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Potential biosecurity breaches in poultry farms: Presence of free-ranging mammals near laying-hen houses assessed through a camera-trap study

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ABSTRACT

Diligent application and implementation of biosecurity measures stand as the most effective measures to prevent disease transmission through direct or indirect interactions between poultry and free-ranging animals. Among these, free-ranging mammals can be hosts or disseminators of several pathogens relevant to poultry and of public health concern. Moreover, evidence of susceptibility to avian influenza virus infection in non-human mammals has raised questions about their potential role in the virus' epidemiology at the domestic animal-wildlife interface. Given this background, this study aimed to identify mammal species occurring near laying-hen houses and characterize the spatiotemporal patterns of these visits. Seven camera traps were deployed for a year-long period in three commercial poultry farms in a densely populated poultry area in Northern Italy. Various methods, including time series analysis and generalized linear models, were employed to analyze daily mammal visits. A total of 1,867 camera trap nights yielded 567 videos of seven species of wild mammals, and 1,866 videos showed domestic pet species (cats and dogs). Coypus (*Myocastor coypus*) and cats were the two mammals more frequently observed near poultry houses. For wild mammals, visits significantly increased at night, and slightly decreased during the spring season. Overall, the data hereby provided lay the groundwork for designing novel surveillance and intervention strategies to prevent cross-species disease transmission. Moreover, the utilization of visual evidence depicting free-ranging animals approaching poultry houses could assist health authorities in educating and raising awareness among stakeholders about potential risks of pathogen spillover.

1. Introduction

The risk of transmission of infectious diseases in intensive poultry farming, amplified by factors such as densely populated poultry areas and poor management of the flocks, represents a continuous challenge for birds' health and welfare Hagenaars, Boender, Bergevoet & van Roermund (2018); Muir et al. (2008); Van Limbergen et al. (2020). Furthermore, direct and indirect interactions between wild and farmed animals can mediate pathogens' spillover or spillback at the domestic animal-wildlife interface Wiethoelter, Beltran-Alcrudo, Kock & Mor (2015). Farm biosecurity stands as one of the most effective tools for mitigating the risk of introduction and spread of diseases within and in-between farms Robertson (2020). Among these diseases, highly pathogenic avian influenza (HPAI) has devastating consequences to the poultry industry in multiple countries. Since 2020, an unprecedented number of outbreaks due to the HPAI H5Nx viruses of clade 2.3.4.4b has been reported worldwide, following the 2021 intercontinental spread of this Eurasian lineage to the American continent Bevins et al. (2022); Caliendo et al. (2022); Leguia et al. (2023). A high number of HPAI infections in wild birds has also been observed in the same period (EFSA et al., 2023a), with mortality events and die-offs reported for a broad range of bird species Kleyheeg et al. (2017); Rijks et al. (2022). Besides this, HPAI H5Nx virus infections of clade 2.3.4.4b have also affected both wild and domestic mammals in Europe, United States of America, Canada, South America, and Japan EFSA et al. (2023b); APHIS (2023). The majority of the affected species belonged to the Carnivora order, coming into contact with naturally infected wild birds or poultry through a predator-prey relationship. This is the case for both terrestrial

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and aquatic wild mammals Bordes et al. (2023); Floyd et al. (2021); Plaza et al. (2024); Leguia et al. (2023); Murawski et al., 2024; Puryear et al. (2023); Rijks et al. (2022); Shin et al. (2019); Tammiranta et al. (2023); Thorsson et al. (2023). Farmed wild species raised for fur production in Europe were also found infected Agüero et al. (2023); Lindh et al. (2023). Recent HPAI H5N1 virus detections in cats and dogs prompted further concerns public health, due to their close contacts with humans (EFSA et al., 2023a). Overall, evidence of susceptibility to infection has raised concerns about the potential involvement of wild or domestic mammals in the epidemiology of avian influenza viruses (AIVs) in or near poultry farms Root & Shriner (2020). Given the rising number of HPAI detections in mammals, an increased passive surveillance in wild and free-roaming domestic carnivores has been therefore recommended EFSA et al. (2023a). Additionally, knowledge gained from a deeper understanding of the domestic bird-wildlife interface could enhance the selection of targeted mammal species to be included in epidemiological surveys of AI and mammalian-borne poultry diseases. This, in turn, could contribute to the development of more effective disease surveillance and prevention strategies Barasona, VerCauteren, Saklou, Gortazar & Vicente (2013). Notably, free-ranging mammals have been primarily recognized as hosts or disseminators of other pathogens relevant to poultry, particularly bacteria. Among these, virulent avian serovars of *Pasteurella multocida*, the causative agent of fowl cholera, were isolated in a number of healthy wild mammals captured in turkey farms' premises Snipes et al. (1988). Zoonotic agents such as *Salmonella* or *Leptospira* have also been isolated from rodents sampled in chicken farms Domańska-Blicharz, Opolska, Lisowska & Szczotka-Bochniarz (2023); Manabella Salcedo et al. (2021).

Previous research on the characterization of the poultry-wildlife interface has primarily concentrated on wild bird data, combining field observations and camera trap recordings Burns et al. (2012); Elbers & Gonzales (2020); Le Gall-Ladevèze et al. (2022); Martelli et al. (2023); Veen et al. (2007). As results, rodents and wild carnivores have been predominantly reported among mammals Elbers & Gonzales (2020); Scott et al. (2018).

This study aimed to characterize visits of domestic and wild mammal to three commercial poultry facilities in Northern Italy, through a yearlong camera trap survey. Specifically, the research focused on identifying the species frequenting the vicinity of the poultry houses and describing their behavior and detection patterns over time, as a preliminary step to identify potential disease transmission routes between free-ranging mammals and poultry.

