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USUV exposure, determinants and immunity after the 2018 epizootic among captive birds in French zoos

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Abstract

Background

The Usutu virus (USUV) was first detected in France in 2015. In 2018, this mosquito-borne zoonotic flavivirus caused an outbreak across the French mainland, leading to high mortality in both wild and captive bird populations. Following this epizootic, several key questions remained unresolved: were some species more susceptible to USUV exposure? Could environmental or individual factors be identified as determinants of infection? Did surviving birds develop an acquired immunity, and if so, how long did it persist? To address these questions, we sampled captive birds from 13 zoological institutions following the 2018 outbreak. Opportunistic sampling enabled us to collect and analyse blood samples from 366 birds (70 species, 12 orders), 350 of which were included in a multivariate regression logistic model to identify determinants of USUV exposure. Ultimately, USUV antibody titres were monitored from 2018 to 2020 in 67 birds (four orders, 25 species).

Results

Overall seroprevalence among birds was 35% (95% CI: 30%-40%), with rates generally not exceeding 70% across all the species and orders tested. Among orders with more than five individuals analysed, Strigiformes and Passeriformes showed significantly higher exposure than Falconiformes. Zoos in western France were significantly more infected than those in central France. Bird body weight, age (year of birth prior to 2015), use of mosquito nets and past exposure to USUV were also identified as significant determinants of USUV exposure. Overall, USUV antibody titres remained stable over the three-year follow-up period and stayed above the neutralising titre threshold in Accipitriformes, Cathartiformes, Passeriformes and Strigiformes.

Conclusions

Our results highlight the role of immune responses and biosafety measures in limiting USUV circulation among captive bird populations.

Background

The Usutu virus (USUV) is a mosquito-borne virus belonging to the *Flaviviridae* family, *Orthoflavivirus* genus and to the Japanese encephalitis virus (JEV) serocomplex [1]. It was discovered in the *Culex neavei* mosquito for the first time in 1959 in Swaziland (now Eswatini) [2]. Until the late 1900s, this pathogen remained confined to the African continent [1]. However, this spatial distribution was challenged when it was detected in 2001 in Austria [3] and retrospectively in Italy in 1996 [4]. Since then, USUV has been regularly detected in several other European countries [2, 5–7]. In some of them, including France, USUV is now considered endemic but epizootics can still occur [8, 9].

The Usutu virus is primarily maintained in the environment through a transmission cycle involving birds and mosquitoes [10]. In Europe, the *Culex pipiens* mosquito is the primary vector for USUV [11]. Common blackbirds (*Turdus merula*), great grey owls (*Strix nebulosa*) [12–14], house sparrows (*Passer domesticus*) [15, 16] and other Passeriformes [16, 17] are more specifically infected and particularly sensitive, with high mortality rates following USUV infection [14]. In blackbirds, hepatomegaly and splenomegaly are the main macroscopic lesions [18], while histopathological examination generally reveals coagulative necrosis, lymphoplasmacytic inflammation and vasculitis [19]. Several other species have also been found to be infected (e.g. blue tits (*Parus coeruleus*) [12], black-headed warblers (*Sylvia atricapilla*) or magpies (*Pica pica*) [20]).

The zoonotic potential of USUV is now well established, and human cases are mainly asymptomatic [21] or characterised by mild symptoms [14]. Rare severe neurological complications such as encephalitis or meningoencephalitis can occur, most often in immunocompromised individuals [8, 22]. However, the recent death of an immunocompromised patient infected with USUV has raised renewed concern about its pathogenic potential [23]. Even though *Cx. pipiens* are ornithophilic mosquitoes, epizootic phases with numerous infected birds could pose a risk of transmission to bridge mosquitoes and the risk of USUV among humans should be considered, particularly in patients with meningoencephalitis [15].

Captive avian species maintained in zoological collections are susceptible to USUV infection and represent a significant epidemiological concern, both because of the potential impact on their conservation and their possible role in viral maintenance and circulation. The infection can lead to death in captive birds such as western capercaillies (*Tetrao urogallus*) (Lahoreau, personal communication), canaries (*Serinus canaria domestica*) or great grey owls. Passeriformes and Strigiformes are primarily affected by USUV [15, 24]. Various studies have confirmed that USUV seroprevalence varies depending on the bird order: USUV antibodies have been detected in captive birds exposed to the virus in Austria, Hungary and Switzerland among Sphenisciformes, Phoenicopteriformes, Ciconiiformes, Anseriformes, Accipitriformes, Galliformes and Coraciiformes. But the highest seroprevalence was observed in the Strigiformes order [25]. In France, USUV antibodies were detected in Bucerotiformes, Pelecaniformes, Ciconiiformes and Galliformes. The highest seroprevalence values were observed in Rheiformes and Casuariiformes [10]. Hypotheses to account for these results included greater susceptibility to orthoflavivirus infection or higher rate of vector feeding. Antibodies have been detected in several species in the absence of overt clinical symptoms or other evidence of active viral circulation. For instance, some studies demonstrated that some birds can develop and maintain detectable antibodies without ever exhibiting clinical symptoms [5, 26].

