Poultry Science

Characterizing the domestic-wild bird interface through camera traps in an area at risk for avian influenza introduction in Northern Italy

Giulia Graziosi ,*[,1,2] Caterina Lupini,*[,1] Francesco Dalla Favera,* Gabriella Martini,† Geremia Dosa,† Giacomo Trevisani,‡ Gloria Garavini,‡ Alessandro Mannelli ,§ and Elena Catelli *

*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna 40064, Italy; †Veterinary Services, Local Health Unit of Imola (A.U.S.L. di Imola), Imola, Bologna 40026, Italy; ‡Veterinary Services of Eurovo Group, Imola, Bologna 40026, Italy; and §Department of Veterinary Sciences, University of Torino, Grugliasco, Turin 10095, Italy

ABSTRACT Direct or indirect interactions between sympatric wildlife and poultry can lead to interspecies disease transmission. Particularly, avian influenza (**AI**) is a viral epidemic disease for which the poultry-wild bird interface shapes the risks of new viral introductions into poultry holdings. Given this background, the study hereby presented aimed to identify wild bird species in poultry house surroundings and characterize the spatio-temporal patterns of these visits. Eight camera traps were deployed for a year (January to December 2021) in 3 commercial chicken layer farms, including free-range and barn-type setups, located in a densely populated poultry area in Northern Italy at high risk for AI introduction via wild birds. Camera traps' positions were chosen based on wildlife signs identified during preliminary visits to the establishments studied. Various methods, including time series analysis, correspondence analysis, and generalized linear models, were employed to analyze the daily wild bird visits. A total of 1,958 camera trap days yielded 5,978 videos of wild birds from 27 different species and 16 taxonomic families. The animals were predominantly engaged in foraging activities nearby poultry houses. Eurasian magpies (*Pica pica*), ring-necked pheasants (*Phasianus colchicus*), and Eurasian collared doves (*Streptopelia decaocto*) were the most frequent visitors. Mallards (*Anas platyrhynchos*), an AI reservoir species, were observed only in a farm located next to a fishing sport lake. Time series analysis indicated that wild bird visits increased during spring and winter. Farm and camera trap location also influenced visit frequencies. Overall, the results highlighted specific species that could be prioritized for future AI epidemiological surveys. However, further research is required to assess their susceptibility and infectivity to currently circulating AI viruses, essential for identifying novel bridge hosts.

Key words: camera-trap survey, poultry farm, avian influenza, domestic-wild bird interface, BRIDGE HOST

2024 Poultry Science 103:103892
https://doi.org/10.1016/j.psj.2024.103892

INTRODUCTION

Direct or indirect interactions between wildlife and domestic animals may lead to interspecies disease transmission (Craft, 2015), resulting in animal health issues and economic losses for the livestock and poultry sector (Daszak, 2000; Gortázar et al., 2007; Wiethoelter et al., 2015). Factors such as human population growth, spatial overlap between hosts and vectors, land-use, or environmental changes play crucial roles in increasing the risk of disease emergence at the wildlife-livestock interface (Hassell et al., 2017; Vanwambeke et al., 2019). From a One Health perspective, 60% of globally reported emerging infectious diseases in humans are zoonoses, with 70% of these originating from wild animals (Cleaveland et al., 2001; Jones et al., 2008). Therefore, characterizing the wildlife-domestic animal-human interface is pivotal in preventing and controlling both animal and zoonotic infectious diseases (Lloyd-Smith et al., 2009).

For poultry, wild birds serve as vectors or reservoirs for a wide range of bacterial and viral pathogens (Ayala et al., 2020; Franklin et al., 2021; Graziosi et al., 2022a; Graziosi et al., 2022b; Tucciarone et al., 2022). Among these, the avian influenza virus (**AIV**) finds its reservoir in waterbirds belonging to the Anseriformes (ducks, swans, and geese) and Charadriiformes orders (shorebirds, terns, and gulls) (Webster et al., 1992). Since 2020, highly pathogenic avian

Received January 10, 2024.
Accepted May 20, 2024.

[1]These authors equally contributed to this work.

[2]Corresponding author: giulia.graziosi2@unibo.it

influenza (**HPAI**) H5Nx viruses of clade 2.3.4.4b of the H5 goose/Guangdong (Gs/Gd) lineage have been persistently circulating in wild aquatic birds, leading to their global spread (Caliendo et al., 2022; European Food Safety Authority et al., 2023d). Primary introductions from wild birds, as well as secondary spread between farms, have caused an unprecedented number of HPAI virus outbreaks in rural and commercial poultry in Eurasia, Africa, and North and South America (European Food Safety Authority et al., 2022b; European Food Safety Authority et al., 2022d; European Food Safety Authority et al., 2023a; European Food Safety Authority et al., 2023c). HPAI viruses of clade 2.3.4.4b are also of public health concern due to the presence of molecular signatures in their genomes that indicate adaptation to mammals, making them a zoonotic and potentially pandemic risk (Kuiken et al., 2023).

In Italy, multiple incursions of H5Nx HPAIVs of clade 2.3.4.4b have resulted in 358 outbreaks in poultry between October 2021 and July 2023. The most affected region has been the northeastern part of the Country, where Densely Populated Poultry Areas (**DPPAs**) coexist with numerous wetlands and lagoons. Interestingly, recent H5Nx HPAI outbreaks in Italy and Europe have been linked to primary introductions from wild birds (European Food Safety Authority et al., 2023b; European Food Safety Authority et al., 2023c). Waterfowl and shorebirds, which have distinct ecological requirements, are considered to have limited direct contact with domestic birds (Caron et al., 2014). However, the role of synanthropic wildlife as bridge hosts in transmitting AI from reservoir species to poultry or between poultry farms has recently been suggested with respect to the virus epidemiology (Caron et al., 2014; Root et al., 2020; Shriner et al., 2020; Verhagen et al., 2021). Nevertheless, there is a lack of comprehensive information on wild birds visiting patterns in poultry farms. To date, such assessments in Italy have been previously conducted through visual bird counts carried out between January and May on 7 mixed-species backyard farms and 2 commercial poultry holdings (Veen et al., 2007).

Given this background, the present study aimed to characterize the domestic-wild bird interface in 3 commercial layer farms located within a DPPA of Northern Italy, where the Italian poultry production is concentrated. This was achieved through a yearlong camera trap survey. The study specifically focused on identifying the wild bird species that visited the surroundings of the poultry houses and describing their behavior and detection patterns over time. Additionally, the study examined whether these visits varied according to season and type of areas monitored. Overall, the data hereby obtained could be utilized to parameterize disease transmission models for pathogens that have a reservoir in free-range avifauna, such as the AIV.

MATERIALS AND METHODS

Study Design

The study was conducted on 3 commercial layer farms, located in the Emilia-Romagna region, namely Farm 1, Farm 2 and Farm 3 (Figure 1). These were randomly selected from a group of a total of 20 laying hen farms that reported wildlife presence within farms' boundaries, also preliminarily assessed through on-site visits by the authors. This assessment involved conducting short interviews with farmers, making direct observations of wildlife, and identifying indirect signs such as tracks, scats, fecal droppings. Prior permission was obtained from the owner to install camera-traps before commencing the study.

Farm 1 housed approximately 130,000 hens distributed across 5 multi-tier aviaries and included an egg packing plant. The farm was located in close proximity to a fishing sport lake (100 meters away) and quarry lakes formed from dismissed cave systems (less than 1 kilometers away). Chicken manure was collected every 3 d without being stored on-site. Waterfowl were sporadically observed in the farm area. Moreover, during the 2016/2017 H5Nx HPAI epidemic in Italy, an H5N8 HPAIV outbreak was reported in Farm 1, and it was determined that the virus had been introduced through direct or indirect contacts with infected wild aquatic birds (Istituto Zooprofilattico Sperimentale delle Venezie, 2018). Farm 2 was an organic layer farm that housed 140,000 hens in 6 poultry houses. The farm had outdoor spaces accessible to the animals, which were surrounded by water channels and arable fields. In these outdoor areas, there were no poultry feeding points, and water was sheltered to prevent access by wild birds. Farm 3 consisted of 5 multi-floor sheds and 12 multi-tier aviaries, making it one of the largest poultry holdings in the Emilia-Romagna region, with 1.4 million raised hens. The farm included an egg packing facility and a poultry manure pelleting facility. The farm area was surrounded on 3 sides by arable fields and on one side by a small waterway. In Farm 2 and 3, specific sites were used for manure storage before further processing. The 3 facilities were surrounded by fences; however, several breaches were present. Pest control through rodenticide baits was routinely applied. During the study period, the layers in the 3 farms were tested once for the detection of antibodies against AIV, as part of the National Avian Influenza Surveillance Plan 2021 (https://www.izsvenezie.it/documenti/temi/influenza-aviaria/piani-sorveglianza/piano-nazionale-influenza-aviaria-2021.pdf) and the Commission Delegated Regulation (EU) 2020/689. All serological tests yielded negative results.

Overall, the 3 facilities are located in the Po Valley, within a DPPA (more than 2 million birds located in the Bologna province) with a high risk of introduction of HPAI from wild birds due to the presence of waterways and natural or artificial wetlands used for purposes such as water storage for cropland irrigation, gamebirds hunting grounds, or wastewater plants ("zone B" at high risk