2. Materials and methods

2.1. Camera trap survey

The study area was set in the Bologna province, Emilia-Romagna region, within a densely populated poultry area at high risk of HPAI introduction 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 of AI introduction and higher risk of AI spread according to the DGSAF protocol number 29,049 dated November 20, 2019, https://www.trovanorme.salute.gov.it/norme/dettaglioAtto?id=71728). The selected area also borders the Argenta valleys of the Delta Po Regional Park, among the largest freshwater wetlands in northern Italy.

Three commercial layer farms, namely Farm 1, Farm 2 and Farm 3, were selected at random 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. The assessment involved conducting short interviews with farmers, making direct observations of wildlife, and identifying indirect signs such as tracks and scats. Prior permission was obtained from the owner to install camera-traps before commencing the study.

Farm 1 and Farm 3 were two conventional in-door aviaries, housing approximately 130,000 and 1.4 million hens, respectively. Farm 2 was an organic layer farm that housed 140,000 hens with outdoor spaces accessible to the animals. In these outdoor areas, there were no poultry feeding points, and water was sheltered to prevent access by wild birds. The three facilities were surrounded by fences; however, several breaches were noticed. Pest-control through rodenticide baits was routinely applied. Water channels, arable fields and, in case of Farm 1, a fishing sport lake, were located in the vicinity of the farms (< 500 m).

Seven motion-sensing infrared digital cameras (Dark-Ops Pro XD Dual Lens, Browning Trail Cameras, Utah, U.S.A.) were deployed in the three above-mentioned facilities at sites where signs of wildlife were observed, within the farms' boundaries. Cameras were set to operate from 6 a.m. – 6 a.m. and programmed to record 30-second-long videos after detecting movement, with a lag period of 30 s to avoid continuous triggers. Cameras were deployed across the three farms (Fig. 1) at feed silos area (FSilo) (location C, D, F) and chicken manure collection point (CMan) (location A, E, G). Drainage ditches were located nearby location G, within the camera's field of view. On Farm 1 an additional camera was placed near the air inlets of a poultry house (PHouse) (location B), to monitor the area adjacent to the fishing sport lake. On Farm 1 and 2, the study ran from January 2021 – December 2021, whereas on Farm 3 from February 2021 – November 2021. Standard camera-trap survey's guidelines were followed to set up the study Wearn & Glover-Kapfer (2017). An average distance of 30 cm between the camera sensor and the ground was applied, in order to detect small to medium-sized mammals (McCallum, 2013; Molyneux, Pavey, James & Carthew, 2017), and the camera vertical angle was perpendicular to the ground surface. Prior to deployment, time and date displayed on the camera traps were synchronized, and a unique code was assigned to identify each camera location ID. Throughout the study, camera traps' batteries and SD cards were replaced every three weeks. The operations performed, such as setup, battery and SD card replacements, and malfunctions, were recorded in a data sheet using Microsoft Excel 2021, version 16.49.

2.2. Data processing and analysis

This study exclusively considered videos capturing wild or domestic mammals, while wild bird visits were characterized elsewhere (Graziosi et al., 2024). Visual analysis of the recordings was conducted using the Timelapse Image Analyzer (Greenberg & Godin, 2012) by two authors independently (G.G. and F.D.F.). If a disagreement in species identification occurred, the visual analysis was conducted jointly and resolved.

A wild or domestic mammal visit was defined as an observation of at least one individual of a given species within a 30-minute time interval from previous observation of the same species. Duplicate footages, defined as recordings of the same species and number of animals within 30 min from the previous detection, were removed from the analysis to avoid probable detection of the same individual Payne, Chappa, Hars, Dufour & Gilot-Fromont (2016); Scott et al. (2018). Clips featuring humans were removed and excluded from further analysis, following current privacy regulations. Metadata extraction, data visualization and time-series analysis were performed utilizing R software version 4.0.4. Team (2020). The 'camtrapR' package was used for dataset exploration and computation of activity patterns (relative frequency by time) of wild mammals overall observed $\geq$ 40 times. Daily mean detection rates (MDR) of visits were calculated based on data collected from three farms over a year, with 95 % confidence intervals (95 % CI) computed using the 'poisson.test' function. Daily visit counts were transformed into a time-series object, and seasonal patterns were analyzed by examining three-month rolling averages through the 'zoo' package (version 1.8–12) Zeileis, Grothendieck, Ryan, Andrews & Zeileis (2014). For wild mammals, observed actions were categorized as reported in Table 1 (Kappeler, 2021; Varela-Castro, Sevilla, Payne, Gilot-Fromont & Barral, 2021), and the occurrence percentages of each behavior concerning the

