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## Transmission dynamics of highly pathogenic avian influenza among multiple waterfowl species and backyard poultry: the impact of the stopover period

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Spillover of Highly Pathogenic Avian Influenza (HPAI) to backyard poultry via migratory birds threatens the poultry industry and public health. To improve the understanding of spillover events, we developed a stochastic compartmental mathematical model of HPAI transmission dynamics at the waterfowl-backyard poultry interface in a high-risk area for HPAI introduction into poultry. The model described the infection spread among resident and migratory waterfowl and backyard poultry farms and was validated with historical outbreak data in backyard poultry farms and swan mortalities. We used the model to assess the impact of the timing and duration of migratory birds' stopover period on the probability of HPAI infection in backyard poultry farms. Additionally, we predicted mortality in a sentinel bird species and assessed the impact of HPAI virulence and immunity in a resident reservoir species on the HPAI transmission dynamics. The stopover duration of the reservoir species predicts the HPAI infection probability in backyard poultry farms from waterfowl communities, but the stopover timing has no effect. HPAI virus virulence and immunity against the virus impact the transmission risk to backyard poultry. Understanding factors influencing reservoir species' migration stopover duration in a location will aid HPAI outbreak forecasting and control in backyard poultry farms.

Keywords Emerging disease, Mathematical modeling, Outbreak prediction, Monte Carlo, Zoonosis

The widespread dissemination of Highly Pathogenic Avian Influenza (HPAI) H5N1 clade 2.3.4.4b and the establishment of infections in novel geographical areas severely threaten wild animal populations, poultry production, and human health<sup>1,2</sup>. The frequent occurrence of HPAI outbreaks in poultry has made culling infected flocks a growingly unsustainable approach to control viral spread among farms and reduce the risk of human infection<sup>2</sup>. As the occurrence of HPAI outbreaks accelerates<sup>3</sup>, identifying ecological factors contributing to its dissemination between wild birds and poultry becomes pivotal to predicting HPAI outbreaks better and devising effective strategies for surveillance and control. Backyard poultry farms are particularly vulnerable to HPAI introduction due to their often lacking biosecurity measures and thereby represent an opportunity for HPAI spillover to poultry and humans<sup>4,5</sup>.

Previous mathematical modeling efforts have provided valuable insights into the ecology and epidemiology of HPAI at the wild bird-poultry interface. These efforts have explored how HPAI transmission is influenced by seasonality<sup>6</sup>, cross-immunity between Low Pathogenic Avian Influenza (LPAI) and HPAI strains<sup>7</sup>, environmental transmission<sup>8</sup>, interactions between wild birds and poultry through habitat overlap<sup>9</sup>, infection control and prevention measures for wild birds and poultry<sup>8,10,11</sup>, and mutation of LPAI into HPAI strains<sup>12,13</sup>. To a more limited extent, mathematical models have explored the role of avian migration in HPAI introduction to domestic birds. Liu et al.<sup>8</sup> developed a deterministic model of HPAI transmission between poultry, wild birds, and the environment and determined that migratory wild birds with low susceptibility to the virus could play a key role in HPAI spatial dissemination. Although valuable, these insights did not consider the influence of migratory birds' phenology on HPAI dissemination or species-specific responses to HPAI infection in wild birds. Rao et

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al.<sup>14</sup> developed a spatially explicit model to predict the population-level global spread of H5N1 among waterfowl, poultry, and humans via migratory birds. Their model linked outbreaks in poultry farms to the annual migratory patterns of waterfowl, providing valuable information about the global dynamics of H5N1 spread. However, the model simplifies the dynamics of HPAI transmission at the local level by disregarding the varying susceptibility of different waterfowl species to the virus and the role of resident waterfowl populations in the local spread of H5N1. Additionally, it assumes that migratory flocks cannot recover from infection, which might overestimate the dissemination of HPAI to poultry farms once the virus is introduced to resident waterfowl communities. In their comparison of compartmental models, Tuncer and Martcheva<sup>6</sup> determined that wild bird migration has an important impact on the seasonality of H5N1 cases in poultry and humans. One of their proposed models used a single, time-varying parameter for H5N1 transmission from migratory birds and poultry, without representing migratory birds as a model compartment. Bourouiba et al.<sup>15</sup> developed a model detailing HPAI dynamics during wild bird migration from breeding to wintering grounds, as well as the transmission of H5N1 between wild birds and poultry farms in Poyang Lake, China. Assuming the virus is endemic in poultry farms, their model predicted that the presence of H5N1 in these farms is responsible for sustaining infection among migratory wild bird populations in this region. Although these modeling efforts contribute to a better understanding of the importance of avian migration in the HPAI occurrence in poultry, the influence of the timing and stopover duration of migratory reservoir species have not been assessed as possible determinants of HPAI infection in domestic birds. In particular, factors influencing HPAI spread to backyard poultry farms, which have a higher risk of involvement in spillover events, have been scarcely explored, which is concerning.

Here, we developed a mathematical model representing the spread of HPAI at the waterfowl-backyard poultry interface after the initial introduction of the virus into a waterfowl community by migratory birds. The objective of our study was to determine the impact of the avian migration timing and the duration of the fall migration stopover period of an HPAI reservoir species on the probability of infection in backyard poultry farms.

#### Methods

#### Model structure and parameterization

To model the transmission of HPAI among wild birds and backyard poultry, we developed a Susceptible-Infected-Recovered (SIR) compartmental model structure based on ordinary differential equations (ODEs) in R v4.2 using the deSolve package<sup>16</sup>. To parameterize the model, we searched articles in PubMed and Google Scholar from August 8, 2022, to December 25, 2023, using the terms "HPAI" AND "mallards" OR "mute swans" OR "environmental persistence" as keywords. We also reviewed articles found under the terms "HPAI" OR "climate change" OR "avian migration" OR "phenology" to inform our research goal. We chose Crna Mlaka, a high-risk area for HPAI transmission (i.e., high density of species more likely to carry HPAI and visit poultry farms) from waterfowl to poultry in Croatia<sup>17</sup>, as a model system. Among components, the model included (i) migratory mute swans (Cygnus olor), (ii) migratory mallards (Anas platyrhynchos), (iii) resident mallards, and (iv) backyard poultry farms. In the model, migratory mute swans arrive between September and November to winter (parameter  $In_w$  in the model) and return to their breeding grounds between February and April ( $Out_w$ ), while migratory mallards arrive in Croatia from October to November  $(In_m)$  and stay for 7 to 28 days  $(T_m; Fig. 1)$ . At the end of their stay, all migratory birds leave Croatia (represented as parameters  $e_w$  and  $e_m$  for mallards and mute swans, respectively). Resident mallards breed from May to August, with births occurring at a rate  $\theta$  (per day) during that period. Parameters related to waterfowl's phenology were based on available information in the literature from multiple locations in Eurasia. Piecewise functions were implemented in ODEs to establish the arrival and departure of migratory mallards and mute swans. Mortality due to causes unrelated to HPAI was represented by parameters  $\mu_w$  and  $\mu_{mal}$  for mute swans and mallards, respectively. The conditions in Crna Mlaka included a resident population of 750 resident mallards, the arrival of 2,250 migratory mallards and 118 mute swans, and 192 backyard poultry farms near the reserve (Town of Jastrebarsko, 12.5 km from Crna Mlaka<sup>18</sup>). Notations, definitions, and values for parameters included in the model are indicated in Supplementary Table S1.