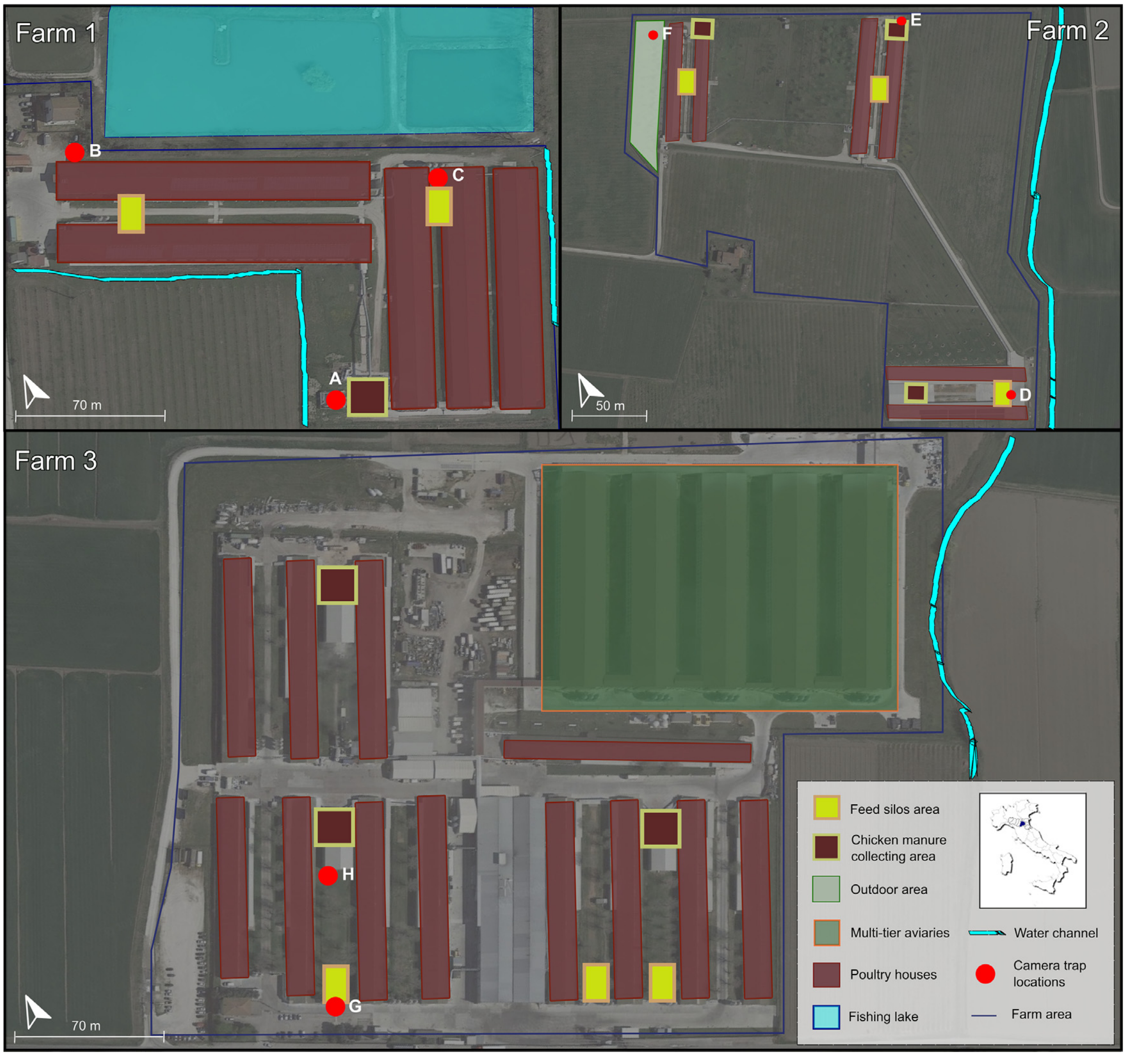

Figure 1. Schematic representation of Farm 1, 2, and 3. The red circles represent the camera trap locations (A to H). The figure also indicates the positions of poultry houses, chicken manure collection areas, and feed silos for each farm. Map realized with QGIS software (version 3.26).

of AI introduction and higher risk of AI spread according to the DGSAF protocol number 29049 dated November 20, 2019, https://www.trovanorme.salute.gov.it/norme/dettaglioAtto?id=71728). Furthermore, within a 20-kilometer radius of the 3 sites, the Argenta valleys of the Delta Po Regional Park is situated. These valleys are among the largest freshwater wetlands in northern Italy and included in the Ramsar Convention of Wetlands of International Importance (https://www.ramsar.org/sites/default/files/documents/library/sitelist.pdf). The area encompasses the Campotto/Bassarone and Vallesanta expansion reservoirs, as well as the Traversante hygrophilous woodland, covering a total of approximately 1600 hectares and providing a suitable habitat for over 300 resident, wintering, or breeding wild bird species (https://ambiente.regione.emilia-romagna.it/it/parchi-natura2000/rete-natura-2000/siti/it4060001).

Camera Trap Survey

The camera trap type used for the study hereby presented was selected based on information available on a reference website (https://www.trailcampro.com) and the cost-effectiveness of the instrument. The main requirements for selection included fast triggers and good video quality. The chosen camera trap system (Dark-Ops Pro XD Dual Lens, Browning Trail Cameras, UT) had a detection range of 27 m, a fast video trigger (0.72 s), a quick recovery speed (1.9 s) and a wide detection angle (41.7°; field of view of 41.8°). Additionally, the system featured two lenses, one for day recordings and one for night recordings, ensuring good overall footage quality that was crucial for identifying animals at the species level, even in low-light conditions.

The study was conducted from January 2021 until December 2021. Considering that various variables, including detection distance, body mass, temperature, and the frontal or lateral approach of the animal, could influence positive triggers (Randler et al., 2018), a 4-wk preliminary test was carried out to evaluate the detection probability of birds, using 2 motion-sensing infrared digital cameras. Based on the results of the pilot study, a total of 8 infrared motion-triggered camera traps were deployed in the studied layer farms. Camera traps were located within farm boundaries, at sites where signs of wild birds were seen during preliminary visits or at locations likely to attract wild birds, such as chicken manure collecting sites, drainage ditches nearby poultry houses or feed silos areas (Figure 1).

On Farm 1, 3 cameras (locations A to C in Figure 1) were deployed from January 2021 to December 2021. One camera was positioned near a feed silo (**FSilo**) (A), another was placed near the air inlets of a poultry house (**PHouse**) (B), facing the area adjacent to the fishing sport lake, and the third camera was placed in front of the chicken manure collection point (**CMan**) (C). On Farm 2, 2 cameras (locations D and E in Figure 1) were deployed from January 2021 to December 2021. One camera was positioned to capture the feed silos area (**FSilo**) (D), and the other was placed at a chicken manure collection point (CMan) (E). Since Farm 2 was an organic farm, an additional camera was placed within 10 meters from the poultry house entrance, facing the fenced outdoor area accessible to hens and located near the outdoor poultry drinking station (**Out**) (location F in Figure 1). The camera at location F was operational only from July 2021 to mid-October 2021, which coincided with the period when Italian poultry was reallowed to outdoor access after restriction implemented during the H5N1 HPAI epidemic in poultry (European Food Safety Authority et al., 2022c). On Farm 3, 2 cameras (locations G and H in Figure 1) were deployed from February 2021 to November 2021. One camera was positioned at the feed silos area, and the other was placed near one of the farm's chicken manure collection point, nearby drainage ditches.

The camera traps at locations A to E and G and H were set to operate from 6 am to 6 pm and programmed to record 30-s-long videos after detecting movement, with a lag period of 30 s to avoid continuous triggers. The camera at location F was operational from 5 pm to 6 am, with the same setup, to avoid continuous detections of chickens during daylight. Prior to deploying the camera traps, all instruments were synchronized to the correct time and date, and a unique code was assigned to identify each camera location ID. Throughout the study, batteries and SD cards were replaced every 3 wk. Camera trap operations, such as setup, battery and SD card replacements, and malfunctions, were recorded in a data sheet using Microsoft Excel 2021, version 16.49.

Data Processing and Analysis

Temporal Pattern of Wild Bird Visits and Behaviors. Data were stored and managed in accordance with previously published guidelines on camera trap studies (Wearn et al., 2017). Footages analysis was performed using Timelapse Image Analyzer (Greenberg et al., 2012). Data visualization and further analyses were conducted using GraphPad Prism (version 9) and R software (version 4.0.4). After each SD card collection, videos were renamed based on the camera location ID and the date of recording using the imageRename function from the "camtrapR" package (version 2.2.0) (Niedballa et al., 2016).

Table 1. Category of behaviors exhibited by wild birds in the poultry farms studied.

Behavior	Description
Moving through	Moving from one side to another of the camera's field of view, on land or by flight, without exhibiting other behaviors
Observing surroundings	Explorative behavior expressed as observing surroundings
Vocalizing	Emitting sounds to communicate with other individuals of the same species or other species
Grooming/bathing	Rubbing the beak to remove dirt and dust from feathers or dust bathing
Excreting	Defecating
Foraging	Eating or drinking
Territorial behavior	Attacking or charging an intruder of the same species or other species

A wild bird visit to poultry farms was defined as an observation of at least one individual of a given wild bird species on the camera's field of view. Two authors with a background in ornithology (G.G. and F.D.F.) screened the videos, and the following information was recorded from each footage clip: date, time (hh:mm:ss), type of visitor (human, poultry, wild bird, wild mammal, cat or dog), species, behavior, and time spent in front of the camera. Subsequent footages of the same species, number of animals recorded, date, and time (hh:mm) up to 4 min from the previous detection were considered duplicates and therefore removed from the daily wild bird counts. In cases where more than one species of wild bird was present in the same footage, the event was identified as a separate visit (Scott et al., 2018). Footage clips with humans or domestic animals were excluded from further analysis and removed, following current privacy regulations (Repubblica Italiana, 2018).

The behaviors exhibited by wild birds were classified into several categories (Miller, 1988; Payne et al., 2016), as reported in Table 1. Different behaviors displayed by a single individual within the same footage were considered as separately.

Daily mean detection rates (**MDR**) and 95% confidence interval (**CI**) were calculated for each species for the overall farms studied, each farm, camera trap location (except for location F, as described later), and for seasons defined as spring (from March 1st), summer (from June 1st), autumn (from September 1st), and winter (from December 1st). Since wild bird counts showed overdispersion and aggregation over time, to estimate the 95% confidence interval an intercept-only model was fitted using a negative binomial distribution (Zuur et al., 2009). The number of visits by calendar month was further formatted into a time-series object, and the seasonal pattern of visits was assessed by inspecting the 3-month rolling averages using the "zoo" package (version 1.8-12) (Zeileis et al., 2014). Activity patterns of wild birds on poultry farms were computed through the "camtrapR" package only when $\geq$ 40 visits/year were recorded for a given species.

Influence of Farm, Location, and Seasons on Wild Bird Counts. To investigate whether the number of wild birds varied across farms (Farm 1, 2, and 3) and camera trap locations (silos area and chicken manure collecting point) during different season, a generalized linear model (**GLM**) was applied. All the statistical analyses were performed using the R software (version 4.0.4). Two camera trap locations, specifically the one positioned nearby the air inlets in Farm 1 (location B) and the one in the outdoor area in Farm 2 (location F), were excluded from the analysis as they were not present in all 3 farms, making them noncomparable. Results of camera traps at location B and F were therefore only included in the qualitative synthesis of results. Before conducting the analysis, the data from each camera trap were aggregated by date, camera trap location (chicken manure collection point or feed silos area), farm monitored, and season, using the "aggregate" function. Regarding the season variable, after inspecting the overall 3-month rolling averages, the ones with the highest number of counts, namely winter and spring, were aggregated as season 1, and summer and autumn as season 0. A negative binomial distribution of counts ("glm.nb" function of the 'MASS' package) was employed to account for over-dispersion and aggregation of counts over time (Zuur et al., 2009). The model utilized in the analysis included camera trap locations, farms, and seasons as predictors, along with 2-way interactions between farms and camera trap locations, farms and seasons, and camera trap locations and seasons. Results were expressed as rate ratios (RR) with their 95% confidence intervals (95% CI), after the exponentiation of the estimated parameters. As model diagnostics, Pearson Chi-square Goodness-of-Fit test and the plotting of Pearson residuals by fitted values for each observation were used (Brown & Prescott, 2014). Additional information on R codes used and model diagnostics are provided in S1 and Figure S1 of Supplementary Materials 1.