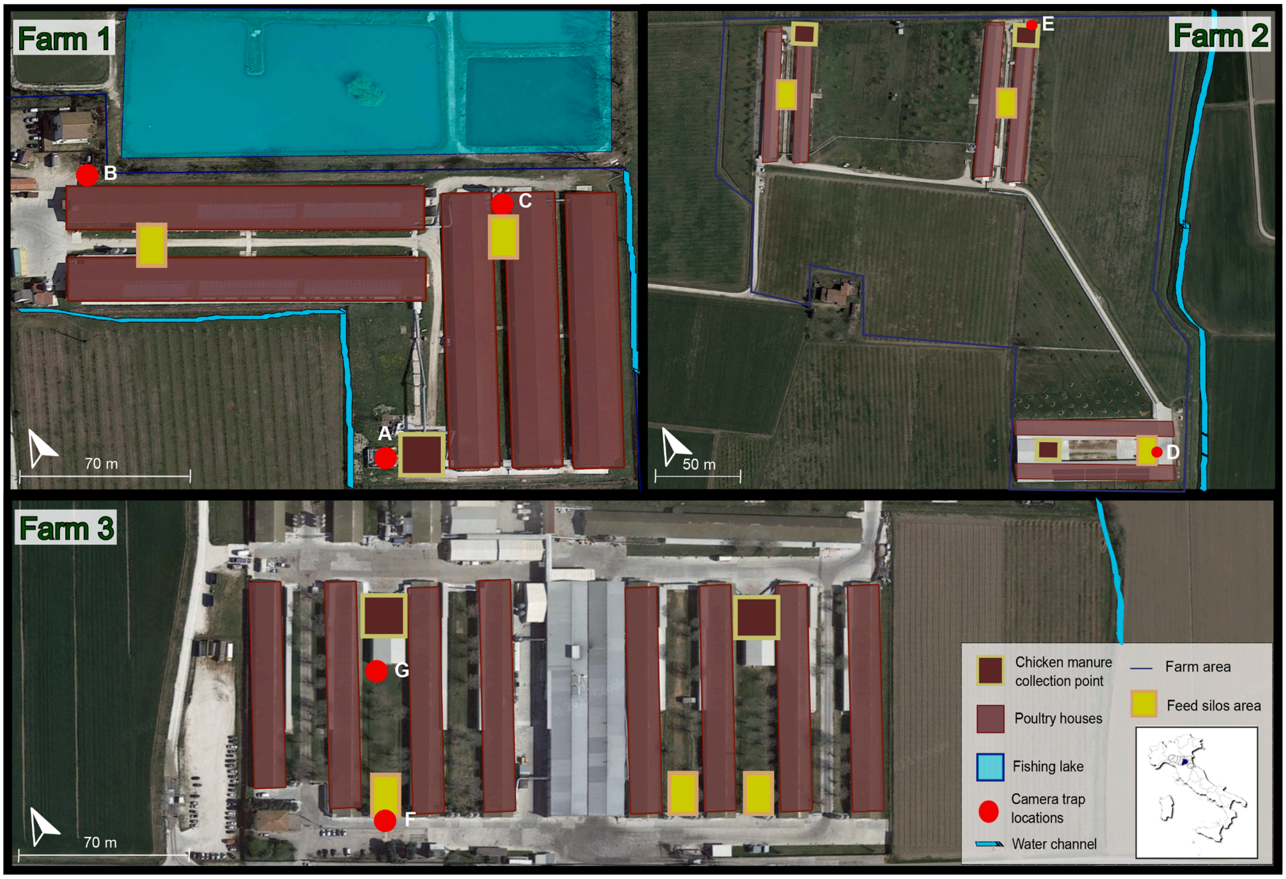

Fig. 1. Satellite images of Farm 1, 2, and 3. The red circles represent the camera trap locations (A to G). Poultry houses, chicken manure collection points, feed silos, and presence of waterways are shown. Map data: Google Earth Pro (2022).

Table 1
Categorization of behaviors exhibited by wild mammals observed in the three poultry farms monitored through camera-traps.

Behavior	Description
Moving through	Passing from one side to another of the camera fields, without exhibiting other behaviors
Observing surroundings	Explorative behavior expressed as observing surroundings
Grooming/ scratching	Applying paws to the body in repetitive movements, scratching
Excreting	Urinating or defecating
Foraging	Eating or drinking
Territorial behavior	Attacking or charging an intruder of the same species or other species

total visits of a species were calculated.

Data recorded by different cameras were considered as independent. Prior to further analysis, data from each camera trap were aggregated based on date, monitored farm, season, and time of the day (Day: from 6.00 a.m. – 5:59 p.m; Sunset: from 6 p.m. – 8:59 p.m.; Night: from 9 p.m. – 5:59 a.m) using the 'aggregate' function. Normality testing using the Shapiro-Wilk test revealed a non-normal distribution in the aggregated dataset Shapiro & Wilk (1965). To assess potential variations in mammal visits among farms, camera trap locations (chicken manure collection point and feed silos area), seasons, and time of the day, a Kruskal-Wallis test was employed. Generalized linear models (GLMs) utilizing a Poisson distribution, implemented via the 'MASS' package ('glm' function), were employed to explore the link between daily wild mammal visit frequencies and several predictor variables such as: monitored farm (Farm 1, 2 or 3), location of the camera traps (CMan or FSilo), season (defined as spring from March 1st, summer from June 1st, autumn from September 1st, and winter from December 1st), and time of the day (night, day, sunset).

3. Results

3.1. Overview of the survey

During the study period, the camera traps were operational for a combined total of 877 trap days on Farm 1, with a monthly camera trap effort ranging from 20 – 26 trap days. On Farm 2, there were 532 trap days, with a monthly camera trap effort varying from 17 – 24 trap days. Farm 3 had 458 trap days, with monthly camera trap effort ranging from 17 – 31 trap days. In total, 31,774 recordings were captured, as detailed in Table 2, requiring a cumulative review time of 442 h for individual

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

Farm	Location monitored	Total days surveyed	Total number of videos recorded (N)	Wild mammals (% of N)	Domestic mammals (% of N)
1	CMan[a] (A)	309	3863	296 (7.6)	101 (2.6)
	PHouse[b] (B)	267	3910	11 (0.3)	125 (3.2)
	FSilo[&] (C)	301	3755	72 (1.9)	256 (6.8)
2	FSilo (D)	301	5072	53 (1.0)	28 (0.5)
	CMan (E)	231	8551	13 (0.15)	24 (0.3)
3	FSilo (F)	211	2363	37 (1.56)	224 (9.5)
	CMan (G)	247	4260	85 (2.0)	1108 (26)

[a] Chicken manure collection point.
[b] Side of the poultry house, adjacent to air inlets.
[c] Feed silos area.

analysis.