The model describes the dynamics of HPAI among waterfowl and backyard poultry farms after the initial introduction of the virus via migratory mallards stopping in Croatia during their fall migration (Fig. 2). It considers the time unit to be a day. In the model, mallards and mute swans were represented as populations, with individual birds as the unit of measurement. In the European Union member countries, "backyard poultry' means chickens, turkeys, and other species belonging to the Galliformes order and ducks, geese, and other species belonging to the Anseriformes order which are kept by their owners for their own consumption or use or as pets<sup>19,20</sup>. For purposes of our model, a backyard poultry farm was defined as a uniform unit without distinction between poultry species (e.g., chickens, ducks, or other poultry), and the population size within each farm was not considered as a model parameter. Backyard poultry was included in the model at the farm level (i.e., unit for backyard poultry is the whole farm) to represent poultry clustering on farms since those flocks are typically kept in separately owned yards. The probability of a migratory mallard being infected on arrival ( $\rho_m$ ) was defined based on reported data about HPAI prevalence in European mallards during October and November of 2021<sup>21</sup>. On the contrary, migratory mute swans arriving in Croatia for wintering and resident mallards were assumed to be fully naïve to HPAI before migratory mallards introduced the infection. Waterfowl can become infected with HPAI through direct contact with individuals from the same species (intraspecific transmission) at a rate  $\beta_w$  and  $\beta_{mal}$  for mute swans and mallards, respectively. Interspecific transmission (between mute swans and mallards) occurs only via the contaminated environment at a rate  $\alpha$  (per HPAI effective infectious dose (EID<sub>50</sub> per bird per day)) and considering an effective infectious dose  $\eta_w$  for mute swans (EID<sub>50</sub> per mL) and  $\eta_{mal}$  for mallards (EID<sub>50</sub> per mL). Once infected, mallards and mute swans shed HPAI into the environment at rates  $\varepsilon_{mal}$  and  $\varepsilon_w$ (EID<sub>50</sub> per mL per day), respectively. In the environment, HPAI is quickly dispersed through the water body and inactivated at a rate  $\tau$  (per day). Mallards recover from HPAI infection at a rate  $\gamma_{mal}$  (birds per day), based on the duration of the infectious period ( $p_{map}$  in days), and die from infection at a rate  $\delta_{mal}^{mal}$  (birds per day). Information



**Fig. 1**. Phenological events of the mallard and mute swan's life cycle. Migratory mute swans start their winter migration between September and October, overwinter in Croatia between November and January, and begin their spring migration out of Croatia between February and March. Migratory mallards stop in Croatia from October to November during their fall migration through the Mediterranean-Black Sea flyway. The breeding period for the resident mallard population spans from May to August. The model considers migratory mallards as responsible for introducing Highly Pathogenic Avian Influenza (HPAI) into waterfowl communities in Croatia.

about shedding levels, probability of death after infection, and duration of the infectious period were obtained from experimental studies assessing HPAI infection in mallards and mute swans (Supplementary Table S1). In the case of mallards, values for parameterization were obtained from mallard contact-exposure to the virus in these experiments (i.e., mallard exposed to an individual previously inoculated with HPAI) as they likely better represent natural conditions compared to direct viral inoculation. This was not possible for mute swans due to a lack of information (i.e., direct inoculation experiments were considered). The experimental studies used for parameterization included the assessment of infections with H5N1, H5N5, and H5N8 HPAI subtypes from various clades (Supplementary Table S1). Once recovered, mallards remain immune to reinfection for the remainder of the simulation based on the strong elicited immunity after recovering from HPAI infection<sup>22,23</sup>. The absence of information about immunity against reinfection with the same (homosubtypic) or different (heterosubtypic) HPAI subtypes precluded incorporating partial immunity in HPAI dynamics in the baseline model; however, the impact of immunity was assessed in scenario analysis. Mute swans are highly susceptible to HPAI with all individuals dying after becoming infected at a rate  $\delta_{u}$  (birds per day). In the model, mallards are responsible for introducing HPAI into backyard poultry farms through a density-dependent transmission rate  $\beta_{0}$  (per farm per day) due to their classification as medium- to high-risk species for HPAI dissemination to poultry farms in Europe<sup>17,24</sup>. Once affected by HPAI, backyard poultry farms are culled at a rate  $\delta_{0}$  (farms per day). Our modeling approach considers the direct impact of waterfowl infections on backyard poultry farms, without accounting for the indirect effects of farm-to-farm transmission. We assume that rapid culling of infected backyard poultry flocks is effectively implemented, thus minimizing farm-to-farm spread of the virus. If this assumption does not hold, our model likely provides a conservative overestimation of the probability of backyard poultry infection resulting from spillover events. The outcomes of interest for the model were (i) the probability of HPAI infection in backyard poultry and (ii) mute swan mortality by the end of a 1-year simulation period starting on September 1st (i.e., before mute swans' winter migration).

The model was successfully validated by comparing model outcomes with independent historical data. The locations and their characteristics used for model validation are provided in Supplementary Table S2. The predicted probability of HPAI infection in backyard poultry farms matched the historical outbreak data in backyard poultry in Mlaka Antiska, Croatia. Likewise, the predicted mute swan mortality approximated values in real HPAI outbreak events that occurred among mute swans in Lonjsko Polje Nature Park, Croatia, and Lake Balaton, Hungary (further details about the calibration and validation process are explained below).

Stochastic processes in the model were accounted for via Monte Carlo simulation. Model predictions were generated with a total of 1,000 iterations as this number of iterations provided results that only marginally



Fig. 2. Diagram depicting the Highly Pathogenic Avian Influenza (HPAI) transmission dynamics at the waterfowl-backyard poultry farm interface. (1a) Migratory mallards introduce HPAI into a natural area during their stopover period. (2–3a) Infectious mallards transmit HPAI to their conspecifics (mallard-to-mallard transmission) and contribute to HPAI dissemination through the environment. (4a) Mallards visit backyard poultry farms, providing an opportunity for HPAI introduction into these farms. (1b) Migratory mute swans arrive in the natural area in winter. (2b) Mute swans cohabitating with mallards can acquire the infection by ingesting the virus from the environment. (3b) Due to their high susceptibility to HPAI, mute swans die from the infection.

differed from predictions obtained using a higher number of iterations. Partial rank correlation coefficient (PRCC) analysis was performed to understand the influence of model parameters on the outcomes of interest. We also assessed multiple scenarios to elucidate HPAI dynamics, including scenarios where part of the resident mallard population is infected prior to migratory mallards' arrival, migratory mute swans introduce HPAI into the waterfowl community instead of mallards, and the environmental transmission of HPAI occurs at different rates, among other scenarios (see the "Scenario analysis" section for further details).