Lastly, to better visualize the model outcome with respect to the seasonal pattern of observations and species detected, a correspondence analysis (**CA**) was performed (Elbers et al., 2020). The R packages "FactoMineR" and "factoextra" were used to evaluate the dependency of the 3 bird orders most frequently observed (Passeriformes, Galliformes, and Columbiformes) and the month of the year.

Observations in the Outdoor Area of the Free-Range Hen Farm. Since the camera trap at location F in Farm 2 was operational for a shorter duration and was the only one situated in an outdoor area accessible to poultry, the data from this camera were analyzed separately. An indirect contact between wild birds and poultry was defined as the camera detection of a wild species in the outdoor area, regardless of the presence of poultry. Overall mean indirect contact rate (**ICR**) between wild birds and free-range hens and their 95% confidence interval were calculated as abovementioned for the MDR. Direct contacts between wild birds and poultry were not considered for statistical analysis, as the camera was only active from sunset to sunrise when chickens were rarely in the outdoor space area.

Behaviors observed during the analysis of the footages were categorized as previously described (Table 1).

RESULTS

Overview of the Survey, Wild Bird Population and Behaviors

Throughout the study period, the camera traps were operational for a total of 877 trap days on Farm 1 (with a monthly camera trap effort ranging from 20 to 26 trap days), 623 trap days on Farm 2 (with a monthly camera trap effort ranging from 17 to 24 trap days), and 458 trap days on Farm 3 (with a monthly camera trap effort ranging from 17 to 31 trap days). A total number of 33,519 footages were recorded, as shown in Table 2, and a cumulative review time of 492 h was spent analyzing the recordings.

Among the videos, 5,978 (17.8%) displayed wild birds, with a range of 0 to 46 visits per day. In total, 27 different species of birds were detected, belonging to 16 taxonomic families: Anatidae, Ardeidae, Columbidae, Corvidae, Falconidae, Fringillidae, Motacillidae, Muscicapidae, Passeridae, Phasianidae, Rallidae, Strigidae, Sturnidae, Threskiornithidae, Turdidae, and Upupidae. The majority of species observed were synanthropic corvids and passerines, with pheasants, pigeons, and doves also being observed, albeit to a lesser extent (Figure 2). Among these observations, there were relatively fewer sightings of aquatic wild birds. The Eurasian magpie (*Pica pica*) was the most frequently observed species in the poultry farm area across all the facilities, with a total of 3,249 recorded observations. It was followed by the Eurasian collared dove (*Streptopelia decaocto*) which was observed 887 times, and the common pheasant (*Phasianus colchicus*), which was observed 589 times. A comprehensive list of the recorded species for each monitored location is provided in Table S1 of Supplementary Materials 2. The number of individuals per species detected per month is illustrated in Figure 2G and further discussed for each bird order in the respective specific sections. The highest diversity of wild bird species

Table 2. Number of videos recorded by each camera trap during the study period.

Farm	Location monitored	Total number of videos recorded (N)	Wild bird counts (% of N)
1	CMan[1](A)	3,863	583 (15)
	PHouse[2] (B)	3,910	225 (5.8)
	FSilo[3] (C)	3,755	684 (18)
2	FSilo (D)	5,072	1,571 (31)
	CMan (E)	8,551	2,446 (28.6)
	Out[4](F)	1,745	22 (1.3)
3	FSilo (G)	2,363	380 (1.6)
	CMan (H)	4,260	69 (1.6)

[1]Chicken manure collection point.
[2]Side of the poultry house, adjacent to air inlets.
[3]Feed silos area.
[4]Outdoor area.

Figure 2. A selection of wild bird species observed in the poultry farms studied (A to F) and overall wild bird visits detected per month (G). (A) Eurasian magpie (*Pica pica*), (B) European jackdaw (*Corvus monedula*), (C) Eurasian-collared dove (*Streptopelia decaocto*), (D) wood pigeon (*Columba palumbus*), (E) wild pheasant (*Phasianus colchicus*), (F) and a male (right) and female (left) mallards (*Anas platyrhynchos*). (G) Number of wild birds per species per month detected by camera traps on the 3 farms studied.

was observed in the chicken manure collection area for Farm 1 (13 species) and Farm 2 (11 species), and nearby feed silos (7 species) for Farm 3.

Corvids and Other Passerines (Passeriformes Order). The wild birds most observed belonged to the Passeriformes order. A total of 14 species were recorded, listed from most to least frequent: Eurasian magpie (3,249 visits), common blackbird (*Turdus merula*; 379 visits), white wagtail (*Motacilla alba*; 310 visits), Western jackdaw (*Corvus monedula*; 103 visits), grey wagtail (*Motacilla cinerea*; 56 visits), Eurasian jay (*Garrulus glandarius*; 46 visits), black redstart (*Phoenicurus ochruros*; 37 visits), Eurasian tree sparrow (*Passer montanus*; 31 visits), common chaffinch (*Fringilla coelebs*; 25 visits), house sparrow (*Passer domesticus*; 18 visits), common starling (*Sturnus vulgaris*; 18 visits), European robin (*Erithacus rubecula*; 14 visits), European greenfinch (*Chloris chloris*; 1 visit) and Western yellow wagtail (*Motacilla flava*; 1 visit). Among the corvids, Eurasian magpies and Western jackdaw were the species most frequently observed. Regarding other passerines, the common blackbird and white wagtail were the 2 most frequent visitors observed in the surroundings of the poultry houses. The activity patterns of these 4 species are presented in Figures 3A−3D. Eurasian magpies were regularly observed from 5:30 am to 7:30 pm, while common blackbirds, white wagtails, and Western jackdaws mostly visited the poultry farms between midday and 6:00 pm.

In terms of the temporal distribution of the visits (Figure 2G), Eurasian magpies were observed throughout the year, common blackbirds were frequently observed during the spring and early summer months, white wagtails were mainly seen from December to March, and Western jackdaws were recorded only from April to May. The predominant behaviors displayed were feeding (46.3% of total observations for common blackbirds, 56% for Eurasian magpies, and 70.9% for Western jackdaws) or moving through the camera's field of view (73.9% for white wagtails) (Figure 4). Furthermore, magpies were observed foraging on eggs fallen from the longitudinal belt of the automated egg transport system to convey eggs from the hen houses to the egg packing plant of Farm 1.

Pigeons, Doves (Columbiformes Order), and Pheasants (Galliformes Order). Among columbids, Eurasian collared doves were regularly present in the surroundings of the poultry houses throughout the year (887 visits), except for the month of June. During the spring migration and breeding season, the migratory European turtle dove (*Streptopelia turtur*; 77 visits) was also seen (Figure 2G). Both species of doves were primarily observed engaging in feeding behavior (51.8% and 51.9% respectively) or moving through the camera's field of view (46.2% and 46.8%) (Figure 4). A small proportion of the recorded footages captured instances of Eurasian collared doves (0.1%) and European turtle doves (1.3%) in the act of defecating. The activity pattern of the doves exhibited consistent occurrences throughout daylight hours for the Eurasian collared dove, while the European turtle dove was predominantly seen in the afternoon, specifically from 4 to 6 pm (Figures 3E−3F). Conversely, sightings of rock pigeons (*Columba livia*) and wood pigeons (*Columba palumbus*) were infrequent (7 and 3 visits, respectively).

The common pheasant stood out as one of the most frequently observed species across all the sites monitored, except for the feed silos area (location G) on Farm 3. Pheasant visits displayed a consistent distribution throughout the entire year, with a notable peak in April (Figure 2G). The observed behaviors primarily encompassed foraging activities (53.4%) and moving through the camera's field of view (44%), while 0.5% of the recorded instances captured pheasants in the act of defecating (Figure 4). The daily activity pattern of pheasants exhibited 2 peaks in counts: a smaller one around sunrise (4:30 am−7:00 am) and a more pronounced one from 4 pm to 8 pm (Figure 3E−3F).

Wild Aquatic Birds (Anseriformes, Gruiformes and Pelecaniformes Orders). Among the farms studied, the presence of wild aquatic birds was primarily evident in Farm 1, situated in close proximity to a fishing sport lake. However, there were also sightings of cattle egrets (*Bubulcus ibis*) in Farm 2. The observed bird species, listed in descending order of frequency (Figure 2G), included mallards (*Anas platyrhynchos*; 49 visits), cattle egrets (8 visits), common moorhens (*Gallinula chloropus*; 5 visits), a grey heron (*Ardea cinerea*; 1 visit) and an African sacred ibis (*Threskiornis aethiopicus*; 1 visit).

Mallards were spotted in Farm 1 during the late winter and breeding season (February to June) across all camera trap locations (A to C), primarily moving through the camera's field of view (87.8% of observations) (Figure 4). Notably, ducklings were observed in late June near camera location B, which is close to the air inlets of a poultry house facing the area adjacent to the fishing sport lake. About 8.2% of the recorded mallard observations showed them foraging near feed silos or drinking from rainwater ponds, with peak observations occurring around 6:00 am and 6:00 pm (Figure 3G).

On the other hand, cattle egret observations were restricted to the chicken manure collection area (location E) in Farm 2. Their behavior was predominantly categorized as either moving through the camera's field of view (62.5%) or feeding (37.5%) in groups (Figure 4), primarily during midday (Figure 3H).

Temporal and Spatial Patterns of Visits The MDRs (95% CI) of total wild bird counts across the 3 farms are presented in Table 3, and these of each wild bird species are in Table S2 of Supplementary Materials 2.

Among the locations, the chicken manure collection point of Farm 2 (location E) exhibited the highest MDR (8.7; 6.6−10.8 95% CI), followed by the feed silos area (location D) (4.6; 2.5−6.6 95% CI), and the feed silos area (location C) of Farm 1 (2.2; 0.1−4.3 95% CI). Different effects of camera trap locations across farms on the number of wild birds' counts resulted in significant farm by locations interactions as shown in GLM results reported in Table 4.

