KJVR
Korean Journal of Veterinary Research

Original Article

Virology

pISSN 2466-1384 · eISSN 2466-1392
Korean J Vet Res 2024;64(2):e13
https://doi.org/10.14405/kjvr.20240016

*Corresponding author:
Chung-Young Lee
Department of Microbiology, Kyungpook National University School of Medicine, 680 Gukchaebosang-ro, Jung-gu, Daegu 41944, Korea
Tel: +82-53-420-4840
E-mail: cylee87@knu.ac.kr
https://orcid.org/0000-0003-3037-7581

Conflict of interest:
The authors declare no conflict of interest.

Received: May 31, 2024
Revised: Jun 7, 2024
Accepted: Jun 9, 2024

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

Chung-Young Lee[1,2,*], Ilhwan Kim[3], Hyuk-Joon Kwon[4,5]

[1]Department of Microbiology, Kyungpook National University School of Medicine, Daegu 41944, Korea
[2]Untreatable Infectious Disease Institute, Kyungpook National University, Daegu 41944, Korea
[3]Division of Emerging Infectious Diseases, Bureau of Infectious Disease Diagnosis Control, Korea Disease Control and Prevention Agency, Cheongju 28159, Korea
[4]Laboratory of Poultry Medicine, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea
[5]Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea

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].

During the period of 2013 to 2014, clade 2.3.4.4 HPAIs first emerged in East Asia [12–14]. Subsequently, these viruses sequentially spread to Europe, Africa, and America, occasionally causing spillover events into mammalian hosts including humans [15–18]. While migratory birds are widely considered to be the primary vectors for the global spread of these viruses, the potential contribution of intermediate species in viral spread within individual countries remains poorly investigated. In this study, wild rodents were captured twice in the spring of 2013 and 2014 in Gyeonggi province near poultry farms and migratory bird habitats. Antibody detection and IAV isolation experiments were performed on the wild rodent specimens to monitor for potential IAV infection. Among 189 samples, we found no evidence of IAV infection and could not isolate IAVs from lung specimens from wild rodents.

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 procedure 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-destroying 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: $[1-(OD_{sample}/\text{mean } OD_{negative\ control})] \times 100$. 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

https://doi.org/10.14405/kjvr.20240016

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.

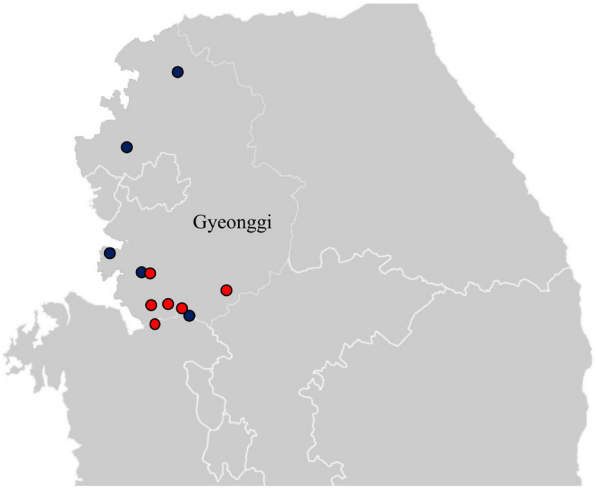

Fig. 1. Geographical distribution of the captured wild rodents in 2013 and 2014. Blue circles denote the locations where wild rodents were captured in 2013, and red circles indicate the capture sites in 2014.

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.

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

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

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 rodent 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.

ORCID

Chung-Young Lee, https://orcid.org/0000-0003-3037-7581
Ilhwan Kim, https://orcid.org/0000-0003-3566-5523
Hyuk-Joon Kwon, https://orcid.org/0000-0001-9107-7860

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.

https://doi.org/10.14405/kjvr.20240016

Funding

This research was supported by Kyungpook National University Research Fund, 2022

Acknowledgments

We extend our thanks to Baek-Jun Kim and Jong-Hun Kim at the National Institute of Ecology for their exceptional work in capturing wild rodents.