We modeled HPAI transmission dynamics considering the following assumptions adopted under the principle of parsimony:

- i. Transmission of HPAI through the environment follows the assumption of homogenous mixing. In other words, all waterfowl have the same chance of contacting HPAI from the environment. Direct intraspecific HPAI transmission between mallards and mute swans was assumed to be frequency dependent.
- ii. Naïve swans and mallards have not previously experienced HPAI or LPAI infection and remain fully susceptible prior to infection.
- iii. No other wild bird species besides mallards and mute swans are implicated in the dynamics of HPAI.
- iv. Waterfowl shed HPAI into the environment through feces, while airborne dissemination is accounted for through direct intraspecific interactions.
- v. Mallard migration is the sole source of HPAI infection for resident waterfowl, while natural areas adjacent to backyard poultry farms are the only source of infection for backyard poultry.

The transmission dynamics of HPAI at the waterfowl-backyard poultry interface are represented in Fig. 3 and described in the ODEs as follows:

$$\frac{dS_r}{dt} = \theta \left( S_r + I_r + R_r \right) - \beta_{mal} \frac{(I_m + I_r)S_r}{S_r + I_r + R_r + S_m + I_m + R_m} - \alpha S_r \frac{E}{\eta_{mal}} - \mu_{mal}S_r$$
(1)

$$\frac{dI_r}{dt} = \beta_{mal} \frac{(I_m + I_r)S_r}{S_r + I_r + R_r + S_m + I_m + R_m} + \alpha S_r \frac{E}{\eta_{mal}} - (\mu_{mal} + \delta_{mal} + \gamma_{mal})I_r$$
(2)

$$\frac{dR_r}{dt} = \gamma_{mal} I_r - \mu_{mal} R_r \tag{3}$$

$$\frac{dS_m}{dt} = -\beta_{mal} \frac{(I_m + I_r)S_m}{S_r + I_r + R_r + S_m + I_m + R_m} - \alpha S_m \frac{E}{\eta_{mal}} - \mu_{mal}S_m$$
(4)

$$\frac{dI_m}{dt} = \beta_{mal} \frac{(I_m + I_r)S_m}{S_r + I_r + R_r + S_m + I_m + R_m} + \alpha S_m \frac{E}{\eta_{mal}} - (\mu_{mal} + \delta_{mal} + \gamma_{mal})I_m$$
(5)

$$\frac{dR_m}{dt} = \gamma_{mal} I_m - \mu_{mal} R_m \tag{6}$$

$$\frac{dS_w}{dt} = -\beta_w \frac{I_w S_w}{S_w + I_w} - \alpha S_w \frac{E}{\eta_w} - \mu_w S_w \tag{7}$$

$$\frac{dI_w}{dt} = \beta_w \frac{I_w S_w}{S_w + I_w} + \alpha S_w \frac{E}{\eta_w} - (\mu_w + \delta_w) I_w$$
(8)

$$\frac{dS_p}{dt} = -\beta_p (I_m + I_r) S_p \tag{9}$$

$$\frac{dI_p}{dt} = \beta_p (I_m + I_r) S_p - \delta_p I_p \tag{10}$$

$$\frac{dE}{dt} = \epsilon_{mal} \left( I_m + I_r \right) + \epsilon_w I_w - \tau E \tag{11}$$

**HPAI transmission at the waterfowl level** ( $\theta_{mal}$  and  $\theta_w$ ) The transmission rate of HPAI between mallards ( $\beta_{mal}$ ) was estimated by considering information from Wood et al.<sup>25</sup>, personal communications with Dr. Kevin Wood, and information about HPAI transmission in Ahrens et al.<sup>26</sup>. În detail, we divided the number of agonistic intraspecific interactions recorded for mallards per day by the average number of mallards in the study area, both reported by Wood et al.<sup>25</sup> to estimate the probability of a mallard-to-mallard encounter. As agonistic behaviors only represent a small fraction of mallard behavior during the non-breeding season<sup>27</sup>, we increased the number of interactions by 145% to account for courtship interactions during that season<sup>28,29</sup>. The resulting probability was multiplied by the time that mallards remain active each day<sup>30</sup> to obtain a rate of intraspecific contacts for each mallard per day. Then  $\beta_{mal}$  was calculated by multiplying the rate of intraspecific contact by the probability of infection per mallard per day after susceptible



Fig. 3. Highly Pathogenic Avian Influenza (HPAI) transmission dynamics at the waterfowl-backyard poultry farm interface. S = Susceptible, I = Infectious, R = Resistant, E = Environment. Subscripts were included to refer to specific components of the model, namely w for mute swans, r for resident mallards, m for migratory mallards, mal for all mallards (both resident and migratory), and p for backyard poultry farms. Parameters  $Ar_{p}$ and  $e_w$  are included in the diagram to indicate the arrival and departure of migratory mute swans, respectively. Similarly,  $Ar_m$  and  $e_m$  represent the arrival and departure of migratory mallards. All mallards and mute swans migrate out after their time in the area.

mallards are exposed to an infected seeder mallard calculated from the experimental study by Ahrens et al.<sup>26</sup>. We also considered the transmission of HPAI among mute swans ( $\beta_w$ ) to be 2.5 times lower than mallards based on the lower population density in mute swan compared to mallard colonies<sup>24</sup>. Furthermore, we assumed that mute swans, like chickens, reduce feces production when infected with HPAI due to a decrease in food intake<sup>31</sup>.