Among the recorded videos, 567 (1.8 %) featured wild mammals with a daily visit range of 0–31, while 1866 videos (5.8 %) showed domestic mammals, with an overall daily visit range of 0–99. Seven wild mammal species were identified during the study period (Fig. 2A). These were ranked in descending order of frequency: coypu (*Myocastor coypus* (Molina, 1782); 437 visits), European hedgehog (*Erinaceus europaeus* Linnaeus, 1758; 57 visits), rats (*Rattus* spp.; 56 visits), European hare (*Lepus europaeus* Pallas, 1778; 7 visits), red fox (*Vulpes vulpes* (Linnaeus, 1758); 5 visits), mice (*Apodemus* spp. or *Mus* spp.; 4 visits) and beech marten (*Martes foina* (Erxleben, 1777); 1 visit). Examining the temporal pattern of the three wild mammals more frequently detected by cameras (Fig. 2B), coypus were observed year-round, rats during autumn and winter, and European hedgehogs were predominantly observed from July to October. The remaining wild mammals were sporadically observed in different months. Regarding domestic mammals, cats were more frequently recorded than dogs (1815 times *versus* 51, 97 % of observations), with visits spanning the entire study year. Both cats and dogs were feral. Notably, the majority of cat sightings occurred on Farm 3, constituting 73.4 % of all cat observations, attributed to the presence of a feral cat colony.

The behavior most commonly exhibited by wild mammals was moving through the camera's field of view (88.5 % of all observations). Feeding behavior was specifically noted in coypus (43 % of observations), mice (2 %), and European hedgehogs (2 %) (Fig. 3). Among these, coypus, being herbivores, directed their feeding behavior towards terrestrial vegetation in meadow areas within the cameras' range. Additionally, coypus were observed engaging in ingesting fecal matter (coprophagia), as evidenced by a video reported in Supplementary Material 1.

3.2. Temporal and spatial patterns of observations

Wild mammals were predominantly sighted at the chicken manure collection area (location A) of Farm 1 (296 visits), followed by the chicken manure collection area (location G) of Farm 3 (85 visits) and the silos area (location C) of Farm 1 (72 visits). The silos area of Farm 3 (location G) had the highest number of visits (1108) of domestic mammals, succeeded by the silos area (location C) of Farm 2 (256 visits) and the silos area (location F) of Farm 3 (224 visits). The daily mean detection rate (MDR) of wild mammal visits across the three farms was 0.30 (95 % CI: 0.28–0.33) (Table 3), while for domestic mammals was 1.0 (95 % CI: 0.95–1.04) (Table 4). The number of mammal visits significantly varied between farms (Kruskal-Wallis χ^2= 127.36, df = 2, $p < 0.001$) and between cameras located at silos or chicken manure collection areas (Kruskal-Wallis χ^2= 75.284, df = 1, $p < 0.001$). Among the three most frequently observed wild mammal species, the visits of coypus did not differ significantly between farms but did vary significantly based on the camera trap locations (Kruskal-Wallis $\chi^2 = 34.97$, df = 1, $p < 0.001$), season (Kruskal-Wallis $\chi^2 = 12.295$, df = 3, $p = 0.006$), and time of the day (Kruskal-Wallis $\chi^2 = 20.059$, df = 2, $p < 0.001$). Observations of rats and hedgehogs did not show significant differences regarding farms, locations, season, or time.

Being coypus, rats, hedgehogs, cats and dogs more frequently observed, the characteristics of their visits to the farm facilities are summarized in Table 5.

The three-month rolling averages displayed in Fig. 4A showed a notable increase in daily domestic mammal visits during winter, reaching its maximum in February. Conversely, the spring, summer, and autumn seasons exhibited a lower average number of detections. For wild mammals, the rolling averages of daily visits of coypus, rats and hedgehogs are provided in Fig. 4B.

Being these three wild species observed ≥ 40 times, their daily activity patterns are presented in Fig. 5 (5A-5C). Coypus were observed consistently throughout the day, with a slight decrease in frequency around mid-day. Hedgehogs and rats, being nocturnal species, were observed exclusively from 9:00 p.m. – 7:00 a.m.

The frequency of wild mammal visits was statistically analyzed in relation to the monitored farm, camera trap location, season, and time of day. The presence of domestic mammals on poultry farms, which was predominantly associated with cat colonies and feral dogs, was not included in the modeling. The fitted model accounted for 14.1 % of the