Environmental factors may influence the epidemiology of USUV, similarly to what is observed for WNV [25, 27]. Several studies have highlighted a relationship between increased WNV activity and increased temperature [28, 29] and precipitation [29]. Temperature and precipitation are also positively correlated with the abundance of *Cx. pipiens*, with soil and land use interacting with precipitation only [30]. Given its vector-borne transmission, several measures may be proposed to mitigate the risk of infection: e.g. elimination of artificial container habitats, management of aquatic mosquito breeding sites, use of

approved mosquito larvicides and adulticides [31] or use of mosquito nets or protective caging during periods of high mosquito density [32].

In France, USUV emerged as a clinically apparent pathogen in 2015 [18], after having circulated silently for several years [33]. A large-scale epizootic occurred only three years later, in 2018, causing significant mortality in wild common blackbirds as well as in captive avian populations [32]. Between 2018 and 2020, anti-USUV antibodies were evaluated in several captive bird species kept in French zoological gardens to investigate the range, intensity and dynamics of USUV exposure. The aims of this prospective study were consequently (i) to evaluate the seroprevalence of USUV among birds in French zoological gardens after the 2018 outbreak, (ii) to identify individual and environmental determinants associated with their USUV exposure and (iii) to monitor the dynamics of the antibody response over a three-year period at the individual level.

Methods

Study population

Thirteen French zoological institutions, identified from A to M, participated in the study (Figure 1). Opportunistic sampling was performed in all these institutions for three consecutive years (2018-2020), following the major 2018 USUV epizootics in France and after the mosquito-prone and USUV at-risk periods. Bird capture and sampling were performed concurrently with the avian influenza vaccination campaigns (from October to January). Species from the orders Strigiformes, Accipitriformes and Falconiformeswere more specifically targeted and sampled exhaustively. Blood samples were collected from the brachial vein by veterinarians and centrifuged (3000-5000 rpm x10 min) within 12 h. Retrieved sera were frozen at -20°C until they were shipped by each institution to the ANSES laboratory at Maisons-Alfort. For each serum sample, participating institutions were asked to fill out a questionnaire (see additional file 3) compiling data relevant for epidemiological analysis: (i) individual data, (ii) housing conditions, (iii) presence of nearby water, (iv) reports of nearby vectors observed, (v) prophylaxis against vectors, (vi) USUV and/or haemoparasite infection history.

Serology

As an initial screening step, sera were tested using a commercial competitive ELISA (ID Screen® Flavivirus Competition, Innovative Diagnostics, Montpellier, France) based on purified WNV envelope antigens. Due to high orthoflavivirus cross-reactivity, this cELISA enables broad orthoflavivirus antibody screening, including USUV, and detects IgY antibodies in birds. Tests were performed per manufacturer's instructions, with samples classified as positive (S/N ≤40%), doubtful (40–50%), or negative (>50%).

cELISA-positive samples underwent virus microneutralisation tests (VNTs) to identify the infecting orthoflavivirus, as this method specifically detects neutralising antibodies [34]. VNTs were performed for WNV (strain Is98, GenBank AF481864.1) and USUV (strain Italy 2012, GenBank KX816653.1) following Beck et al. [35] protocol. Samples were considered positive when complete cell protection was observed

at 1:10 serum dilution as defined by WOAH [34]. In cross-neutralisation cases, the infecting virus was determined based on the highest neutralising titre with a minimum fourfold difference.

For epidemiological analyses, birds were considered USUV-seropositive if they tested positive at least once by cELISA during the three-year study period and were confirmed positive by USUV VNT. We described the seroprevalence among the species and orders of birds that underwent analysis. All confidence intervals were computed using the Jeffreys interval [36].

Multivariate logistic regression model

For each bird sampled, nine variables were defined based on its individual characteristics and on the answers to the questionnaire. Regarding individual characteristics, four variables were defined: order, body weight, year of birth (before or after 2015, which was the year that USUV was first detected and reported among blackbirds in France) and location of the bird's zoo of origin (zoos in eastern France: A, M and I; zoos in western France: C, F, G, H, K and L; zoos in central France: B, D, E and J; Figure 1). Five variables were defined on the basis of questionnaire data: use of mosquito nets, application of insecticide in the bird's environment, application of insecticide directly on birds, presence of water points and prior exposure to USUV.

To identify variables associated with the USUV status of birds, we developed a multivariable logistic regression model using the USUV infectious status of each bird (positive or negative, see the Serology section) as the outcome variable. Avian orders represented by five or fewer individuals were excluded from the analysis.