#### Calibration of the HPAI transmission rate between mallards and backyard poultry farms $(\theta_n)$

Information regarding mallard-backyard HPAI poultry transmission ( $\beta_p$ ) is currently absent from the literature. We calibrated  $\beta_p$  through a systematic trial-and-error approach in which we compared historical information of HPAI outbreaks in backyard poultry and related ornithological observations with predictions from the model after considering equally spaced  $\beta_p$  values (e.g., 5.2115\*10<sup>-7</sup>, 5.2120\*10<sup>-7</sup>, 5.2125\*10<sup>-7</sup>, 5.2130\*10<sup>-7</sup>, 5.2135\*10<sup>-7</sup>). Based on available historical data on the H5N1 outbreaks reported in 2021-2022 in Croatian backyard poultry farms (obtained from the World Organisation for Animal Health (WOAH) World Animal Health Information System (WAHIS<sup>32</sup>), we calculated HPAI infection probabilities to be 0.09% and 0.3% for backyard poultry farms in Staro Pračno (located in the vicinity of Natural Park Lonjsko Polje) and City Beli Manastir and Banjin Vrh (located near the Natural Park Kopački Rit), respectively. We then selected  $\beta_n$  values that resulted in a predicted median and associated interquartile range (IQR) infection probabilities that closely resemble the probability of infection in backyard poultry farms in Staro Pračno and City Beli Manastir and Banjin Vrh. The estimated  $\beta_p$  for each one of the locations were then averaged to obtain a final  $\beta_p$  value that was used for model predictions ( $\beta_p$  for Staro Pračno = 0.0000001425,  $\beta_p$  for City Beli Manastir and Banjin Vrh = 0.0000009000; average = 0.00000052125). In the model, the initial conditions for the Staro Pračno – Natural Park Lonjsko Polje were 760 resident mute swans, 57 resident mallards, 512 migratory mallards, and 1,130 backyard poultry farms. For City Beli Manastir and Banjin Vrh - Natural Park Kopački Rit, the initial conditions in the model were 137 resident mute swans, 1,382 resident mallards, 12,438 migratory mallards, and 583 backyard poultry farms. These conditions were set based on data from the Croatian Bureau of Statistics<sup>18</sup> and observations from personal communications with ornithologists. In both Natural Parks, we assumed that 10% of the migratory mallard population were residents, as in most cases ornithological observations about mallards did not distinguish between resident and migratory mallards. The median probability of HPAI infection in backyard poultry farms and the associated IQR for model estimations with different  $\beta_p$  are indicated in Supplementary Table S3.

#### Model validation

Our model was developed to predict two epidemiologically relevant outcomes: (i) the probability of HPAI infection in backyard poultry farms and (ii) mortality in mute swans. Both components were separately validated using available independent data (not used in the model development).

The probability of HPAI infection in backyard poultry was validated by comparing epidemiological data observed in Mlaka Antiska, Croatia (infection probability: 0.18%; 5 farms affected out of 2,713 backyard poultry farms in the area), about outbreaks affecting backyard poultry farms between 2016 and 2017, with model predictions. Ornithological observations in the surrounding areas (River Drava and Bijelo Brdo) indicate the presence of 1,497 mallards and the absence of mute swans (historically present only in the area in very small numbers). Model predictions indicate a 0.13% (IQR=0.07-0.21%) probability of HPAI infection in backyard poultry farms in Mlaka Antiska, closely resembling historical outbreak data in that area (0.18%).

Model predictions about the mortality in mute swans were validated by comparing historical outbreaks of HPAI in mute swans in Natural Park Lonjsko Polje (obtained from WAHIS<sup>32</sup>) and the median number of mute swan deaths and associated IQR predicted by our model. Information from Natural Park Lonjsko Polje indicates outbreaks from 2016 to 2017 with seven mute swan deaths reported. Our model projected that the 59th percentile of mute swan death predictions (median = 4.1, IQR = 1.4-29.3) matches the outbreak data observed for that specific area and year. Further validation of the mute swan mortality predicted by the model was done with historical outbreak data (obtained from WAHIS<sup>32</sup>) and ornithological observations from Lake Balaton, Hungary<sup>33</sup>. In January 2017, 26 mute swan deaths due to HPAI across five separate outbreaks were identified in Lake Balaton. Our model predicted 26 mute swan deaths occurring in the area in the 91.5th percentile (median = 4.8, IQR = 2.0-11.5), roughly aligning with real-world data.

#### Model outcomes

The model outcomes assessed in this study were (i) the probability of HPAI infection in backyard poultry (i.e., the number of affected farms in an outbreak among the total number of farms in the area summarized over 1,000 iterations) and (ii) the mortality in mute swans (i.e., number of dead mute swans in an outbreak among the total number of mute swans in the modeled population summarized over 1,000 iterations). Predictions were obtained using 1,000 iterations as this value provided results that were only marginally different from predictions produced using a higher number of iterations (Supplementary Table S4). Findings revealed that the stopover duration of mallards during their fall migration correlates with an increased probability of HPAI infection in backyard poultry (Supplementary Fig. S1).

#### Scenario analysis

To better understand the transmission dynamics of HPAI at the waterfowl-backyard poultry interface, we assessed scenarios to determine the influence of the initial HPAI prevalence among mallards and mute swans, natural immunity in mallards against HPAI, presence of a resident mute swan population (instead of being migratory), different HPAI infectious doses in mute swans, different HPAI transmission mechanisms, environmental transmission of HPAI, and efficiency in the transmission of HPAI among mute swans on the probability of HPAI infection in backyard poultry and mute swan mortality (Supplementary Table S5). To assess the influence of immunity in mallards against HPAI on the probability of infection in backyard poultry and mortality in

mute swans, we evaluated the effects of varying initial prevalence of immunity among migratory mallards and analyzed scenarios involving complete (baseline), partial, and no protection among recovered mallards. For the latter analysis, we included an adjusted HPAI transmission rate ( $\beta_{mal}$ ) for mallards in the Recovered compartment (i.e., recovered mallards are now susceptible to infection). The modified  $\beta_{mal}$  reflected different levels of partial protection: 100% (baseline), 90%, 70%, 50%, 30%, 10%, and 0%, where 0% (no) protection means the full susceptibility to infection while in the Recovered compartment.

#### Results

Model predictions indicate that the median probability of HPAI infection to backyard poultry in the Crna Mlaka area is 0.31% (Interquartile range (IQR) = 0.21–0.43%) by the end of the 1-year simulation period, while the median mortality among mute swans was predicted to be 1.18% (IQR=0.60–2.47%). Based on results from the sensitivity analysis (Fig. 4a), the duration of migratory mallards' stopover period in Crna Mlaka positively correlated with the probability of HPAI infection in backyard poultry ( $\rho$ =0.60). This is evident when assessing the influence of varying stopover durations for mallards on the probability of HPAI infection in backyard poultry (Fig. 5a). Nonetheless, the timing of the arrival and departure of migratory mallards and mute swans did not relate to infection in backyard poultry (Fig. 4a). The impact of the mallards' stopover period on mute swan mortality was comparatively less relevant than its impact on the probability of HPAI infection in backyard poultry (Figs. 4b and 5b). Mallards' recovery and HPAI-related death rates were strongly and negatively correlated with HPAI infection probability in farms ( $\rho$ =-0.84 and  $\rho$ =-0.85, respectively). Findings also indicate that HPAI shedding by mallards and HPAI-related death rate in mute swans strongly correlated with mute swan mortality ( $\rho$ =0.91 and  $\rho$ =-0.94, respectively), while deaths in mute swans were unrelated to migratory mallards' phenology.