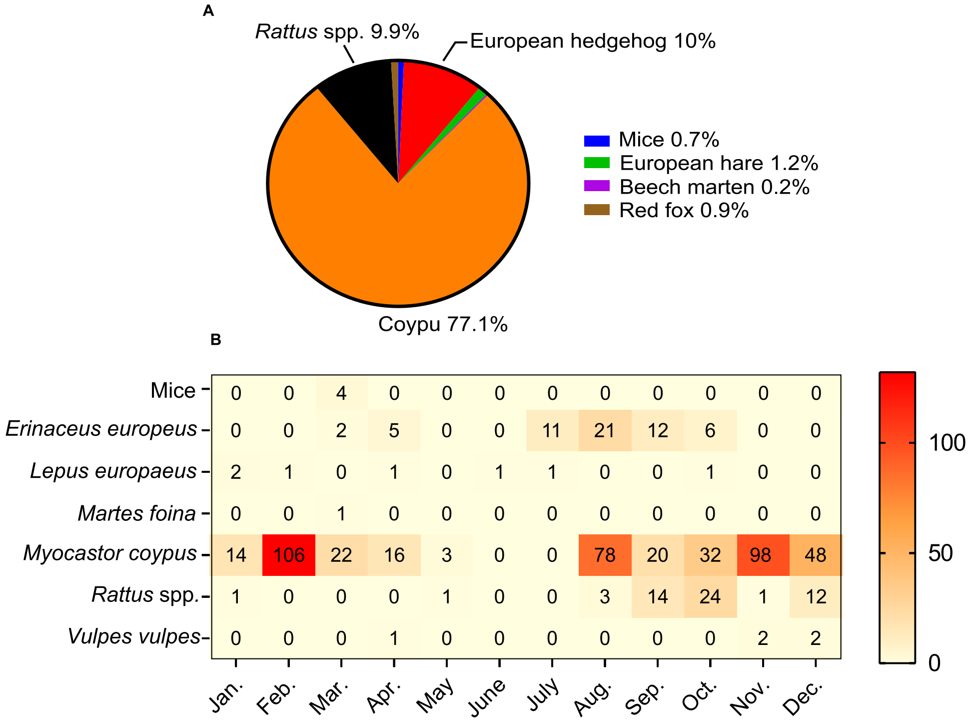

Fig. 2. Overview of wild mammal species observed in the studied poultry farms. (A) Pie chart of wild mammal species; (B) number of wild mammals observed per month.

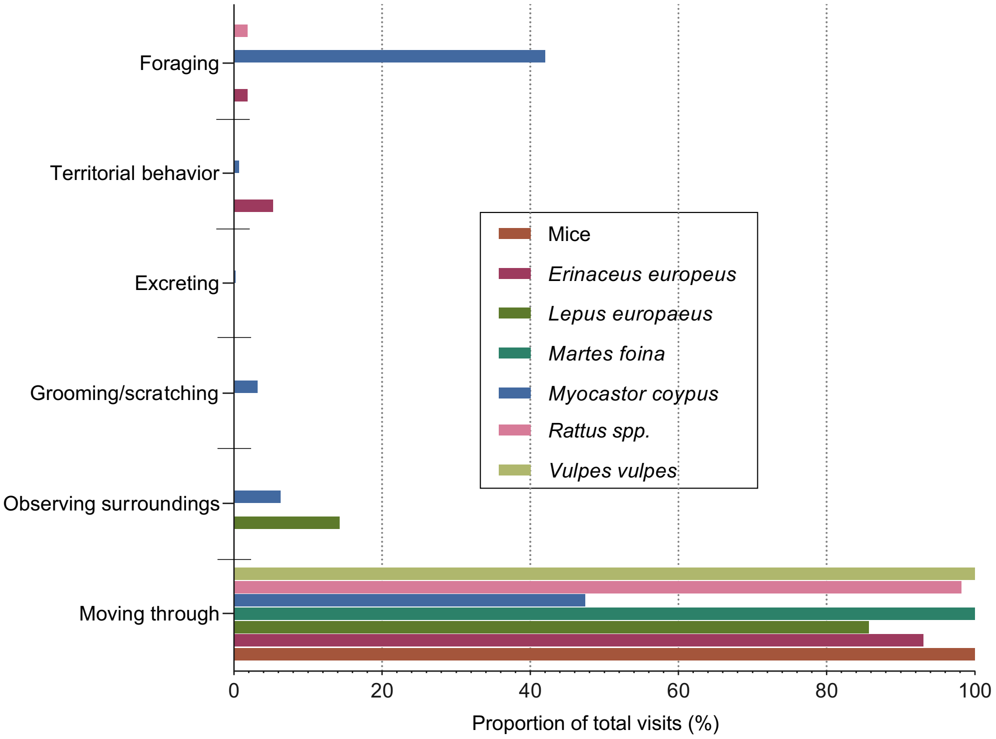

Fig. 3. Behaviors displayed by wild mammals as recorded in the three studied poultry farms.

Table 3
Detection rates (mean number of visits in the three farms over a period of one year) and 95 % confidence intervals (95 % CI) of wild mammals' 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	0.4 (0.4 – 0.5)	CMan (A)	0.9 (0.8 – 1.1)
	267		PHouse (B)	0.04 (0.02 – 0.1)
	301		FSilo (C)	0.2 (0.2 – 0.3)
2	301	0.2 (0.16 – 0.23)	FSilo (D)	0.2 (0.1 – 0.2)
	231		CMan (E)	0.05 (0.03 – 1.0)
3	211	0.2 (0.15 – 0.23)	FSilo (F)	0.2 (0.1 – 0.2)
	247		CMan (G)	0.3 (0.3 – 0.4)

Table 4
Detection rates (mean number of visits in the three farms over a period of one year) and 95 % confidence intervals (95 % CI) of domestic mammals' 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	0.55 (0.5 – 0.6)	CMan (A)	0.3 (0.3 – 0.4)
	267		PHouse (B)	0.5 (0.4 – 0.55)
	301		FSilo (C)	0.85 (0.75 – 1.0)
2	301	0.1 (0.1 – 0.1)	FSilo (D)	0.1 (0.06 – 0.13)
	231		CMan (E)	0.1 (0.06 – 0.15)
3	211	3.0 (2.75 – 3.1)	FSilo (F)	1.1 (0.9 – 1.2)
	247		CMan (G)	4.5 (4.2 – 4.8)

Table 5
Summary of the characteristics of visits of coypus, rats, hedgehogs, cats, and dogs in the three studied poultry farms.

