all samples collected in each respective year.

**Results**

The wild rodents were captured twice in the spring of 2013 and 2014 in Gyeonggi province near poultry farms and wild bird habitats (Fig. 1). According to a public data provided by the Ministry of Agriculture, Food and Rural Affairs, Gyeonggi province is the region with the second largest number of poultry farms in Korea. Moreover, Anseong, Hwaseong, and Pyeongtaek rank 2nd, 3rd, and 4th, respectively, as regions with the largest number of poultry farms after Gyeonggi province. During the H5N8 outbreaks in 2014, the total number of reported cases from January to July was 212, and 23 cases were reported in Gyeonggi province [14]. Therefore, the capturing region and period are likely to be appropriate to assess the potential role of wild rodents in avian IAV dissemination.

In proximity to poultry farms and the Sihwa Lake region, striped field mice (*Apodemus agrarius*) were the most dominant rodent species among those captured. These mice constituted 91% of the total rodent abundance in the areas within Gyeonggi province (Table 1). In addition, other rodent species including house mice (*Mus musculus*, 2.6%), Eurasian harvest mice (*Micromys minutus*, 2.1%), lesser shrews (*Crocidura suaveolens*, 3.7%), and Norway rats (*Rattus norvegicus*, 0.5%) were identified among the captured rodents.

After conducting species analyses, we collected serum samples from the wild rodents and performed ELISA to detect influenza NP antibodies. None of the 137 collected serum samples showed the presence of influenza NP antibodies (Table 1).

To further investigate the potential presence of IAV in these wild rodents, lung lysates were inoculated into 10-day-old ECEs, and virus propagation was confirmed by the HA test after 3 days post-inoculation. Similarly, no virus was isolated from the lung lysate samples from the wild rodents (Table 1). These results collectively suggest that wild rodents are unlikely to play a significant role in the ecology or potential transmission pathways of avian IAVs within these regions.

![Fig. 1. Geographical distribution of the captured wild rodents in 2013 and 2014. Blue circles denote the locations where wild rodents were captured in 2013, and red circles indicate the capture sites in 2014.](image)

Table 1. Serological testing and virus isolation for influenza A viruses in wild rodents captured for the study

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Sampling region a</th>
<th>Striped field mouse (<em>Apodemus agrarius</em>)</th>
<th>House mouse (<em>Mus musculus</em>)</th>
<th>Eurasian harvest mouse (<em>Micromys minutus</em>)</th>
<th>Lesser shrew (<em>Crocidura suaveolens</em>)</th>
<th>Norway rat (<em>Rattus norvegicus</em>)</th>
<th>Total no. of samples</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013 March–2013 April</td>
<td>Hwaseong</td>
<td>18 (94.7)</td>
<td>1 (5.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>19 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Anseong</td>
<td>18 (94.7)</td>
<td>0 (0)</td>
<td>1 (5.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>19 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Sihwa Lake</td>
<td>16 (94.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5.9)</td>
<td>0 (0)</td>
<td>17 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Paju</td>
<td>42 (87.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (12.5)</td>
<td>0 (0)</td>
<td>48 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Yeoncheon</td>
<td>18 (85.7)</td>
<td>0 (0)</td>
<td>3 (14.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>21 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2014 March–2014 April</td>
<td>Hwaseong</td>
<td>11 (68.8)</td>
<td>4 (25.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (6.3)</td>
<td>16 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Pyeongtaek</td>
<td>24 (100.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>24 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Anseong</td>
<td>25 (100.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>25 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>172 (91.0)</td>
<td>5 (2.6)</td>
<td>4 (2.1)</td>
<td>7 (3.7)</td>
<td>1 (0.5)</td>
<td>189 (100.0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Values are presented as number (%).

a The sampling regions were near poultry farms except for Sihwa Lake, a wild bird habitat.
Discussion