Findings from the scenario analysis indicate that the exposure to HPAI among resident mallards prior to the introduction of an HPAI strain via migratory mallards slightly reduced the median probability of infection in backyard poultry. The likelihood of an HPAI introduction to backyard poultry farms shifted from 0.31% (IQR=0.23-0.41%) with an initial HPAI prevalence of 0.1% among resident birds to 0.28% (IQR=0.21-0.37%) when the initial infection prevalence among resident mallards was 0.5%, and slightly decreased to 0.26% (IQR=0.21-0.35%) when 10% of resident mallards were initially infected. When assessing the relevance of mallards' immunity to HPAI, the probability of HPAI infection in backyard poultry farms decreases as the protection provided by immunity in recovered mallards increases. In the scenario with complete protection



**Fig. 4**. Findings from the Partial Rank Correlation Coefficient (PRCC) analysis about model parameters' influence on (**a**) the probability of Highly Pathogenic Avian Influenza (HPAI) infection in backyard poultry and (**b**) mute swan mortality.



**Fig. 5**. Influence of the mallard stopover period on the (**a**) proportion of backyard poultry farms affected by Highly Pathogenic Avian Influenza (HPAI) and (**b**) mute swan mortality. The importance of the mallard stopover period on HPAI transmission dynamics at the waterfowl-backyard poultry interface was evaluated at lengths of 7, 14, 21, and 28 days. Shaded areas represent the interquartile range.

(baseline), the median probability of farm infection was 0.31% (IQR=0.21-0.43%), compared to the higher infection probabilities observed for partial protection at 50% (median=1.22%, IQR=0.80-1.78%) and no protection (median=1.61%, IQR=0.98-2.74%). A similar trend was observed for mortality in mute swans (Supplementary Fig. S2). Furthermore, a higher prevalence of migratory mallards in the Recovered compartment

at their arrival meaningfully reduces the likelihood of infection in both backyard poultry and mute swans (Supplementary Table S5). When assessing the introduction of HPAI by migratory mute swans instead of mallards, the infection was able to propagate in the waterfowl community and resulted in a lower probability of HPAI infection in backyard poultry (0.14%, IQR=0.12-0.16%) compared to an introduction via mallards. Model simulations suggest that the environment plays a rather minor role in the dissemination of HPAI among mallards compared to direct bird-to-bird transmission (Supplementary Table S5).

#### Discussion

We developed a mathematical model of HPAI transmission dynamics at the waterfowl-backyard poultry interface to improve our understanding of the HPAI spillover events. Using the model, we identified and characterized the length of the mallard stopover period as an ecological determinant of HPAI infection risk in backyard poultry farms. From this finding, we can infer that understanding the factors influencing the stopover duration of a reservoir species in a location can enhance HPAI outbreak forecasting and improve the implementation of surveillance and control strategies to prevent infection risk in backyard poultry. Model findings also indicate (i) the crucial role of HPAI virulence in the infection risk in backyard poultry farms and waterfowl communities, (ii) the role of immunity in mallards against HPAI reinfection, (iii) and critical knowledge limitations and model gaps that require further research. We expect that findings from this study will improve our understanding of the effect of ecological and epidemiological determinants of HPAI dissemination on the probability of the infection spillover to backyard poultry farms, thus aiding in HPAI outbreak forecasting and control. Ultimately, we hope our contribution will aid future efforts to safeguard poultry health and reduce the risk of spillover to humans.

The added value of our study comes from combining findings from diverse scientific documents to develop a model representing HPAI spread at the waterfowl-backyard poultry interface in a high-risk area for HPAI introduction into backyard poultry. Our model distinguishes itself from previous modeling efforts by predicting infection risk in backyard poultry farms, which often lack biosecurity measures to prevent the infection<sup>4,5</sup>. Furthermore, our model divides waterfowl into two key epidemiological categories common in waterfowl-poultry systems worldwide<sup>34</sup>: reservoir species (represented by mallards), which maintain and spread HPAI, and sentinel species (represented by mute swans), which are relevant for surveillance due to their high susceptibility to infection. Our model also differs from previous studies by estimating a transmission rate for viral dissemination between mallards and then calibrating HPAI transmission between mallards and backyard poultry. Additionally, it incorporates species-specific responses to HPAI infection and phenology, considering seasonal breeding by resident waterfowl and waterfowl migratory patterns. Unlike Bourouiba et al.<sup>15</sup> and Rao et al.<sup>14</sup>, who assessed HPAI dynamics during avian migration, we focused our assessment on the risk of HPAI introduction into backyard poultry farms as waterfowl arrive in the area for resting or wintering. This approach avoids drawing assumptions about HPAI transmission dynamics before and during migration, allowing us to assess epidemiological and ecological determinants of infection in backyard poultry farms once the virus has been introduced into a nearby waterfowl community. Parameter calibration and model validation steps were taken to allow the model to be used as a prediction tool for infection in backyard poultry. The transmission of HPAI between mallards and backyard poultry was calibrated using outbreak data in backyard poultry farms in the modeled ecological area in Croatia and ornithological observations in natural areas close to these outbreaks. This is relevant, as this parameter was not previously described in the literature while being key in understanding waterfowl-to-backyard poultry HPAI transmission. This process ensured that the model reflects the transmission dynamics between waterfowl and backyard poultry in a real-world scenario. Furthermore, the model was validated using independent historical data about HPAI infections in backyard poultry farms in Croatia and mute swan deaths in Croatia and Hungary.

Previous studies have indicated the urgent need to identify ecological cues to better understand HPAI transmission dynamics and help prevent HPAI introduction to poultry and its potential spillover to humans<sup>2,3,35</sup>. Predictions from our model revealed that the time spent at stopover sites by migratory mallards was a determinant factor of HPAI spillover to domestic backyard birds. While intuitive, this finding highlights the importance of further studies to understand the factors influencing the duration of the stopover period of HPAI reservoir species to improve HPAI forecasting and control. An example of these factors is climate change-induced alterations in waterfowl phenology and migratory patterns, as they can affect the timing of migratory events in waterfowl species involved in HPAI dissemination<sup>36</sup>. For instance, snow conditions and low temperatures have been found to trigger mallards' fall migratory movements and limit mid-winter movements<sup>37,38</sup>, possibly altering HPAI dynamics in areas where waterfowl communities and domestic birds intersect. This is particularly important in the context of climate change, which continues to exacerbate and affect the ecological interactions, phenology, and distribution of migratory birds<sup>39,40</sup>. Furthermore, the stopover location chosen by migratory waterfowl is also influenced by the presence of suitable natural resources for feeding, resting, and sheltering<sup>41-43</sup>. Therefore, we can expect that improving our understanding of the impact of weather factors on waterfowl migration patterns and the application of land use mapping will help predict specific high-risk areas and seasons where HPAI dissemination to backyard poultry farms is more likely to occur due to the expected prolonged presence of migratory reservoir birds.