	Coypus	Rats	Hedgehogs	Cats	Dogs
Daily mean detection rate (95% CI)	0.23 (0.21 −0.26)	0.03 (0.02 – 0.04)	0.03 (0.02 – 0.04)	0.97 (0.93 – 1.02)	0.03 (0.02 – 0.03)
Mean number of individuals per visit (range)	1.1 (1–6)	1.05 (1–2)	1.0 (1)	1.0 (1)	1.0 (1)
Favorite season	Winter	Autumn	Summer	n.a.[a]	n.a.
Favorite site	CMan	FSilo	CMan	FSilo	PHouse

[a] n.a. not applicable – the favorite season variable was not considered for domestic mammals, as their presence in poultry farms was not attributable to natural events.

data variance based, as indicated by the pseudo R^2, and showed an overdispersion value of 0.93 (Zuur, Ieno, Walker, Saveliev & Smith, 2009). A summary of the results is provided in Table 6. Specifically, the feed silos area displayed a significantly lower number of wild mammal visits compared to the chicken manure collection point (RR: 0.58, 95 % CI: 0.46 - 0.73, $p < 0.001$). When comparing the number of visits during spring to the number of visits during autumn, the observations during spring were 0.64 times lower (0.45 - 0.91, 95 % CI: $p = 0.01$). Lastly, observations during nighttime were 1.35 times higher than during the daytime (95 % CI: 1.04 - 1.76, $p < 0.05$).

4. Discussion

A yearlong camera-trap study was conducted on three laying-hen farms located in a densely populated poultry area. As a result, a total of 567 wild mammal visits and 1866 cat and dog visits were recorded during 1867 camera trap days. The GLM's results showed that daily observations of wild mammals were significantly related to the location of the camera traps, time of the day and season. With respect to the cameras' locations, the chicken manure collection points were positively related to the frequency of visits. As this location was situated near perimeter areas (Farm 1 and Farm 2) or close to drainage ditches (Farm 3), the animals observed moving through the camera's field of view were likely engaged in further exploration of the farm area or seeking shelter. Visits increased at night, primarily due to the nocturnal activity

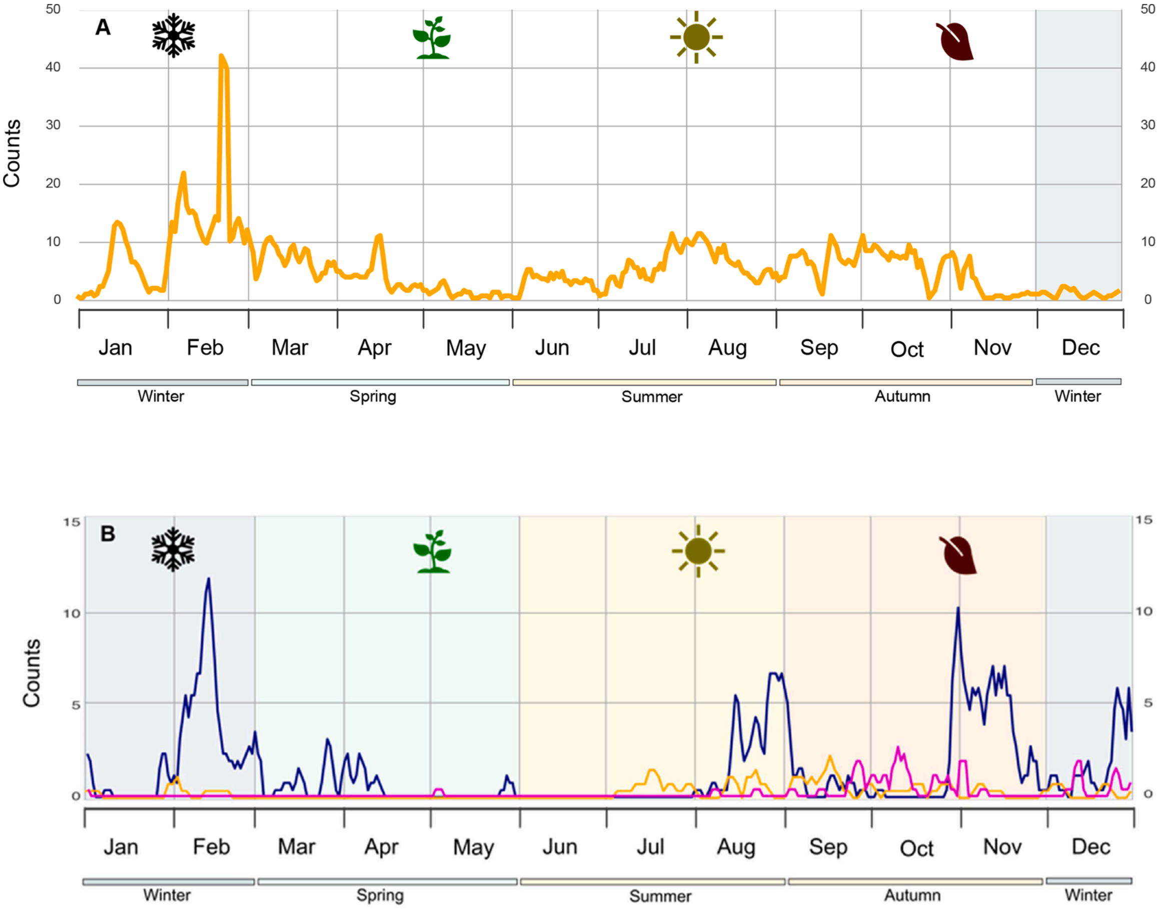

Fig. 4. Temporal distribution of mammal visits across the study period (camera traps at locations A to G). (A) Three-month-long rolling average of daily counts for domestic mammals; (B) Three-month rolling averages of the most frequently observed wild mammals. Coypus are represented by the blue line, rats by the pink line, and hedgehogs by the ocher line.