As illustrated in Figure 5A, the 3-month rolling averages of daily wild birds' visits exhibited an increase during late autumn and early winter, reaching its peak in December and January. The spring season displayed the

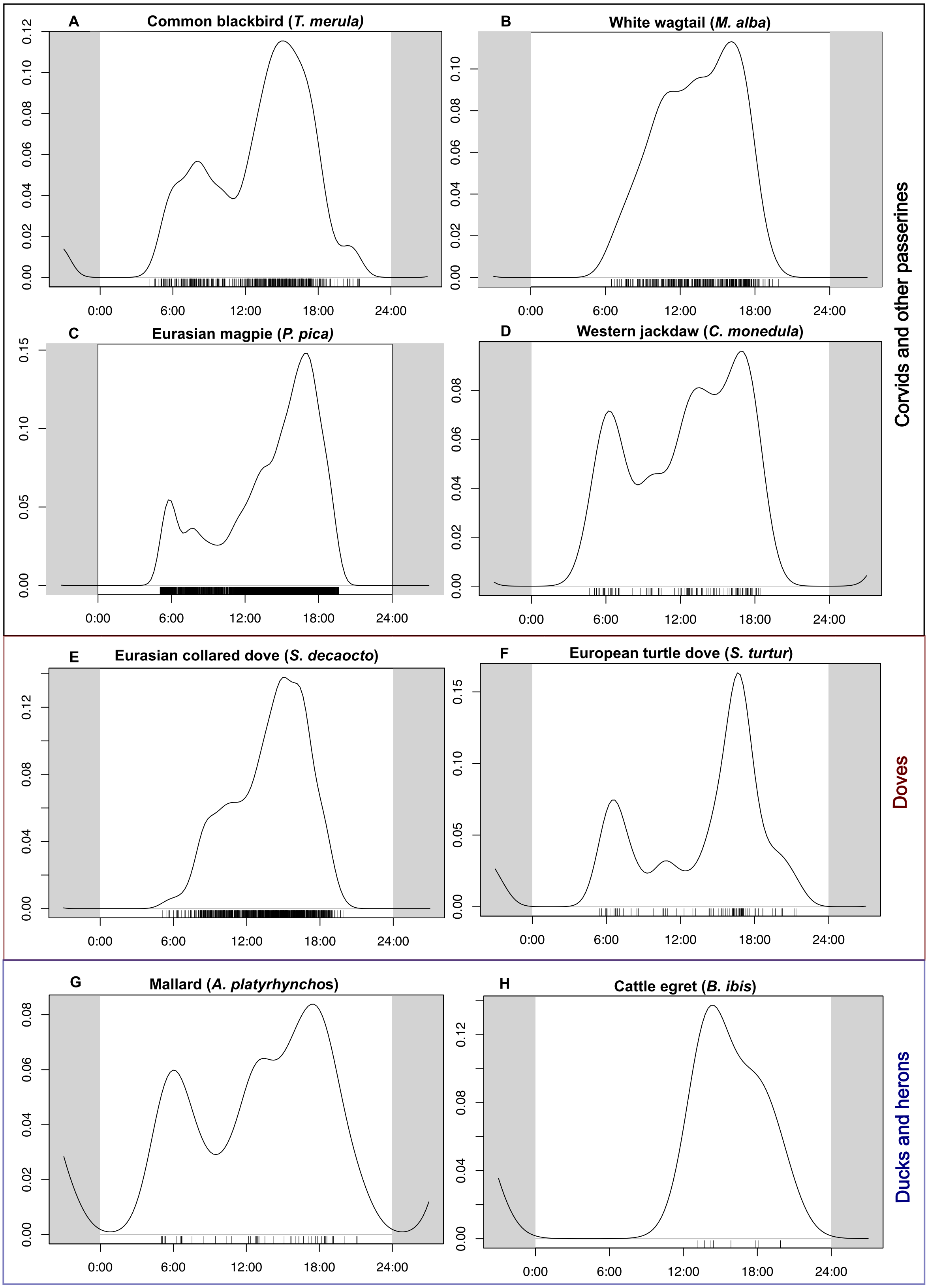

Figure 3. Activity patterns (relative frequency by time) of selected wild bird species visiting the poultry farms studied. (A) Common blackbird (*Turdus merula*), (B) white wagtail (*Motacilla alba*), (C) Eurasian magpie (*Pica pica*), (D) Western jackdaw (*Corvus monedula*), (E) Eurasian collared dove (*Streptopelia decaocto*), (F) European turtle dove (*Streptopelia turtur*), (G) mallard (*Anas platyrhynchos*), and (H) cattle egret (*Bubulcus ibis*).

highest number of consecutive wild bird detections, while the lowest peaks were observed during the summer and autumn months. In the case of Farm 1, the periods with the highest daily visitation rates were spring (20 visits/day) and late autumn/December (12 visits/day) (Figure 5B). For Farm 2 (Figure 5B), notable observation peaks occurred in January 2021 (37 visits/day), December 2021 (30 visits/day), and late summer-early

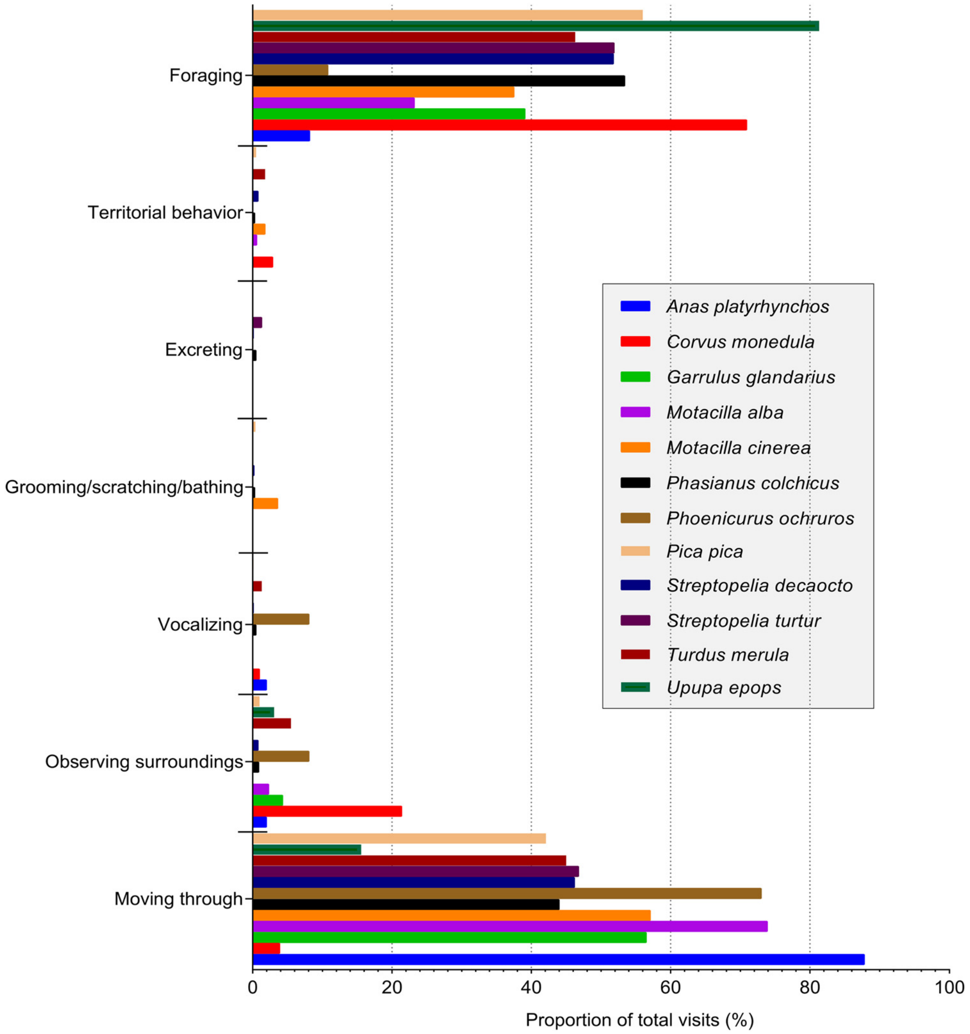

Figure 4. Behaviors displayed by selected wild bird species during the period of study as recorded by camera traps placed in the 3 laying-hen farms.

autumn (~20 visits/day). Despite being monitored for a shorter duration, Farm 3 (Figure 5B) displayed significant peaks in daily wild bird counts during February (11 visits/day) and March (15 visits/day). Overall, Farm 2 consistently recorded a higher frequency of daily wild bird observations compared to Farm 1 and Farm 3.

MDRs and 95% confidence interval for each season are presented in Figure 6A. Farm 1 exhibited the highest average counts during the spring season (MDR 7.4; 5.3 −9.5 95% CI), followed by autumn (3.4; 1.3−5.6), winter (3.4; 1.1−5.6) and summer (2.1; 0−4.3). For Farm 2, the peak mean detection rate occurred in winter (16.1; 14.0 −18.2), followed by summer (10.2; 8.1−12.3), autumn (10; 7.9−12.1) and spring (9.9; 7.9−12.0). Lastly, Farm 3 displayed the highest mean detection rate during winter (6.2; 4.0 − 8.4), followed by spring (4.2; 1.9−6.5), summer (0.2; 0−2.7) and autumn (0.1; 0−3.2). Different seasonality of wild birds' visits between farms was confirmed by significant season by farms interactions in the GLM (Table 4).

The CA results, depicted in Figure 6B, indicate an association between the presence of Passeriformes species and the months of August, September, and December. The presence of wild Galliformes, represented solely

Table 3. Detection rates of wild bird visits on Farm 1, 2, and 3.

Farm	Camera trap days	Overall mean detection rate (95% CI)	Location	Mean detection rate (95% CI)
1	309		CMan[1] (A)	1.9 (0.0 − 4.0)
	257	4.1 (2.0 − 6.2)	PHouse[2] (B)	0.9 (0.0 − 3.1)
	311		FSilo[3] (C)	2.2 (0.1 − 4.3)
2	342	11.6 (9.7 − 13.7)	FSilo (D)	4.6 (2.5 − 6.6)
	281		CMan (E)	8.7 (6.6 − 10.8)
3	211	1.9 (0.0 − 4.1)	FSilo (G)	1.7 (0.0 − 4.0)
	247		CMan (H)	0.3 (0.0 − 2.8)

[1]Chicken manure collection point.
[2]Side of the poultry house, adjacent to air inlets.
[3]Feed silos area.

Table 4. Generalized linear model and explanatory variables considered. The percentage of variation explained (**V.E.**) by fixed terms, the exponentiated estimates (95% CI), and the *P*-value of Wald test for contrasts between the reference level and the level considered are displayed.

V.E.	Explanatory variable and levels		Rate ratio (95% CI)	*P*-value
42.6%	Farm monitored[1]	Farm 1	0.22 (0.18–0.27)	<0.001**
		Farm 3	0.08 (0.05–0.11)	<0.001**
	Camera trap location[2]	Feed silos area	0.51 (0.42–0.61)	<0.001**
	Season[3]	Season 0	1.13 (0.71–1.08)	0.225
	Farm monitored × Camera trap location[4]	Farm 1 × Feed silos area	2.39 (1.88–3.04)	<0.001**
		Farm 3 × Feed silos area	10.8 (7.24 - 16.13)	<0.001**
	Farm monitored × Season[5]	Farm 1 × Season 0	0.75 (0.58–0.95)	<0.05*
		Farm 3 × Season 0	0.04 (0.02–0.07)	<0.001**
	Camera trap location × Season[6]	Feed silos area × Season 0	1.09 (0.89–1.34)	0.446

[1]Farm 2 taken as a reference.
[2]Chicken manure collection point as a reference.
[3]Season 1 (winter and spring seasons) taken as a reference; season 0 refers to summer and autumn grouped together.
[4]Farm 2 and Chicken manure collection point as reference.
[5]Farm 2 and Season 1 (winter and spring seasons) as reference.
[6]Chicken manure collection point and Season 1 (winter and spring seasons) as reference.
*$P \leq 0.05$.
**$P \leq 0.001$.

by pheasants, was linked to a higher number of visits in April and June. Additionally, the Columbiformes showed a connection to the month of March.