Frequent occurrences of avian IAV spread to domestic poultry are commonly attributed to the genetic similarity between wild waterfowl and domestic poultry. Some migratory waterfowl are highly susceptible to avian IAVs with high virus shedding and low pathogenicity, making them as distance carriers of avian IAVs [21]. However, the possibility of virus introduction through bridge hosts should not be ruled out. Wild rodents, which are known carriers of human pathogens such as hantavirus, Lassa virus, Leptospira, and Salmonella, could potentially play a role in transmission of infectious pathogens to humans. In this study, the wild rodents were captured in Gyeonggi province, and serological testing and virus isolation were conducted for active surveillance of IAV infections in these wild rodents. To the best of our knowledge, this study represents the first investigation into the potential role of wild rodents in the transmission of IAVs specifically in South Korea.

Our study failed to find evidence of IAV infection in the wild rodents during avian IAV season in Gyeonggi province, Korea. Several researchers undertook the task of capturing wild rodents around poultry farms to evaluate a potential involvement of these rodents as vectors for avian IAV. Similar to our findings, most previous studies were unable to detect any signs of IAV infection in the captured wild rodents—except for one study conducted by Shriner et al. [8,11,22,23]. Shriner et al. [23] presented evidence indicating potential IAV infection in wild rodents. In addition, a cross-sectional study in backyard poultry flocks suggested that effective pest control could potentially reduce the seroprevalence of IAV [8].

One plausible explanation for this discrepancy could be the variation in the species composition of wild rodents captured. In this study, the striped field mouse (A. agrarius) constituted the majority of the captured rodent species, in accordance with its dominance in the country’s small mammal population [24]. In contrast, Shriner et al. [23] predominantly captured house mice. Different species possess different virus-host interaction mechanisms that result in different host susceptibility and permissiveness to pathogens. Notably, wild mice possessing the Mx1 gene generally exhibit resistance to IAV infection, while laboratory mice lacking a functional Mx1 protein are more susceptible [9,25–27]. Interestingly, experimentally infected house mice with IAVs demonstrate moderate viral replication in the lungs [23]. Although data on IAV susceptibility in striped field mice are lacking, it is plausible that different outcomes may be associated with the different rodent species captured.

To evaluate the potential for virus transmission through bridge hosts, the experimental design should consider several compound factors such as geographical factors or the seasonality of the virus. The capture regions and periods in this study were strategically determined based on their proximity to areas of avian IAV prevalence in Korea and the timing of the avian IAV season. However, it is important to note that the study did not encompass a nationwide scope. In addition, the number of rodents captured in this study does not sufficiently elucidate the potential role of wild rodents as bridge hosts in the transmission of avian IAV. Furthermore, this study was unable to assess the possibility of mechanical transfer of IAVs by wild rodents. Given these limitations, the potential for avian IAV transmission via wild rodents cannot be entirely dismissed. Therefore, ongoing and comprehensive nationwide surveillance of wild rodents is necessary to thoroughly evaluate their role as bridge hosts in the spread of avian IAVs.

Understanding the routes of IAV transmission to domestic poultry is vital because these viruses impact not only the poultry industry but also pose zoonotic risks. Furthermore, the potential adaptation of IAVs within rodent populations significantly increases the risk of zoonotic spillover. To the best of our knowledge, this study represents the first surveillance effort focusing on IAV infections among wild rodents in Korea. Although no IAV infections were detected in the wild rodents captured between 2013 and 2014, the necessity for continuous, proactive surveillance to monitor IAV spillover to wild rodents is essential for controlling and preparing potential cross-species transmission of IAVs to humans.

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Author’s Contributions

Conceptualization: all authors; Data curation: Lee CY; Formal analysis: Lee CY; Funding acquisition: Lee CY; Investigation: Lee CY, Kim I; Methodology: Lee CY, Kim I; Project administration: Kwon HJ; Resources: Lee CY, Kim I; Software: Lee CY; Supervision: Lee CY; Validation: Lee CY, Kwon HJ; Visualization: Lee CY; Writing—original draft: Lee CY; Writing—review & editing: all authors.
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