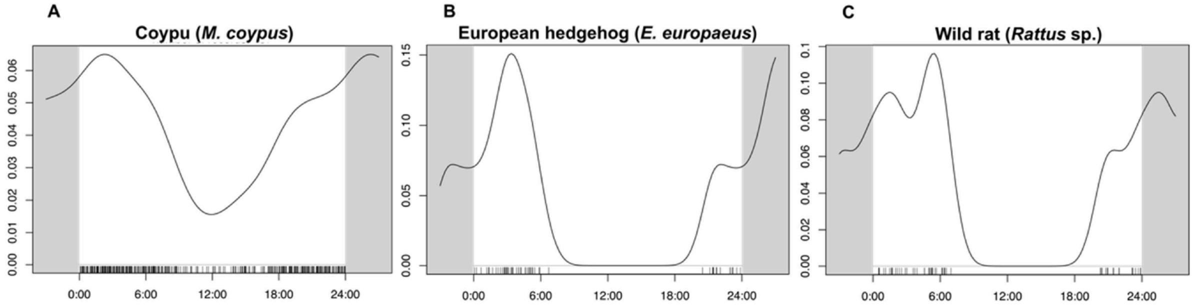

Fig. 5. Activity patterns (relative frequency by time) of coypus, European hedgehogs, and rats visiting the three studied poultry farms.

of the most frequently observed wild species, namely coypus, European hedgehogs, and rats. However, coypus were also observed during daylight hours (from 6:00 a.m. to 6:00 p.m.), likely due to the absence of large wild predators (Mori, Andreoni, Cecere, Magi & Lazzeri, 2020) and minimal human disturbance. The coypus, a large semiaquatic rodent native to the subtropical regions of South America (Woods, Contreras, Willner-Chapman & Whidden, 1992), is now widespread in Northern and Central Italy, as an invasive alien species subjected to population control Cocchi & Bertolino (2021). To the authors' knowledge, this study represents the first documented instance of coypus' activity on commercial animal farms. Coypus were observed throughout the year in all three facilities, and they exhibited feeding behavior in 43 % of the total observations. Unlike wild birds, which are attracted by the food resources offered by the farms (Graziosi et al., 2024; Scott et al., 2018), coypus were only observed foraging on terrestrial vegetation in meadow areas within the camera's range. The presence of these animals was likely facilitated by breaches in the farms' fences, allowing individuals to enter the farm area. Coypus exhibit a range of disease susceptibility, but there are few reports of infectious diseases documented for this species in the wild Bollo et al., (2003); Lim et al. (2019); Martino et al. (2014); Zanzani et al. (2016). Free-ranging coypus have tested positive for various pathogens of poultry, including zoonotic agents, such as *Streptococcus equi* subspecies *zooepidemicus* (Martino et al., 2014), *Chlamydia psittaci* (Howerth, Reeves, McElveen & Austin, 1994; Martino et al., 2014) and *Toxoplasma gondii* Bollo et al. (2003); Howerth et al. (1994). With respect to AIV, there have been no reports of coypus being susceptible to infection. Field or experimental studies are therefore needed, considering the aquatic habits of this rodent and the potential

Table 6

Generalized linear model to explain the frequency of visits of wild mammals. The estimates, rate ratios (exponentiated estimates) (95 % CI), and the *p*-values of Wald test for contrasts between the reference level and the level considered are displayed.

Explanatory variables and levels		Rate ratio (95% CI)	*p*-value
Farm monitored[a]	Farm 2	0.78 (0.56 – 1.07)	0.1251
	Farm 3	0.97 (0.73 - 1.29)	0.8320
Camera trap location[b]	FSilo	0.51 (0.46 - 0.73)	<0.001[***]
Season[c]	Spring	0.64 (0.45 - 0.91)	0.0145[*]
	Summer	0.93 (0.70 – 1.25)	0.6582
	Winter	1.10 (0.86 - 1.42)	0.4473
Time of the day[d]	Night	1.35 (1.05 - 1.76)	0.0219[*]
	Sunset	0.83 (0.60 - 1.14)	0.2680

[a] Farm 1 taken as a reference.
[b] Chicken manure collection point (CMan) as a reference.
[c] Autumn taken as a reference.
[d] Day time taken as a reference.
[*] $p < 0.05$.
[***] $p < 0.001$.

overlap in ecological niche with AIV hosts.

Hedgehogs and rats were observed far less frequently than coypus. Epidemiological studies on wild hedgehogs have reported high prevalence of *Salmonella enterica* subsp. *enterica* serovar Typhimurium or Enteritidis (Handeland et al., 2002; Lawson et al., 2018), which are major food-borne bacteria posing a threat to public health worldwide (EFSA Panel on Biological Hazards et al., 2019). With respect to AIV, to date, hedgehogs have never been reported as being infected, and their susceptibility to the infection remains unknown. On the other hand, AI virus molecular detection in rats has been reported in the USA (Cummings et al., 2019) and China Shao, Zhang, Sun, Liu & Chen (2023). Serological evidence of AIV infection in rats has been found in individuals sampled on a gamebird farm which experienced low pathogenic AIV H5N8, H4N7 and H11N7 outbreaks Shriner et al. (2012). Moreover, several rodent species, such as mice (*Mus musculus* Linnaeus, 1758) and bank voles (*Myodes glareolus* (Schreber, 1780)), have been experimentally infected with non-rodent adapted LPAI and HPAI viruses that efficiently replicated in these hosts Romero Tejeda et al. (2015); Shriner et al. (2012). Although rats, which are highly synanthropic, have been abundantly detected around poultry houses through camera trap surveys (Elbers & Gonzales, 2020; Scott et al., 2018), they were surprisingly not detected in our investigation. Given their small home range (Davis, Emlen & Stokes, 1948), rats and mice could potentially influence the local-scale AIV epidemiology by transitioning from the external environment into poultry houses and actively shed the virus. Additionally, they could act as mechanical vectors through AIV-contaminated coats Velkers, Blokhuis, Veldhuis Kroeze & Burt (2017). However, their actual role in the AI virus epidemiology requires further investigation. Furthermore, a recent paper has reviewed bacterial infections in poultry that can be spread through murids and included *Salmonella* spp., *Escherichia coli, Campylobacter* spp., *Yersinia pseudotuberculosis* and *Y. enterocolitica* Domańska-Blicharz et al. (2023). This evidence emphasizes the importance of implementing efficient on-farm rodent control strategies.