The GLM of the association between wild bird visits and the monitored farm, camera trap location, and the season, successfully converged and accounted for 42.6% of the data deviance based on the pseudo R^2 (Zuur et al., 2009). A summary of the results is provided in Table 4. The significant interaction effects have already been presented above; a non-significant camera trap location by season interaction was obtained.

Wild Birds in the Free-Range Area The camera trap located in the outdoor area of Farm 2 (location F) was operational from July 2021 to mid-October 2021, running from 6 pm to 6 am during 88 camera trap-days. Throughout this timeframe, a total of 1,745 videoclips were captured. Among these, 1,711 videos captured the presence of poultry, which accessed the area between 5:30 pm and 8:30 pm daily or every 2 d,

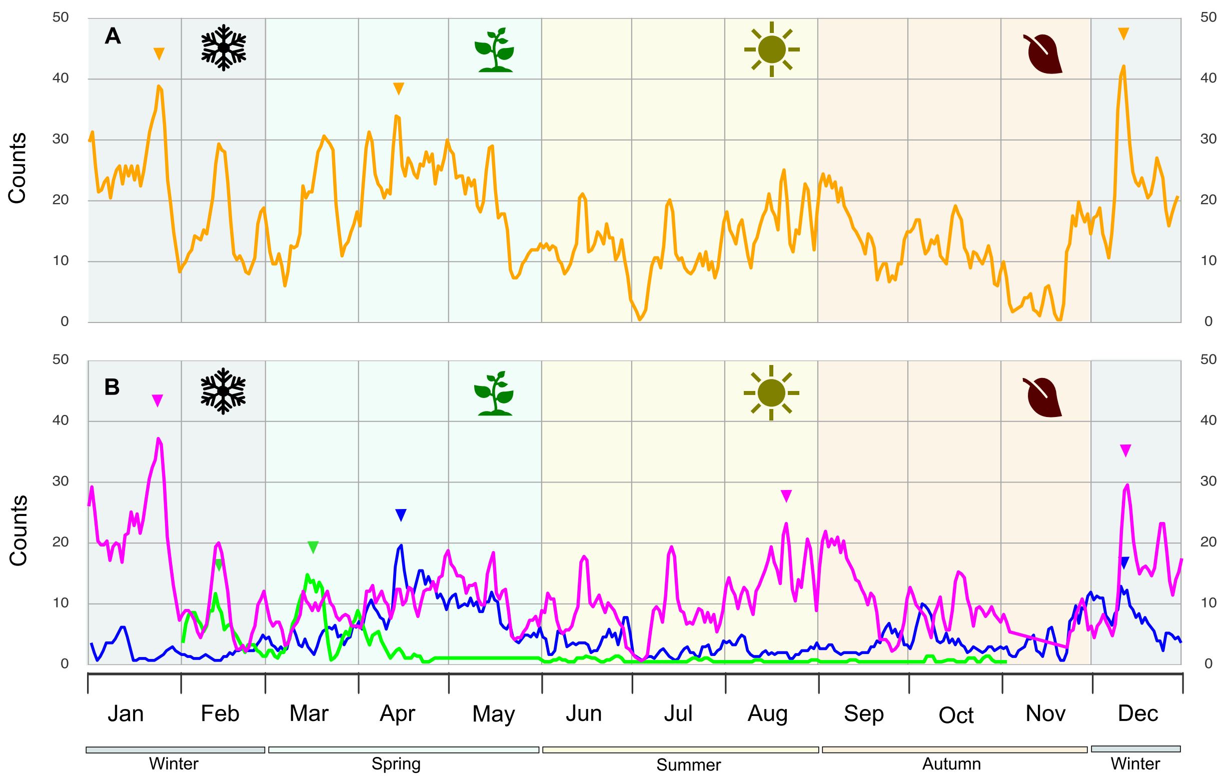

Figure 5. Three-month rolling averages of daily wild birds' visits throughout the period studied (camera traps at location A to E, G, and H). (A) Overall daily counts for Farms 1, 2 and 3; (B) Daily counts displayed separately for the 3 farms (Farm 1, light blue line; Farm 2, magenta line; Farm 3, green line). Color-coded arrows indicate peaks of wild bird observations.

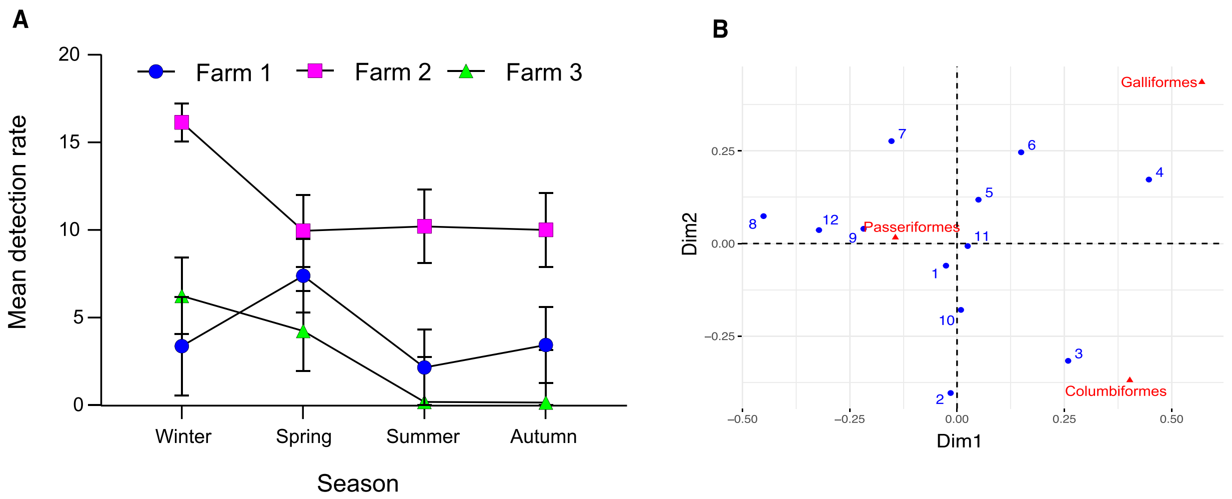

Figure 6. Seasonality of wild birds' visits to the studied laying-hen farms. (A) Seasonal mean detection rates of daily counts of wild birds visiting the 3 poultry farms studied. Farm 1, blue circle; Farm 2, magenta square; Farm 3, green triangle. (B) Correspondence analysis plot between Passeriformes, Galliformes, and Columbiformes and months of the year.

depending on weather conditions. Occasionally, a small group of 2 to 3 hens remained outdoors during the night. Instances of wild bird detection numbered 21 (1.2% of the total number of recordings) and an ICR of 0.5 (0−3.0 95% CI) was calculated over the period of 88 d monitored. The most frequently observed species included the house sparrow and the common pheasant, both observed 7 times. The Eurasian magpie was spotted 6 times, while the common chaffinch 5 times. The Eurasian collared dove appeared 3 times, while the Eurasian hoopoe (*Upupa epops*), wood pigeon, and common blackbird were observed 2, 1, and 1 time(s) respectively.

Behaviors exhibited by these species primarily involved moving through the camera field and foraging, as depicted in Figure 7. Specifically, wood pigeons (100%), house sparrows (100%), Eurasian magpies (60%), and Eurasian collared doves (100%) were predominantly engaged in foraging activities.

Despite the camera trap's primary activity during the period when laying hens were indoors, a total of 8 instances of co-occurrence between wild birds and poultry were recorded, constituting 38% of the observed wild bird visits in the outdoor area. Notably, 3 instances were documented where Eurasian magpies were observed foraging in the meadow. Additionally, 2 separate recordings

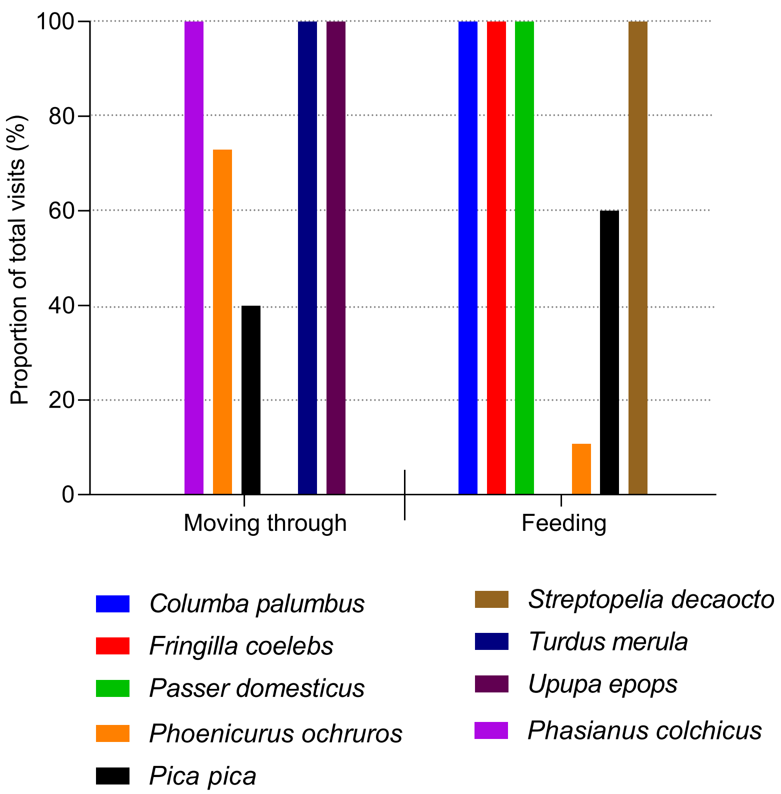

Figure 7. Behaviors displayed by wild birds in the outdoor area of Farm 2 as recorded by the camera trap.

captured common chaffinches engaging in foraging activities within the area. One footage depicted a common pheasant moving through the camera's field of view, while another clip recorded a Eurasian dove foraging. Lastly, occurrences were noted where both common pheasants and Eurasian doves were observed moving through the camera's field of view and engaging in foraging, respectively.