Currently, in the United States and Europe, areas prioritized for HPAI surveillance in waterfowl communities are determined based on species diversity and abundance, migratory patterns, and evidence of previous HPAI infection in the area<sup>44–46</sup>. Findings from our study suggest adjusting this approach by incorporating weather and land use data to predict reservoir birds' phenology for specific seasons and years. This would enable more accurate identification of areas with conditions that favor an extended stay by reservoir waterfowl species during migration, thus increasing the risk of HPAI introduction to domestic birds. These identified areas can then be targeted with enhanced surveillance and preventive measures to mitigate potential outbreaks in backyard

poultry farms, allowing for a more efficient allocation of logistical and financial resources for HPAI prevention and control.

Model predictions indicated that less virulent HPAI strains are more likely to cause outbreaks in backyard poultry farms, as mallards have a higher likelihood of surviving infection. This is supported by the literature as the recently emerged H5N1 2.3.4.4.b clade displays increased adaptation to wild and domestic birds compared to previously dominant HPAI strains<sup>2</sup>, possibly contributing to its establishment in some areas of Europe<sup>47</sup>. However, this also has implications for HPAI prevention as surveillance partly relies on field observations of suspected cases in wild birds and avian mortality events<sup>48</sup>. If wild birds are experiencing less severe presentations of HPAI and surviving infection, then the effectiveness of surveillance efforts to prevent outbreaks in domestic animals and humans might be reduced. In our model, highly susceptible birds, represented by mute swans, were found to be a reliable sentinel of HPAI infection in the waterfowl community. However, reduced virulence in HPAI delayed the occurrence of mass mortality events in the population of migratory mute swans and, in consequence, reduced the likelihood of early detection of the virus. These findings highlight the importance of periodically evaluating the reliability of putative highly susceptible species usually prioritized for testing, such as mute swans, amids the emergence of strains with increased reassortment potential and decreased virulence.

In our model, resident mallards exposed to HPAI developed immunity against future infections with the same virus, effectively limiting viral transmission within the waterfowl community after the arrival of infected migratory mallards and minimizing the subsequent dissemination to backyard poultry farms. This was reflected in the protective effect of the HPAI recovery rate (Fig. 4b) and is in agreement with previous studies reporting that mallard survival from HPAI infection leads to the development of robust immunity against future homosubtypic infections, as well as long-lasting protective effects<sup>22,23</sup>. Furthermore, our scenario analysis revealed that even partial immunity against HPAI can meaningfully contribute to limiting the occurrence of the infection in backyard poultry and mute swans (Supplementary Table S5, Supplementary Fig. S2). Hence, our findings indicate that determining the immune status of resident waterfowl populations against HPAI prior to fall migration could assist in predicting the risk of transmission of the virus to backyard poultry farms during winter.

It is important to note that we did not consider the co-circulation of LPAI strains among waterfowl due to the still limited understanding of heterosubtypic immunity on HPAI dynamics in natural populations. This knowledge limitation is meaningful given evidence of previously acquired LPAI immunity partially protecting against HPAI infection in mallards<sup>22,49</sup>, which might cause our model to overestimate the risk of HPAI (thus presenting a conservative spillover prediction) due to the calibration process being done without considering LPAI-acquired immunity. This is further complicated by, also not modeled, differences in susceptibility to avian influenza exposure according to age, with younger mallards being more susceptible compared to adults<sup>49</sup>. The decision to not categorize waterfowl populations by age in our model stems from the insufficient data on the immune responses triggered by previous infections with either HPAI or LPAI strains, making it challenging to accurately account for age-specific immune pressures. Further research aiming to understand heterosubtypic immunity against HPAI among different age categories will allow an improved in silico assessment of infection dynamics in wildlife communities, particularly considering the introduction of novel strains into natural waterfowl populations.

Findings here suggest that environmental transmission is not an effective mechanism for HPAI dissemination among mallards. This is a consequence of the rapid dispersion of HPAI virions in the environment, compounded by the reduced susceptibility of mallards compared to mute swans. The latter contrasts with what was observed for mute swans, which were highly sensitive to environmental contamination with HPAI. Importantly, the model reveals that even under scenarios with little interaction between mute swans and HPAI in the environment, swans still become infected (Supplementary Table S4). Nonetheless, these observations need to be taken carefully as HPAI inactivation did not consider persistence under different conditions of salinity, pH, and temperature. These factors have been reported to heavily influence HPAI persistence in the environment<sup>50</sup> but were not included in our model as their influence has not been properly explored in natural conditions. This knowledge gap precluded the exploration of climate change consequences on HPAI transmission dynamics in our model, which is particularly relevant given the suggested extended persistence of HPAI in the environment during colder temperatures<sup>51</sup>.

Although mallards and mute swans interact in nature during winter<sup>25</sup>, the interspecific transmission of HPAI between these two species was not considered in the model due to a lack of epidemiological data. This is a relevant knowledge limitation that might underestimate HPAI transmission in the waterfowl community. Furthermore, waterfowl communities are more complex than what was depicted in the model, with a wider variety of species interacting and participating in HPAI dynamics. Nonetheless, incorporating multiple species into the description of HPAI dynamics would increase model uncertainty, as a deeper understanding of each waterfowl species' role as a viral disseminator within the community is essential for effective model parameterization. A relevant limitation of our calibration and validation approaches was not accounting for the influence of sampling intensity (i.e., differences in the effort to monitor HPAI in terms of the number of samples to identify infection in backyard poultry and individual mute swans<sup>52</sup>) on HPAI underreporting, a limitation shared with other modeling efforts attempting to understand HPAI transmission at the waterfowl-poultry interface [e.g.<sup>53</sup>]. Accounting for the sampling intensity in future modeling approaches would improve the accuracy and reliability of predictions by considering variations in detection probabilities and addressing potential biases in reported data.

Our model incorporates key components in HPAI dynamics at the waterfowl-backyard poultry interface, including migratory and resident reservoir populations, sentinel species, and backyard poultry farms. As these components are present in a variety of ecological areas, supported by our validation process based on data from Hungary, we expect the model to effectively predict the probability of HPAI infection in backyard poultry farms

in other locations with homologous conditions. Insights provided by our model are especially timely, given the current emergence of novel HPAI strains with increased potential for reassortment<sup>2</sup>. Particularly important is the influence of the duration of migratory mallard stopover during fall migration in predicting HPAI risk in backyard poultry, highlighting the need to predict migratory patterns of reservoir waterfowl species. Our findings underscore the crucial roles played by prior immunity and HPAI virulence in shaping infection dynamics and recognize relevant limitations in knowledge about the virus. We expect that our model will serve as a decision support tool for purposes of aiding surveillance and prevention of HPAI introduction to backyard poultry farms and thus reducing the spillover risk to humans.

#### Data availability

Data used for model parametrization, calibration, and validation is described in the manuscript and in the Supplementary Material. R code for the model is available at https://github.com/sgllanos/HPAI\_model.