Overall, a limited presence of wild mammals was observed near poultry houses. To further deter wild mammals from entering farm areas, strategies such as fencing the farm and maintaining the fences in good condition are crucial, especially for medium-sized animals. For rodents, implementing pest control through a combination of trapping methods and rodenticide application has been associated with a decreased risk of selecting resistant individuals, which could reproduce and replenish the population Guidobono, León, Gómez Villafañe & Busch (2010). Understanding the behavioral reactions of rodents to baits is also a key element for efficient numerical control of rats and mice on the farm premises Pelz & Klemann (2004).

Results of the camera trap survey hereby presented revealed a high frequency of domestic mammal visits, especially cats. Feral cats can be often found in animal farm areas Coleman & Temple (1993). These were mostly present on Farm 3, where a cat colony was nearby established. Notably, detection of the HPAI H5N1 virus of clade 2.3.4.4b or serological evidence of infection have been recently reported in cats and dogs (EFSA et al., 2023a; Briand et al., 2023; Domańska-Blicharz et al. (2023); Sillman, Drozd, Loy & Harris, 2023), sometimes epidemiologically linked to AI outbreaks in poultry farms Briand et al. (2023); Moreno et al. (2023). The outcomes of HPAI infection in domestic pets can vary from asymptomatic (Moreno et al., 2023) to fatal disease Briand et al. (2023); Canadian Food Inspection Agency (2023); Domańska-Blicharz et al. (2023); Klopfleisch et al. (2007); Songserm et al. (2006). Considering the high number of cat observations reported in this study and their susceptibility to HPAI infection, it is crucial to limit the presence of these mammals on poultry farms to minimize the risk of disease transmission also to humans.

To the best of our knowledge, this study represents the first instance of thoroughly characterizing free-ranging mammal visits on commercial poultry farms using camera trap data. The utilization of visual evidence depicting mammals' activity around poultry houses, such as the footages recorded through camera traps, could assist health authorities in educating and raising awareness of stakeholders about wildlife presence and potential pathogen spillover risks. In combination with the diligent application and enforcement of biosecurity measures, this awareness stands as one of the most effective preventive measures to prevent pathogens spillover or spillback at the interface between domestic animals, wildlife, and humans.

Among the limitations of our study, the few investigated farms and the inclusion of farms that had already reported wildlife presence within their permits should be highlighted. While it provided essential information on the free-ranging mammals-poultry interface in a densely populated poultry area in Northern Italy, this approach potentially reduces the generalizability of the conclusions to other farm settings and locations. Furthermore, the deployment of cameras was carried out based on the observation of wildlife signs, aiming to maximize chances of detecting free-ranging animals in the vicinity of poultry houses. A potential selection bias of the studied sites could have been therefore introduced and led to overestimation of the results obtained. Additionally, camera placement in areas of high human activity (*e.g.*, feed silos; chicken manure collection points) caused numerous non-relevant triggers, contributing to battery depletion and camera trap failures. This reduced the camera trap effectiveness over the study period, as also reported in other similar studies Bacigalupo, Dixon, Gubbins, Kucharski & Drewe (2022); Engeman, Betsill & Ray (2011). Lastly, for a comprehensive understanding of the factors influencing wild mammal presence and their activity on poultry farms, future research should consider additional variables such as quantitative farm-biosecurity scores (Gelaude, Schlepers, Verlinden, Laanen & Dewulf, 2014), habitat characteristics and environmental factors, not included in the models presented within this study.

5. Conclusions

By defining the species of mammals most frequently observed near poultry houses, the findings presented in this study offer insights into the poultry-wildlife interface. These are important prerequisites for providing scientific guidance in designing disease surveillance and intervention strategies, with the aim of preventing cross-species pathogen transmission. Further studies on the susceptibility to infection of the observed wild and domestic mammal species are essential to fully evaluate their actual role in the epidemiology of various poultry pathogens.

Data availability statement

The data supporting the findings of this study are included within the article. The raw database of camera trap observations obtained during the survey is available upon request to the corresponding author.

Ethical statement

The study did not involve the use of animal or human subjects. Video recordings of humans were discarded in compliance with current national privacy regulations.

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CRediT authorship contribution statement

Giulia Graziosi: Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Caterina Lupini:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Francesco Dalla Favera:** Formal analysis, Data curation. **Gabriella Martini:** Project administration, Funding acquisition, Conceptualization. **Geremia Dosa:** Project administration, Investigation, Funding acquisition, Conceptualization. **Gloria Garavini:** Investigation. **Giacomo Trevisani:** Investigation. **Alessandro Mannelli:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation. **Elena Catelli:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2024.100393.

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