DISCUSSION

A year-long camera-trap study was conducted on 3 laying-hen farms located in a DPPA with a high risk of AIV introduction from wild birds. Through individual observations of the footages recorded, the diversity of wild bird species and the quantification and characterization of their visits were achieved. A total of 5,978 visits from wild birds of 16 taxonomic families were recorded. The Farm 2 (free-range type) showed a higher number of visits, recorded by camera traps at feed silos areas and chicken manure collection points, compared to the ones recorded in conventional Farms 1 and 3. Potential factors influencing this variation could be related to the farm-type characteristics, such as the eutrophication of the soil in the outdoor area of the free-range farm due to laying hens' defecation activity (Zoli et al., 2023), to anthropogenic practices affecting wild bird presence, such as the cleanliness of the farm area (Hinsley et al., 1995), or to environmental factors (Bestman et al., 2018), such as surrounding landscape characteristics. Furthermore, the interaction effect between the monitored farm and the camera trap location significantly impacted wild bird counts, highlighting the importance of studying the domestic-wild bird interface in different contexts. Seasons also appeared to play a role in wild bird presence, with fewer visits in Farm 1 and 3 during summer and autumn, in contrast to increased visits in Farm 2 during winter and spring. Increased feeding requirements of wild species during the breeding spring period and colder months could favor bird presence within farm areas (Kuenzel et al., 1999). Given that camera locations near feed silos and chicken manure collection points predominantly captured wild birds during foraging, this underscored the attractiveness of these sites to wild species. The feed silos area, subjected to feed spills, and the chicken manure collection point, with uncleaned litter rich in organic material and prone to insects' colonization (Retamales et al., 2011), offer direct food sources, thus attracting both granivorous and omnivorous/carnivorous birds (Scott et al., 2018). Despite Farms 2 and 3 being in close proximity to lowland areas with nearby irrigation canals, no waterfowl species have been sighted. In contrast, mallards were observed in Farm 1, situated near a fishing sport lake. This aligned with findings from previous studies (Elbers et al., 2020; Velkers et al., 2021), that documented the presence of wild aquatic birds around farms located near water-rich areas. With respect to the seasonal pattern of mallards' visits, these were solely noted during spring, with peaks of observations at sunrise and sunset. Mallards were observed in pairs or with their offspring. Additionally, 8.2% of the recorded instances in this study captured mallards foraging near feed silos or drinking from rainwater ponds. The appeal of in-farm water ponds, formed after a prolonged period of rains, was found to be decisive for observing dabbling ducks during the autumn/winter period in a poultry farm in the Netherlands (Elbers et al., 2020). Since waterfowl tend to exhibit strong site fidelity even toward livestock facilities (McDuie et al., 2022), it becomes crucial to minimize the likelihood of attracting these species near poultry houses through regular cleaning of the farm area and preventing the accumulation of rainwater. Additionally, other wild aquatic birds were sporadically identified in Farm 1, including a grey heron and an African sacred ibis, each observed once, likely due to farm proximity to the fishing sport lake where these species used to reside.

The wild bird species most frequently observed in this survey were synanthropic corvids, specifically Eurasian magpies and Western jackdaws, along with small passerines like white wagtails and common blackbirds. Foraging activities were prevalent, with Eurasian magpies even observed foraging on eggs fallen from the automated egg-transport system. With respect to AI infection, corvids have tested positive for HPAI viral detection linked to outbreaks in poultry farms (Shriner et al., 2020), possibly due to their synanthropic behavior and scavenging habits. Both Eurasian magpies and Western jackdaws are susceptible to HPAI infection (Department for Environment Food & Rural Affairs and Animal and Plant Health Agency, 2022), with Eurasian magpies even included in the target list of wild birds for passive surveillance of H5 HPAI viruses (European Food Safety Authority et al., 2017). Moreover, immunohistochemistry analyses of the digestive tracts of deceased HPAI H5N8-naturally infected Eurasian magpies revealed widespread virus antigen presence, suggesting cloacal virus shedding in the environment (Caliendo et al., 2020). The frequency of farm visits by Passeriformes birds strongly and positively correlated with the months of September and December. This coincides with the period when migratory wild ducks, known AIV reservoirs, populate nearby wetlands (Spina et al., 2008b). Given susceptibility to AIV infection, coupled with their frequent activity on poultry farms and nonmigratory habits (Spina et al., 2008a), magpies and jackdaws could potentially serve as bridge hosts (Caron et al., 2014) in the local-scale AIV epidemiology. In the case of other passerines, various species have been demonstrated to be susceptible to natural (Gronesova et al., 2008; Peterson et al., 2008; Slusher et al., 2014; European Food Safety Authority et al., 2022a), or experimental AIV infection (Brown et al., 2009; Fujimoto et al., 2015). Since both white wagtails and common blackbirds have been commonly observed also in other European poultry farms (Veen et al., 2007; Elbers et al., 2020; Le Gall-Ladevèze et al., 2022), their specific role in the context of HPAI virus epidemiology requires further investigation.

Pheasants and doves were frequently reported in the monitored farms. Pheasants' visits were positively associated with the spring months (April, May, and June). A study by Tammiranta et al. (2023) highlighted how mass mortalities in HPAI H5N1-infected wild pheasants were spatially and temporally linked to AIV cases in non-human wild mammals, illustrating rapid disease spread among these birds. Due to their territorial behavior, small home range (maximum 4.5 km) (Turner, 2007), and high susceptibility to HPAI infection (Brookes et al., 2022), pheasants might therefore act as AI bridge hosts, particularly in DPPAs. Given their limited flight ability, strategies such as fencing the farm area, coupled with regular maintenance on fences, could prevent pheasants' access to poultry farms. Lastly, columbids have been proved susceptible to HPAIV infection, with varying clinical outcomes based on the virus considered (Perkins et al., 2002; Abolnik, 2014; Liu et al., 2020; Peters et al., 2022). Experimental studies with different AIV subtypes (H5N8 LPAI, H5N8 HPAI, H5N2, H4N6) or human influenza virus (H7N9) (Shriner et al., 2016; Kwon et al., 2017; Uchida et al., 2017; Bosco-Lauth et al., 2019) infections in pigeons resulted in limited or absent viral shedding, with no transmission to contact individuals. In contrast, an experimental infection of pigeons with H5N6 HPAIVs of clade 2.3.4.4b revealed a high viral shedding from the oropharynx and cloaca (Liu et al., 2020). Eurasian collared doves were the most frequently observed columbid species in this camera trap survey, with their presence strongly associated with the month of April. While their susceptibility to H5Nx HPAIV strains has not been studied experimentally yet, deceased individuals tested during AI surveillance activities in Europe resulted positive for HPAI infection (Capua et al., 2000; Istituto Zooprofilattico Sperimentale delle Venezie, 2023). Considering the frequent presence of doves nearby poultry houses, further research is recommended to clarify their actual role in the transmission of currently circulating HPAI viruses at the interface between domestic and wild birds.

Regarding the camera placed in the free-range area of the organic farm (Farm 2), 1.2% of the collected recordings featured wild birds, yielding a mean ICR of 0.5 (0−3.0 95% CI). The species most frequently observed in the free-range setting were small passerines like house sparrows and common chaffinches, along with Eurasian magpies, aligning with previous observations (Veen et al., 2007; Elbers et al., 2020). Furthermore, 38% of the wild bird recordings displayed the concurrent presence of wild species, particularly Eurasian magpies, alongside the laying hens. This underscored the heightened risk of AIV introduction via wild birds in organic or free-range farms, given the increased potential for contacts between infectious wild birds and domestic poultry (Bouwstra et al., 2017; Holt, 2021). Hence, it is crucial to diminish the attractiveness of free-range areas to wild birds. Achieving this may involve regular inspections of the outdoor space, swift removal of chicken carcasses and eggs, and implementation of measures to discourage wild bird habituation (Elbers et al., 2020). The use of cover panels and netted sides in the outdoor space could serve a dual purpose: preventing wild bird access to the area and providing enrichment to encourage hens to explore a broader part of the range. Indeed, hens tend to congregate in a limited area nearby the poultry house if supplementary artificial shelters or vegetation are absent (Zeltner et al., 2003; Gilani et al., 2014).

Effective biosecurity farming practices hinge not only on the implementation of established management protocols but also on the farmers' awareness of the consequences of improper behaviors on pathogens' introduction and transmission to domestic birds (Hinjoy et al., 2023). The utilization of visual evidence depicting wild birds around poultry houses, such as the footages recorded through camera traps, could facilitate health authorities in educating and raising awareness between stakeholders regarding wild bird presence and potential pathogen spillover opportunities. Several limitations inherent to this study setting should be acknowledged. Primarily, the camera-trap monitoring encompassed a limited number of poultry farms and sites, possibly limiting the generalizability of the conclusions to other farm settings. Additionally, the placement of cameras in areas of heightened human activity (e.g., feed silos; chicken manure collection points) resulted in numerous non-relevant triggers, contributing to battery depletion and camera trap failures, thereby reducing the camera trap effort over the study period, a phenomenon documented in other studies as well (Bacigalupo et al., 2022; Engeman et al., 2011). Lastly, for a comprehensive grasp of the factors influencing wild bird activity on poultry farms, future research should encompass additional variables such as habitat characteristics and environmental factors, aspects that were not considered in the models presented within this study.

Overall, the data hereby provided regarding the characteristics of the poultry-wildlife interface lay the groundwork for designing novel surveillance and intervention strategies to reduce risks of cross-species disease transmission (Hassell et al., 2017). Considering the absence of data on AIV infection among the birds observed in this study, the exact role of specific wild bird species in the AI epidemiology at the wild-domestic bird interface cannot be concluded. Among the wild birds more frequently observed, the assessment of their susceptibility to infection with currently circulating HPAI viruses and of their infectivity, are necessary steps to fully evaluate their actual role as bridge hosts (Caron et al., 2014). Given the continuous evolution of HPAIV through genetic drift and reassortment events between different subtypes and genotypes, potentially giving rise to strains with novel biological traits (Lycett et al., 2019; European Food Safety Authority et al., 2023d), it is paramount for farmers to be cognizant of the presence of potentially high-risk wild birds around poultry houses. Coupled with the diligent application and enforcement of biosecurity measures, this awareness stands as one of the most effective preventive measures to avert spillover or spillback at the interface between domestic and wild bird populations.

ACKNOWLEDGMENTS

The authors are grateful to the directors and staff of the Eurovo Group for granting permits for the camera trap survey in their poultry farms and providing logistical support.

This research was partially supported by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT) to E.C. The PhD grant of G.G. was funded by the Local Public Health Unit of Imola (A.U.S.L di Imola) through the "Fondo per Emergenza Avicola" (Decreto Ministero della Salute 14 marzo 2018) assigned to the Emilia-Romagna region to develop innovative programs for Avian influenza surveillance and prevention in poultry farms.

Data Availability: The data used to support the findings of this study are included within the article and in the Supplementary Materials 1 and Supplementary Materials 2.