References

1. Abdelwhab EM, Mettenleiter TC. Zoonotic animal influenza virus and potential mixing vessel hosts. Viruses 2023;15:980.
2. AbuBakar U, Amrani L, Kamarulzaman FA, Karsani SA, Hassandarvish P, Khairat JE. Avian influenza virus tropism in humans. Viruses 2023;15:833.
3. Philippon DA, Wu P, Cowling BJ, Lau EH. Avian influenza human infections at the human-animal interface. J Infect Dis 2020;222:528–537.
4. Bos ME, Te Beest DE, van Boven M, van Beest Holle MR, Meijer A, Bosman A, Mulder YM, Koopmans MP, Stegeman A. High probability of avian influenza virus (H7N7) transmission from poultry to humans active in disease control on infected farms. J Infect Dis 2010;201:1390–1396.
5. Alexander DJ. An overview of the epidemiology of avian influenza. Vaccine 2007;25:5637–5644.
6. Ssematimba A, Hagenaars TJ, de Jong MC. Modelling the wind-borne spread of highly pathogenic avian influenza virus between farms. PLoS One 2012;7:e31114.
7. Yoo DS, Chun BC, Kim Y, Lee KN, Moon OK. Dynamics of inter-farm transmission of highly pathogenic avian influenza H5N6 integrating vehicle movements and phylogenetic information. Sci Rep 2021;11:24163.
8. Velkers FC, Blokhuis SJ, Veldhuis Kroeze EJB, Burt SA. The role of rodents in avian influenza outbreaks in poultry farms: a review. Vet Q 2017;37:182–194.
9. Bouvier NM, Lowen AC. Animal models for influenza virus pathogenesis and transmission. Viruses 2010;2:1530–1563.
10. Shortridge KF, Gao P, Guan Y, Ito T, Kawaoka Y, Markwell D, Takada A, Webster RG. Interspecies transmission of influenza viruses: H5N1 virus and a Hong Kong SAR perspective. Vet Microbiol 2000;74:141–147.
11. Shriner SA, Root JJ, Lutman MW, Kloft JM, VanDalen KK, Sullivan HJ, White TS, Milleson MP, Hairston JL, Chandler SC, Wolf PC, Turnage CT, McCluskey BJ, Vincent AL, Torchetti MK, Gidlewski T, DeLiberto TJ. Surveillance for highly pathogenic H5 avian influenza virus in synanthropic wildlife associated with poultry farms during an acute outbreak. Sci Rep 2016;6:36237.
12. Hu T, Song J, Zhang W, Zhao H, Duan B, Liu Q, Zeng W, Qiu W, Chen G, Zhang Y, Fan Q, Zhang F. Emergence of novel clade 2.3.4 influenza A (H5N1) virus subgroups in Yunnan Province, China. Infect Genet Evol 2015;33:95–100.
13. Lee DH, Bertran K, Kwon JH, Swayne DE. Evolution, global spread, and pathogenicity of highly pathogenic avian influenza H5Nx clade 2. 3.4.4. J Vet Sci 2017;18(S1):269–280.
14. Yoon H, Moon OK, Jeong W, Choi J, Kang YM, Ahn HY, Kim JH, Yoo DS, Kwon YJ, Chang WS, Kim MS, Kim DS, Kim YS, Joo YS. H5N8 highly pathogenic avian influenza in the Republic of Korea: epidemiology during the first wave, from January through July 2014. Osong Public Health Res Perspect 2015;6:106–111.
15. Global Consortium for H5N8 and Related Influenza Viruses. Role for migratory wild birds in the global spread of avian influenza H5N8. Science 2016;354:213–217.
16. Caliendo V, Lewis NS, Pohlmann A, Baillie SR, Banyard AC, Beer M, Brown IH, Fouchier RA, Hansen RD, Lameris TK, Lang AS, Laurendeau S, Lung O, Robertson G, van der Jeugd H, Alkie TN, Thorup K, van Toor ML, Waldenström J, Yason C, Kuiken T, Berhane Y. Transatlantic spread of highly pathogenic avian influenza H5N1 by wild birds from Europe to North America in 2021. Sci Rep 2022;12:11729.
17. Rimondi A, Vanstreels RET, Olivera V, Donini A, Lauriente MM, Uhart MM. Highly pathogenic avian influenza A(H5N1) viruses from multispecies outbreak, Argentina, August 2023. Emerg Infect Dis 2024;30:812–814.
18. Quan C, Wang Q, Zhang J, Zhao M, Dai Q, Huang T, Zhang Z, Mao S, Nie Y, Liu J, Xie Y, Zhang B, Bi Y, Shi W, Liu P, Wang D, Feng L, Yu H, Liu WJ, Gao GF. Avian influenza A viruses among occupationally exposed populations, China, 2014-2016. Emerg Infect Dis 2019;25:2215–2225.
19. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th ed. National Academies Press (US), Washington, DC, 2011.
20. World Health Organization (WHO). WHO Manual on Animal Influenza Diagnosis and Surveillance. WHO, Geneva, 2002.
21. Tian H, Zhou S, Dong L, Van Boeckel TP, Cui Y, Newman SH, Takekawa JY, Prosser DJ, Xiao X, Wu Y, Cazelles B, Huang S, Yang R, Grenfell BT, Xu B. Avian influenza H5N1

viral and bird migration networks in Asia. Proc Natl Acad Sci U S A 2015;112:172–177.
22. Nettles VF, Wood JM, Webster RG. Wildlife surveillance associated with an outbreak of lethal H5N2 avian influenza in domestic poultry. Avian Dis 1985;29:733–741.
23. Shriner SA, VanDalen KK, Mooers NL, Ellis JW, Sullivan HJ, Root JJ, Pelzel AM, Franklin AB. Low-pathogenic avian influenza viruses in wild house mice. PLoS One 2012;7:e39206.
24. Kim YR, Kim HR, Kim JY, Myeong HH, Kang JH, Kim BJ, Lee HJ. Spatio-temporal genetic structure of the striped field mouse (Apodemus agrarius) populations inhabiting national parks in South Korea: implications for conservation and management of protected areas. Front Ecol Evol 2023;11:1038058.
25. Staeheli P, Haller O, Boll W, Lindenmann J, Weissmann C. Mx protein: constitutive expression in 3T3 cells transformed with cloned Mx cDNA confers selective resistance to influenza virus. Cell 1986;44:147–158.
26. Grimm D, Staeheli P, Hufbauer M, Koerner I, Martínez-Sobrido L, Solórzano A, García-Sastre A, Haller O, Kochs G. Replication fitness determines high virulence of influenza A virus in mice carrying functional Mx1 resistance gene. Proc Natl Acad Sci U S A 2007;104:6806–6811.
27. Tumpey TM, Szretter KJ, Van Hoeven N, Katz JM, Kochs G, Haller O, García-Sastre A, Staeheli P. The Mx1 gene protects mice against the pandemic 1918 and highly lethal human H5N1 influenza viruses. J Virol 2007;81:10818–10821.

https://doi.org/10.14405/kjvr.20240016