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#### References

- 1. Leguia, M. et al. Highly pathogenic avian influenza A (H5N1) in marine mammals and seabirds in Peru. Nat. Commun. 14 https:// /doi.org/10.1038/s41467-023-41182-0 (2023)
- 2. Xie, R. et al. The episodic resurgence of highly pathogenic avian influenza H5 virus. Nature 622, 810-817. https://doi.org/10.1038 /s41586-023-06631-2 (2023)
- 3. Ramey, A. M., DeLiberto, T. J., Berhane, Y., Swayne, D. E. & Stallknecht, D. E. Lessons learned from research and surveillance directed at highly pathogenic influenza a viruses in wild birds inhabiting North America. Virol 518, 55-63. https://doi.org/10.101 6/j.virol.2018.02.002 (2018).
- 4. Biswas, P. K. et al. Risk for infection with highly pathogenic avian influenza virus (H5N1) in backyard chickens, Bangladesh. Emerg. Infect. Dis. 15, 1931-1936. https://doi.org/10.3201/eid1512.090643 (2009).
- 5. Conan, A., Goutard, F. L., Sorn, S. & Vong, S. Biosecurity measures for backyard poultry in developing countries: a systematic review. BMC Vet. Res. 8, 240. https://doi.org/10.1186/1746-6148-8-240 (2012).
- 6. Tuncer, N. & Martcheva, M. Modeling seasonality in avian influenza H5N1. J. Biol. Syst. 21 https://doi.org/10.1142/S02183390134 00044 (2013).
- 7. Tuncer, N., Torres, J., Martcheva, M., Barfield, M. & Holt, R. D. Dynamics of low and high pathogenic avian influenza in wild and domestic bird populations. J. Biol. Dyn. 10, 104-139. https://doi.org/10.1080/17513758.2015.1111449 (2016).
- 8. Liu, R., Duvvuri, V. R. S. K. & Wu, J. Spread pattern formation of H5N1-avian influenza and its implications for control strategies. Math. Model. Nat. Phenom. 3, 161-179. https://doi.org/10.1051/mmnp:2008048 (2008).
- 9. Pandit, P. S., Bunn, D. A., Pande, S. A. & Aly, S. S. Modeling highly pathogenic avian influenza transmission in wild birds and poultry in West Bengal, India. *Sci. Rep.* 3, 2175. https://doi.org/10.1038/srep02175 (2013). 10. Lee, E. K., Liu, Y. & Pietz, F. H. A computational framework for a digital surveillance and response tool: application to avian
- influenza. American Medical Informatics Association (AMIA) Annual Symposium Proceedings 1090-1099 (2017)
- 11. Glass, K. et al. Modelling the impact of biosecurity practices on the risk of high pathogenic avian influenza outbreaks in Australian commercial chicken farms. Prev. Vet. Med. 165, 8-14. https://doi.org/10.1016/j.prevetmed.2019.02.002 (2019).
- 12. Gumel, A. B. Global dynamics of a two-strain avian influenza model. Int. J. Comput. Math. 86, 85-108. https://doi.org/10.1080/00 207160701769625 (2008)
- 13. Agusto, F. B. & Gumel, A. B. Qualitative dynamics of lowly-and highly-pathogenic avian influenza strains. Math. Biosci. 243, 147-162. https://doi.org/10.1016/j.mbs.2013.02.001 (2013).
- 14. Rao, D. M., Chernyakhovsky, A. & Rao, V. Modeling and analysis of global epidemiology of avian influenza. Environ. Model. Softw. 24, 124-134. https://doi.org/10.1016/j.envsoft.2008.06.011 (2009).
- 15. Bourouiba, L., Gourley, S. A., Liu, R. & Wu, J. The interaction of migratory birds and domestic poultry and its role in sustaining avian influenza. SIAM J. Appl. Math. 71, 487-516. https://doi.org/10.1137/100803110 (2011).
- 16. Soetaert, K., Petzoldt, T. & Setzer, R. W. Solving differential equations in R: package deSolve. J. Stat. Softw. 33, 1-25. https://doi.or g/10.18637/jss.v033.i09 (2010).
- 17. Mužinić, J., Pavlak, M., Savić, V., Cvitković, D. & Tadić, M. Ornithological knowledge for preliminary risk assessment of avian influenza (H5N1): the implementation of the European model in Croatia. Avian Biol. Res. 1, 89-96. https://doi.org/10.3184/17581 5508X365853 (2008).
- 18. Croatian Bureau of Statistics. Statistical Database. https://web.archive.org/web/20240327125032/https://dzs.gov.hr/ (2015).
- 19. European Food Safety Authority (EFSA). Avian influenza overview October 2016-August 2017. EFSA J. 15, e05018. https://doi.or g/10.2903/j.efsa.2017.5018 (2017).
- 20. European Food Safety Authority (EFSA) Animal Health and Welfare Panel et al. Urgent request on avian influenza. EFSA J. 15, e04687. https://doi.org/10.2903/j.efsa.2016.4687 (2017).
- 21. European Food Safety Authority (EFSA), Aznar, I., Baldinelli, F., Stoicescu, A. & Kohnle, L. Annual report on surveillance for avian influenza in poultry and wild birds in Member States of the European Union in 2021. EFSA J. 20, e07554. https://doi.org/10.2903/ j.efsa.2022.7554 (2022).
- 22. Koethe, S. et al. Modulation of lethal HPAIV H5N8 clade 2.3. 4.4 B infection in AIV pre-exposed mallards. Emerg. Microbes Infect. 9, 180-193. https://doi.org/10.1080/22221751.2020.1713706 (2020).
- 23. Caliendo, V. et al. Long-term protective effect of serial infections with H5N8 highly pathogenic avian influenza virus in wild ducks. J. Virol. 96, e01233-e01222. https://doi.org/10.1128/jvi.01233-22 (2022).
- 24. Atkinson, P. W. et al. Urgent preliminary assessment of ornithological data relevant to the spread of Avian Influenza in Europe. https ://web.archive.org/web/20240328002203/https://www.wetlands.org/publication/urgent-preliminary-assessment-of-ornithologica l-data-relevant-to-the-spread-of-avian-influenza-in-europe-phase-1-2/ (2006).
- 25. Wood, K. A., Ham, P., Scales, J., Wyeth, E. & Rose, P. E. Aggressive behavioral interactions between swans (Cygnus spp.) and other waterbirds during winter: a webcam-based study. Avian Res. 11, 30. https://doi.org/10.1186/s40657-020-00216-7 (2020).
- 26. Ahrens, A. K., Selinka, H. C., Mettenleiter, T. C., Beer, M. & Harder, T. C. Exploring surface water as a transmission medium of avian influenza viruses-systematic infection studies in mallards. Emerg. Microbes Infect. 11, 1250-1261. https://doi.org/10.1080/2 2221751.2022.2065937 (2022).
- 27. Drilling, N., Titman, R. D. & McKinney, F. Mallard (Anas platyrhynchos), version 1.0, in Birds of the World (ed Billerman, S. M.) (Cornell Lab of Ornithology, 2020).
- 28. Lee, S. D. A time budget study of wintering mallards on the Southern High Plains of Texas, USA. Anim. Cells Syst. 1, 571-576 (1997).