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2024.103892.

REFERENCES

Abolnik, C. 2014. A current review of avian influenza in pigeons and doves (Columbidae). Vet. Microbiol. 170:181–196.

Ayala, A. J., M. J. Yabsley, and S. M. Hernandez. 2020. A review of pathogen transmission at the backyard chicken-wild bird interface. Front. Vet. Sci. 7:539925, doi:10.3389/fvets.2020.539925.

Bacigalupo, S. A., L. K. Dixon, S. Gubbins, A. J. Kucharski, and J. A. Drewe. 2022. Wild boar visits to commercial pig farms in southwest England: implications for disease transmission. Eur. J. Wildl. Res. 68:69.

Bestman, M., W. de Jong, J.-P. Wagenaar, and T. Weerts. 2018. Presence of avian influenza risk birds in and around poultry free-range areas in relation to range vegetation and openness of surrounding landscape. Agrof. Syst. 92:1001–1008.

Bosco-Lauth, A. M., N. L. Marlenee, A. E. Hartwig, R. A. Bowen, and J. J. Root. 2019. Shedding of clade 2.3.4.4 H5N8 and H5N2 highly pathogenic avian influenza viruses in peridomestic wild birds in the U.S. Transbound. Emerg. Dis. 66:1301–1305.

Bouwstra, R., J. Gonzales, S. de Wit, J. Stahl, R. A. M. Fouchier, and A. R. W. Elbers. 2017. Risk for low pathogenicity avian influenza virus on poultry farms, the Netherlands, 2007−2013. Emerg. Infect. Dis. 23:1510.

Brookes, S. M., K. L. Mansfield, S. M. Reid, V. Coward, C. Warren, J. Seekings, T. Brough, D. Gray, A. Núñez, and I. H. Brown. 2022. Incursion of H5N8 high pathogenicity avian influenza virus (HPAIV) into gamebirds in England. Epidemiol. Infect. 150:e51.

Brown, H., and R. Prescott. 2014. Applied Mixed Models in Medicine. John Wiley & Sons, West Sussex, United Kingdom.

Brown, J. D., D. E. Stallknecht, R. D. Berghaus, and D. E. Swayne. 2009. Infectious and lethal doses of H5N1 highly pathogenic avian influenza virus for house sparrows (*Passer domesticus*) and rock pigeons (*Columbia livia*). J. Vet. Diagn. Invest. 21:437–445.

Caliendo, V., L. Leijten, L. Begeman, M. J. Poen, R. A. M. Fouchier, N. Beerens, and T. Kuiken. 2020. Enterotropism of highly pathogenic avian influenza virus H5N8 from the 2016/2017 epidemic in some wild bird species. Vet. Res. 51:117.

Caliendo, V., N. S. Lewis, A. Pohlmann, S. R. Baillie, A. C. Banyard, M. Beer, I. H. Brown, R. A. M. Fouchier, R. D. E. Hansen, T. K. Lameris, A. S. Lang, S. Laurendeau, O. Lung, G. Robertson, H. van der Jeugd, T. N. Alkie, K. Thorup, M. L. van Toor, J. Waldenström, C. Yason, T. Kuiken, and Y. Berhane. 2022. Transatlantic spread of highly pathogenic avian influenza H5N1 by wild birds from Europe to North America in 2021. Sci. Rep. 12:11729.

Capua, I., B. Grossele, E. Bertoli, and P. Cordioli. 2000. Monitoring for highly pathogenic avian influenza in wild birds in Italy. Vet. Rec. 147:640.

Caron, A., V. Grosbois, E. Etter, N. Gaidet, and M. de Garine-Wichatitsky. 2014. Bridge hosts for avian influenza viruses at the wildlife/domestic interface: an eco-epidemiological framework implemented in southern Africa. Prev. Vet. Med. 117:590–600.

Cleaveland, S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 356:991–999.

Craft, M. E. 2015. Infectious disease transmission and contact networks in wildlife and livestock. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 370:20140107.

Daszak, P. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. Science 287:443–449.

Department for Environment Food & Rural Affairs, and Animal and Plant Health Agency 2022. Highly pathogenic avian influenza (HPAI) in the UK and Europe. Update outbreak assessment #23. Accessed Oct. 2023. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1070246/Highly_pathogenic_avian_influenza__HPAI__in_the_UK_and_Europe__outbreak_update_23.pdf.

Elbers, A. R. W., and J. L. Gonzales. 2020. Quantification of visits of wild fauna to a commercial free-range layer farm in the Netherlands located in an avian influenza hot-spot area assessed by video-camera monitoring. Transbound. Emerg. Dis. 67:661–677.

Engeman, R., C. Betsill, and T. Ray. 2011. Making contact: rooting out the potential for exposure of commercial production swine facilities to feral swine in North Carolina. EcoHealth 8:76–81.

European Food Safety Authority. 2022aAznar, I., F. Baldinelli, A. Stoicescu, and L. Kohnle. 2022a. 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.

European Food Safety Authority, European Centre for Disease Prevention and Control, European Union Reference Laboratory for Avian Influenza. 2022bAdlhoch, C., A. Fusaro, J. L. Gonzales, T. Kuiken, S. Marangon, E. Niqueux, C. Staubach, C. Terregino, I. Aznar, I. M. Guajardo, and F. Baldinelli. 2022b. Avian influenza overview March - June 2022. EFSA J 20:e07415.

European Food Safety Authority, European Centre for Disease Prevention and Control, European Union Reference Laboratory for Avian Influenza. 2023aAdlhoch, C., A. Fusaro, J. L. Gonzales, T. Kuiken, S. Marangon, E. Niqueux, C. Staubach, C. Terregino, I. Aznar, I. M. Guajardo, and F. Baldinelli. 2023a. Avian influenza overview September - December 2022. EFSA J 21:e07786.

European Food Safety Authority, European Centre for Disease Prevention and Control, European Union Reference Laboratory for Avian Influenza. 2022cAdlhoch, C., A. Fusaro, J. L. Gonzales, T. Kuiken, S. Marangon, E. Niqueux, C. Staubach, C. Terregino, I. Aznar, I. M. Guajardo, and F. Baldinelli. 2022c. Avian influenza overview May - September 2021. EFSA J 20:e07122.

European Food Safety Authority, European Centre for Disease Prevention and Control, European Union Reference Laboratory for Avian Influenza. 2023bAdlhoch, C., A. Fusaro, J. L. Gonzales, T. Kuiken, G. Mirinaviciute, E. Niqueux, K. Stahl, C. Staubach,

C. Terregino, A. Broglia, and F. Baldinelli. 2023b. Avian influenza overview March - April 2023. EFSA J 21:45.

European Food Safety Authority, European Centre for Disease Prevention and Control, European Union Reference Laboratory for Avian Influenza. 2017Brown, I., T. Kuiken, P. Mulatti, K. Smietanka, C. Staubach, D. Stroud, O. R. Therkildsen, P. Willeberg, F. Baldinelli, F. Verdonck, and C. Adlhoch. 2017. Avian influenza overview September - November 2017. EFSA J 15:e05141.

European Food Safety Authority, European Centre for Disease Prevention and Control, European Union Reference Laboratory for Avian Influenza. 2023cAdlhoch, C., A. Fusaro, J. L. Gonzales, T. Kuiken, S. Marangon, G. Mirinaviciute, E. Niqueux, K. Stahl, C. Staubach, C. Terregino, A. Broglia, and F. Baldinelli. 2023c. Avian influenza overview December 2022 - March 2023. EFSA J 21:e07917.

European Food Safety Authority, European Centre for Disease Prevention and Control, European Union Reference Laboratory for Avian Influenza. 2022dAdlhoch, C., A. Fusaro, J. L. Gonzales, T. Kuiken, S. Marangon, E. Niqueux, C. Staubach, C. Terregino, I. Aznar, I. M. Guajardo, and F. Baldinelli. 2022d. Avian influenza overview December 2021 - March 2022. EFSA J 20:e07289.

European Food Safety Authority, European Centre for Disease Prevention Control, European Union Reference Laboratory for Avian Influenza. 2023dAdlhoch, C., A. Fusaro, J. L. Gonzales, T. Kuiken, A. Melidou, G. Mirinavičiūtė, É. Niqueux, K. Ståhl, C. Staubach, C. Terregino, F. Baldinelli, A. Broglia, and L. Kohnle. 2023d. Avian influenza overview April − June 2023. EFSA J 21:e08191.

Franklin, A. B., S. N. Bevins, and S. A. Shriner. 2021. Pathogens from wild birds at the wildlife−agriculture interface. Pages 207−228 in Infectious Disease Ecology of Wild Birds.

Fujimoto, Y., T. Usui, H. Ito, E. Ono, and T. Ito. 2015. Susceptibility of wild passerines to subtype H5N1 highly pathogenic avian influenza viruses. Avian Pathol 44:243–247.

Gilani, A. M., T. G. Knowles, and C. J. Nicol. 2014. Factors affecting ranging behaviour in young and adult laying hens. Br. Poult. Sci. 55:127–135.

Gortázar, C., E. Ferroglio, U. Höfle, K. Frölich, and J. Vicente. 2007. Diseases shared between wildlife and livestock: a European perspective. Eur. J. Wildl. Res. 53:241–256.

Graziosi, G., E. Catelli, A. Fanelli, and C. Lupini. 2022a. Infectious bursal disease virus in free-living wild birds: a systematic review and meta-analysis of its sero-viroprevalence on a global scale. Transbound. Emerg. Dis. 69:2800–2815.

Graziosi, G., C. Lupini, and E. Catelli. 2022b. Disentangling the role of wild birds in avian metapneumovirus (aMPV) epidemiology: a systematic review and meta-analysis. Transbound. Emerg. Dis. 69:3285–3299.

Greenberg, S., and T. Godin. 2012. Technical Report 2012-1028-11,.

Gronesova, P., P. Kabat, A. Trnka, and T. Betakova. 2008. Using nested RT-PCR analyses to determine the prevalence of avian influenza viruses in passerines in western Slovakia, during summer 2007. Scand. J. Infect. Dis. 40:954–957.

Hassell, J. M., M. Begon, M. J. Ward, and E. M. Fevre. 2017. Urbanization and disease emergence: dynamics at the wildlife-livestock-human interface. Trends. Ecol. Evol. 32:55–67.

Hinjoy, S., P. Thumrin, J. Sridet, C. Chaiyaso, P. Smithsuwan, J. Rodchangphuen, Y. Thukngamdee, and W. Suddee. 2023. Risk perceptions of avian influenza among poultry farmers on smallholder farms along border areas of Thailand. Front. Vet. Sci. 10:1075308.

Hinsley, S. A., P. E. Bellamy, I. Newton, and T. H. Sparks. 1995. Habitat and landscape factors influencing the presence of individual breeding bird species in woodland fragments. J. Avian Biol. 26:94–104.