The nine variables considered were assessed for collinearity, including multi-collinearity analysis, to ensure a mean variance inflation factor (VIF) below five (<5) prior to inclusion in the multivariable models [37]. A multivariable model was then constructed using a generalised linear model (GLM, [38]), with a binomial distribution and logit link function, with all variables initially included as a main effect. The StepAIC function (MASS package, direction="both") [39] was used to select the model with the lowest Akaike information criterion (AIC). Corrected AIC (AICc) values were used as the number of parameters, k, was higher than the number of observations n ($n/k < 40$) [40]. The error threshold (p-value) was set at 0.05. Results were expressed as odds ratios (ORs) and their 95% confidence intervals. We assessed the fit quality of the model using the area under the ROC curve (AUC) (the closest the AUC is to 1, the better the model) [37]. We also performed an internal validation using a leave-one-out approach: the model was fitted repeatedly (n=1000) with one observation left out [37] and the average AUC was compared with the AUC of the model with the full dataset (close values suggesting that the model is reliable). Statistical computations were performed in R 4.5.0 [41].

Temporal evolution of USUV immunity

Once USUV exposure had been identified, the variation in anti-USUV antibody titres over time was monitored during the three-year study period using the specific USUV VNT technique. To account for

changes in antibody titres over this period, only birds sampled on at least two occasions (consecutive years or not) were retained for temporal analysis. We assumed that antibody titres higher than 10 conferred immunity against USUV infection [34].

Results

Study population

A total of 366 birds from 70 different species were sampled at least once during the study: 242 individuals from 61 species in 2018, 226 individuals from 51 species in 2019, and 146 individuals from 29 species in 2020. Twelve orders were represented among the samples: Accipitriformes (32.8% of birds analysed), Anseriformes (0.8%), Cathartiformes (3.0%), Falconiformes (6.8%), Galliformes (3.0%), Gruiformes (0.3%), Musophagiformes (0.3%), Passeriformes (4.1%), Pelecaniformes (1.4%), Psittaciformes (1.6%), Strigiformes (42.9%) and Struthioniformes (3.0%) (Table 1).

Table 1
Number of birds analysed and found USUV positive between 2018 and 2020 by bird order.

Bird order	Number of birds analysed	Number of birds positive[b] for USUV (percentage)
Strigiformes	157	70 (45%)
Accipitriformes	120	33 (28%)
Falconiformes	25	2 (8%)
Passeriformes	15	7 (47%)
Struthioniformes	11	7 (64%)
Cathartiformes	11	5 (45%)
Galliformes	11	1 (9%)
Psittaciformes[a]	6	2 (33%)
Pelecaniformes[a]	5	0 (0%)
Anseriformes[a]	3	0 (0%)
Gruiformes[a]	1	1 (100%)
Musophagiformes[a]	1	1 (100%)
All orders	366	129 (35%)

[a] Excluded from the multivariate logistic regression model because there were fewer than five birds analysed ≤ 5; note that one Psittaciforme has been excluded due to a lack of information, reducing the number of birds in this order to five.

[b] A positive bird was one that was cELISA-positive at least once between 2018 and 2020 and USUV-VNT positive.

Four out of the 13 zoos sampled birds belonging to only one order (C: Strigiformes, D: Passeriformes, G: Strigiformes, K: Accipitriformes) (Fig. 2). Nine zoos sampled birds belonging to at least two orders, and six of them to at least three orders (A, B, E, H, I and M). When more than two orders were present in the samples, Strigiformes and Accipitriformes were always found (Fig. 2).

Within Strigiformes, *Strix nebulosa* showed low USUV exposure with detectable anti-USUV immunity in only 2/24 animals (8.0% seroprevalence) (Table 2). This was significantly lower than *Bubo bengalensis*, *B. bubo*, *B. bubo sibiricus*, *B. capensis*, *B. scandiacus*, *Strix uralensis*, and *Tyto alba* (Fisher's tests, all $p < 0.05$). Within Passeriformes, *Pica pica* and *Sturnus vulgaris* were most impacted (100% seroprevalence) (Table 2). These rates were significantly higher than *Corvus corax* (Fisher's tests, $p = 0.036$ and 0.002,

respectively) but not than *Corvus splendens* or *Garrulus glandarius* (Fisher's tests, p = 0.33 and 0.17, respectively, all three species being negative; Table 3).

Table 2
Proportion of positive birds per species among all zoos participating in the study (seroprevalence; a positive bird was one that tested positive for anti-USUV antibodies at least once between 2018 and 2020).