- 29. Turnbull, R. E. & Baldassarre, G. A. Activity budgets of mallards and American wigeon wintering in east-central Alabama. *Wilson Bull.* **99**, 457–464 (1987).
- Korner, P., Sauter, A., Fiedler, W. & Jenni, L. Variable allocation of activity to daylight and night in the mallard. *Anim. Behav.* 115, 69–79. https://doi.org/10.1016/j.anbehav.2016.02.026 (2016).
- Mulatti, P., Dorotea, T., Vieira, J. T., Bonfanti, L. & Marangon, S. Effect of biosecurity measures and early detection systems, mitigation measures and surveillance strategies on the spread of HPAI and LPAI between farms. *EFSA Supporti. Publ.* 14, 1142E. https://doi.org/10.2903/sp.efsa.2016.EN-1142 (2017).
- 32. WOAH. WAHIS: World Animal Health Information System. https://wahis.woah.org/#/home (2023).
- Pap, K., Nagy, L., Balogh, C., G-Tóth, L. & Liker, A. Environmental factors shaping the distribution of common wintering waterbirds in a lake ecosystem with developed shoreline. *Hydrobiologia* 716, 163–176. https://doi.org/10.1007/s10750-013-1560-3 (2013).
- Hill, N. J. et al. Ecological divergence of wild birds drives avian influenza spillover and global spread. PLoS Pathog. 18, e1010062. https://doi.org/10.1371/journal.ppat.1010062 (2022).
- 35. Verhagen, J. H., Fouchier, R. A. & Lewis, N. Highly pathogenic avian influenza viruses at the wild-domestic bird interface in Europe: future directions for research and surveillance. *Viruses* 13, 212. https://doi.org/10.3390/v13020212 (2021).
- Morin, C. W. et al. Avian influenza virus ecology and evolution through a climatic lens. *Environ. Int.* 119, 241–249. https://doi.org/10.1016/j.envint.2018.06.018 (2018).
- Sauter, A., Korner-Nievergelt, F. R. & Jenni, L. Evidence of climate change effects on within-winter movements of European mallards *Anas platyrhynchos*. *Ibis* 152, 600–609. https://doi.org/10.1111/j.1474-919X.2010.01028.x (2010).
- Weller, F. G. et al. Environmental drivers of autumn migration departure decisions in midcontinental mallards. *Mov. Ecol.* 10, 1. https://doi.org/10.1186/s40462-021-00299-x (2022).
- Brown, V. L. & Rohani, P. The consequences of climate change at an avian influenza 'hotspot'. Biol. Lett. 8, 1036–1039. https://doi. org/10.1098/rsbl.2012.0635 (2012).
- 40. Tian, H. et al. Climate change suggests a shift of H5N1 risk in migratory birds. *Ecol. Modell.* **306**, 6–15. https://doi.org/10.1016/j.e colmodel.2014.08.005 (2015).
- 41. Bengtsson, D. et al. Movements, home-range size and habitat selection of mallards during autumn migration. *PLOS One.* 9, e100764. https://doi.org/10.1371/journal.pone.0100764 (2014).
- Gillespie, C. R. & Fontaine, J. J. Shorebird stopover habitat decisions in a changing landscape. J. Wildl. Manag. 81, 1051–1062. https://doi.org/10.1002/jwmg.21271 (2017).
- Zhang, W., Li, X., Yu, L. & Ši, Y. Multi-scale habitat selection by two declining east Asian waterfowl species at their core spring stopover area. *Ecol. Indic.* 87, 127–135. https://doi.org/10.1016/j.ecolind.2017.12.035 (2018).
- 44. Department of Agriculture, U. S. & Geological Survey, U. S. U.S. Fish and Wildlife Service & National Flyway Council. Early Detection and Monitoring for Avian Influenzas of Significance in Wild Birds. A U.S. Interagency Strategic Plan (2015). https://www.a phis.usda.gov/sites/default/files/wild-bird-strategic-plan.pdf
- Gargallo, G. et al. Development of a prototype early warning system for avian influenza in the EU based on risk-mapping. EFSA Support Publ. 19, 7762E. https://doi.org/10.2903/sp.efsa.2022.EN-7762 (2022).
- 46. Department of Agriculture, U. S. & Geological Survey, U. S. U.S. Fish and Wildlife Service & National Flyway Council. Implementation plan for avian influenza surveillance in waterfowl in the United States, summer FY 2021 – winter FY 2022 (ver. 1.0, March 2021). https://www.aphis.usda.gov/animal\_health/downloads/animal\_diseases/ai/2021-22-wild-bird-ai-surveillance-i mplementation-plan.pdf (2021).
- Pohlmann, A. et al. Has epizootic become enzootic? Evidence for a fundamental change in the infection dynamics of highly pathogenic avian influenza in Europe, 2021. MBio 13, e00609–e00622. https://doi.org/10.1128/mbio.00609-22 (2022).
- European Food Safety Authority (EFSA). Avian influenza overview September–December 2022. EFSA J. 21, e07786. https://doi.org/10.2903/j.efsa.2023.7786 (2023).
- 49. Stallknecht, D. E. et al. Naturally acquired antibodies to influenza a virus in fall-migrating north American mallards. *Vet. Sci.* 9, 214. https://doi.org/10.3390/vetsci9050214 (2022).
- 50. Brown, J. D. et al. Persistence of H5 and H7 avian influenza viruses in water. Avian Dis. 51, 285–289. https://doi.org/10.1637/763 6-042806R.1 (2007).
- Ottaviani, D. et al. The cold European winter of 2005–2006 assisted the spread and persistence of H5N1 influenza virus in wild birds. *EcoHealth* 7, 226–236. https://doi.org/10.1007/s10393-010-0316-z (2010).
- Giacinti, J. A. et al. Assessing avian influenza surveillance intensity in wild birds using a one health lens. One Health. 18, 100760. https://doi.org/10.1016/j.onehlt.2024.100760 (2024).
- Opata, M. R., Lavarello-Schettini, A., Semenza, J. & Rocklöv, J. Predictiveness and Drivers of Highly Pathogenic Avian Influenza Outbreaks in Europe. Preprint at: (2024). https://doi.org/10.2139/ssrn.4983171

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#### Author contributions

Model development was done by SL-S and DY. Model calibration, validation, and formal analysis were carried out by SL-S. MP provided guidance and information about ornithological observations and poultry data. SL-S and RI interpreted the predictions from the model and study findings. SL-S wrote the manuscript draft and designed figures. RI acquired funding and administered and supervised the research study. RI and MP participated in the conceptualization of the project. All authors participated in the review and editing of the manuscript and approved its final version.

### Declarations

#### **Competing interests**

The authors declare no competing interests.

#### Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/1 0.1038/s41598-025-89827-y.

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