Holt, P. S. 2021. Centennial review: a revisiting of hen welfare and egg safety consequences of mandatory outdoor access for organic egg production. Poult. Sci. J. 100:101436.

Istituto Zooprofilattico Sperimentale delle Venezie. 2018. Influenza aviaria: situazione epidemiologica in Italia − HPAI (2016/2018). Accessed Aug. 2023. https://www.izsvenezie.it/temi/malattie-patogeni/influenza-aviaria/situazione-epidemiologica-hpai/

Istituto Zooprofilattico Sperimentale delle Venezie. 2023. Highly pathogenic avian influenza in birds other than poultry in Italy. Epidemiological situation 20 July 2023. Accessed Sept. 2023. https://www.izsvenezie.com/documents/reference-laboratories/avian-influenza/italy-updates/HPAI/2021-1/italy-wildbirds.pdf

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature 451:990–993, doi:10.1038/nature06536.

Kuenzel, W. J., M. M. Beck, and R. Teruyama. 1999. Neural sites and pathways regulating food intake in birds: a comparative analysis to mammalian systems. J. Exp. Zool. 283:348–364.

Kuiken, T., R. A. M. Fouchier, and M. P. G. Koopmans. 2023. Being ready for the next influenza pandemic? Lancet Infect. Dis. 23:398–399.

Kwon, J. H., Y. K. Noh, D. H. Lee, S. S. Yuk, T. O. Erdene-Ochir, J. Y. Noh, W. T. Hong, J. H. Jeong, S. Jeong, G. B. Gwon, C. S. Song, and S. S. Nahm. 2017. Experimental infection with highly pathogenic H5N8 avian influenza viruses in the Mandarin duck (*Aix galericulata*) and domestic pigeon (*Columba livia domestica*). Vet. Microbiol. 203:95–102.

Le Gall-Ladevèze, C., C. Guinat, P. Fievet, B. Vollot, J. L. Guérin, J. Cappelle, and G. Le Loc'h. 2022. Quantification and characterisation of commensal wild birds and their interactions with domestic ducks on a free-range farm in southwest France. Sci. Rep. 12:9764.

Liu, K., R. Gao, X. Wang, W. Han, Z. Ji, H. Zheng, M. Gu, J. Hu, X. Liu, S. Hu, S. Chen, S. Gao, D. Peng, X. A. Jiao, and X. Liu. 2020. Pathogenicity and transmissibility of clade 2.3.4.4 highly pathogenic avian influenza virus subtype H5N6 in pigeons. Vet. Microbiol. 247:108776.

Lloyd-Smith, J. O., D. George, K. M. Pepin, V. E. Pitzer, J. R. Pulliam, A. P. Dobson, P. J. Hudson, and B. T. Grenfell. 2009. Epidemic dynamics at the human-animal interface. Science 326:1362–1367.

Lycett, S. J., F. Duchatel, and P. Digard. 2019. A brief history of bird flu. Philos. Transact. Royal Soc. B 374:20180257.

McDuie, F., E. L. Matchett, D. J. Prosser, J. Y. Takekawa, M. E. Pitesky, A. A. Lorenz, M. M. McCuen, O. C. T. J. T. Ackerman, S. E. W. De La Cruz, and M. L. Casazza. 2022. Pathways for avian influenza virus spread: GPS reveals wild waterfowl in commercial livestock facilities and connectivity with the natural wetland landscape. Transbound. Emerg. Dis. 69:2898–2912.

Miller, E. H. 1988. Description of bird behavior for comparative purposes. Pages 347−394 in Current Ornithology. V. Nolan and E. D. Ketterson, eds. Springer, Bloomington.

Niedballa, J., R. Sollmann, A. Courtiol, and A. Wilting. 2016. camtrapR: an R package for efficient camera trap data management. Methods Ecol. Evol. 7:1457–1462.

Payne, A., S. Chappa, J. Hars, B. Dufour, and E. Gilot-Fromont. 2016. Wildlife visits to farm facilities assessed by camera traps in a bovine tuberculosis-infected area in France. Eur. J. Wildl. Res. 62:33–42.

Perkins, L. E., and D. E. Swayne. 2002. Pathogenicity of a Hong Kong-origin H5N1 highly pathogenic avian influenza virus for emus, geese, ducks, and pigeons. Avian. Dis. 46:53–63.

Peters, M., J. King, P. Wohlsein, C. Grund, and T. Harder. 2022. Genuine lethal infection of a wood pigeon (*Columba palumbus*) with high pathogenicity avian influenza H5N1, clade 2.3.4.4b, in Germany, 2022. Vet. Microbiol. 270:109461.

Peterson, A. T., S. E. Bush, E. Spackman, D. E. Swayne, and H. S. Ip. 2008. Influenza A virus infections in land birds, People's Republic of China. Emerg. Infect. Dis. 14:1644–1646.

Randler, C., and N. Kalb. 2018. Distance and size matters: a comparison of six wildlife camera traps and their usefulness for wild birds. Ecol. Evol. 8:7151–7163.

Repubblica Italiana. 2018. Decreto Legislativo 10 agosto 2018, n. 101. Gazz. Uff. 2018:2–5.

Retamales, J., F. Vivallo, and J. Robeson. 2011. Insects associated with chicken manure in a breeder poultry farm of Central Chile. Arch. Med. Vet. 43:79–83.

Root, J., and S. Shriner. 2020. Avian influenza A virus associations in wild, terrestrial mammals: a review of potential synanthropic vectors to poultry facilities. Viruses 12.

Scott, A. B., D. Phalen, M. Hernandez-Jover, M. Singh, P. Groves, and J. Toribio. 2018. Wildlife presence and interactions with chickens on Australian commercial chicken farms assessed by camera traps. Avian Dis 62:65–72.

Shriner, S. A., and J. J. Root. 2020. A review of avian influenza A virus associations in synanthropic birds. Viruses 12:1209.

Shriner, S. A., J. J. Root, N. L. Mooers, J. W. Ellis, S. R. Stopak, H. J. Sullivan, K. K. VanDalen, and A. B. Franklin. 2016. Susceptibility of rock doves to low-pathogenic avian influenza A viruses. Arch. Virol. 161:715–720, doi:10.1007/s00705-015-2685-7.

Slusher, M. J., B. R. Wilcox, M. P. Lutrell, R. L. Poulson, J. D. Brown, M. J. Yabsley, and D. E. Stallknecht. 2014. Are passering birds reservoirs for influenza A viruses? J. Wildl. Dis. 50:792–809.

Spina, F., and S. Volponi. 2008a. Atlante della migrazione degli uccelli in italia: 2. Passeriformi. Ministero dell'Ambiente e della Tutela del Territorio e del Mare. Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Roma, Italy. Accessed Jun. 2023 https://www.isprambiente.gov.it/it/pubblicazioni/pubblicazioni-di-pregio/atlante-della-migrazione-degli-uccelli-in-italia.

Spina, F., and S. Volponi. 2008b. Atlante della migrazione degli uccelli in italia: Non-passeriformi. Ministero dell'Ambiente e della Tutela del Territorio e del Mare. Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Roma, Italy. Accessed Jun. 2023 https://www.isprambiente.gov.it/it/pubblicazioni/pubblicazioni-di-pregio/atlante-della-migrazione-degli-uccelli-in-italia.

Tammiranta, N., M. Isomursu, A. Fusaro, M. Nylund, T. Nokireki, E. Giussani, B. Zecchin, C. Terregino, and T. Gadd. 2023. Highly pathogenic avian influenza A (H5N1) virus infections in wild carnivores connected to mass mortalities of pheasants in Finland. Infect. Genet. Evol. 111:105423.

Tucciarone, C. M., G. Franzo, M. Legnardi, D. Pasotto, C. Lupini, E. Catelli, G. Quaglia, G. Graziosi, E. Dal Molin, F. Gobbo, and M. Cecchinato. 2022. Molecular Survey on A, B, C and New Avian Metapneumovirus (aMPV) subtypes in wild birds of Northern-Central Italy. Vet. Sci. 9:373.

Turner, C. V. 2007. PhD Diss. Imp. College, London.

Uchida, Y., K. Kanehira, N. Takemae, H. Hikono, and T. Saito. 2017. Susceptibility of chickens, quail, and pigeons to an H7N9 human influenza virus and subsequent egg-passaged strains. Arch. Virol. 162:103–116.

Vanwambeke, S. O., C. Linard, and M. Gilbert. 2019. Emerging challenges of infectious diseases as a feature of land systems. Curr. Opin. Environ. Sustain. 38:31–36.

Veen, J., J. Brouwer, P. Atkinson, C. Bilgin, J. Blew, S. Eksioglu, M. Hoffmann, R. Nardelli, F. Spina, C. Tendi, and S. Delany. 2007. Ornithological data relevant to the spread of Avian Influenza in Europe (phase 2): further identification and first field assessment of Higher Risk Species. Accessed Jun. 2023. https://www.wetlands.org/publication/ornithological-data-relevant-to-the-spread-of-avian-influenza-in-europe-phase-2/.

Velkers, F. C., T. T. M. Manders, J. C. M. Vernooij, J. Stahl, R. Slaterus, and J. A. Stegeman. 2021. Association of wild bird densities around poultry farms with the risk of highly pathogenic avian influenza virus subtype H5N8 outbreaks in the Netherlands, 2016. Transbound. Emerg. Dis 68:76–87.

Verhagen, J. H., R. A. M. Fouchier, and N. Lewis. 2021. Highly pathogenic avian influenza viruses at the wild-domestic bird interface in Europe: future directions for research and surveillance. Viruses 13:212.

Wearn, O. R., and P. Glover-Kapfer. 2017. Camera-Trapping for Conservation: A Guide to Best-Practices. WWF-UK, Woking, United Kingdom.

Webster, R. G., W. J. Bean, O. T. Gorman, T. M. Chambers, and Y. Kawaoka. 1992. Evolution and ecology of influenza A viruses. Microbiol. Rev. 56:152–179.

Wiethoelter, A. K., D. Beltran-Alcrudo, R. Kock, and S. M. Mor. 2015. Global trends in infectious diseases at the wildlife-livestock interface. Proc. Natl. Acad. Sci. U.S.A. 112:9662–9667.

Zeileis, A., G. Grothendieck, J. A. Ryan, F. Andrews, and M. A. Zeileis. 2014. Package 'zoo'. R package version:1.7-12.

Zeltner, E., and H. Hirt. 2003. Effect of artificial structuring on the use of laying hen runs in a free-range system. Br. Poult. Sci. 44:533–537.

Zoli, M., P. Mantovi, P. Ferrari, L. Ferrari, and V. Ferrante. 2023. Soil organic matter and nutrient levels in outdoor runs in organic laying farms. Animals 13:401.

Zuur, A. F., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York, NY.