Order	**Species**	**USUV Seroprevalence (number of positive/analysed birds) (%)**
Accipitriformes	*Aegypius monachus*	6/11 (55%)
	Aquila rapax	1/9
	Gyps africanus	1/1
	Gyps fulvus	14/44 32%)
	Gyps himalayensis	1/1
	Gyps rueppelli	2/6
	Neophron percnopterus	5/8
	Parabuteo unicinctus	1/2
	Sagittarius serpentarius	1/1
	Trigonoceps occipitalis	1/1
Cathartiformes	*Cathartes aura*	3/8
	Vultur gryphus	2/2
Falconiformes	*Caracara plancus*	1/5
	Falco cherrug	1/4
Galliformes	*Tetrao urogallus*	1/10 (10%)
Gruiformes	*Anthropoides virgo*	1/1
Musophagiformes	*Corythaeola cristata*	1/1
Passeriformes	*Pica pica*	2/2
	Sturnus vulgaris	5/5
Psittaciformes	*Nestor notabilis*	2/2
Strigiformes	*Bubo bengalensis*	4/9
	Bubo bubo	5/11 (45%)
	Bubo bubo sibiricus	5/6
	Bubo capensis	2/3
	Bubo lacteus	1/2

Order	Species	USUV Seroprevalence (number of positive/analysed birds) (%)
	Bubo scandiacus	23/42 (55%)
	Ptilopsis leucotis	1/3
	Pulsatrix perspicillata	3/8
	Strix leptogrammica	3/7
	Strix nebulosa	2/24 (8%)
	Strix uralensis	5/12 (42%)
	Tyto alba	16/25 (64%)
Struthioniformes	*Casuarius casuarius*	2/2
	Rhea americana	1/2
	Rhea pennata	3/6
	Struthio camelus	1/1

Table 3
Species where no infection was detected (a negative bird was one that never tested positive for anti-USUV antibodies between 2018 and 2020).

Order	Species	Number of analysed birds
Accipitriformes	*Accipiter gentilis*	2
	Aquila heliaca	1
	Aquila nipalensis	2
	Buteo buteo	3
	Buteo rufinus	1
	Buteo rufofuscus	1
	Geranoaetus polyosoma	1
	Haliaeetus albicilla	2
	Haliaeetus leucocephalus	3
	Harpia harpyja	1
	Milvus migrans	14
	Milvus milvus	3
	Necrosyrtes monachus	1
	Terathopius ecaudatus	1
Anseriformes	*Callonetta leucophrys*	1
	Dendrocygna bicolor	1
	Somateria mollissima	1
Cathartiformes	*Coragyps atratus*	1
Falconiformes	*Falco biarmicus*	3
	Falco jugger	2
	Falco peregrinus	8
	Falco tinnunculus	3
Galliformes	*Meleagris gallopavo*	1
Passeriformes	*Corvus corax*	6
	Corvus splendens	1
	Garrulus glandarius	1

Order	Species	Number of analysed birds
Pelecaniformes	*Eudocimus ruber*	3
	Scopus umbretta	1
	Threskiornis aethiopicus	1
Psittaciformes	*Amazona leucocephala*	2
	Cyanoliseus patagonus	2
Strigiformes	*Bubo africanus*	1
	Bubo bubo X Bubo bubo sibiricus	3
	Bubo virginianus	1

Table 2

Table 3

Average USUV exposure per zoo over three years never exceeded 75%. Central region zoos showed lower seroprevalence than western or eastern regions (Fig. 1). Overall study seroprevalence was 35% (129/366; 95% CI: 30%–40%). Among orders with > 10 birds analyzed, Struthioniformes had highest seroprevalence (64%; 95% CI: 35%–86%), followed by Passeriformes (47%; 95% CI: 24%–71%), Cathartiformes (45%; 95% CI: 20%–73%), Strigiformes (45%; 95% CI: 37%–52%), Accipitriformes (28%; 95% CI: 20%–36%), Galliformes (9%; 95% CI: 1%–35%), and Falconiformes (8%; 95% CI: 2%–23%) (Table 1).

Multivariate logistic regression model

From 366 birds initially analyzed (Table 1), one bird lacking housing information was excluded (*Amazona leucocephala*, Psittaciformes, negative). Five orders with fewer than five birds analyzed (Anseriformes, Gruiformes, Musophagiformes, Pelecaniformes, Psittaciformes) were excluded (Table 1). Analysis included 350 birds from seven orders (Accipitriformes, Cathartiformes, Falconiformes, Galliformes, Passeriformes, Strigiformes, Struthioniformes), with 125 positive (Table 1). Falconiformes and central zoos showed lowest seroprevalence (Fig. 3) and served as reference categories. Strigiformes had highest USUV exposure in western zoos (69%), while Struthioniformes peaked in central and eastern zoos (67% and 62%, respectively) (Fig. 3). Visual inspection revealed higher seroprevalence where insecticides weren't used (40.0%; 95% CI: 34.2–46.0 vs 23.3%, 95% CI: 15.5–32.8 with use) and near water points (44.4%; 95% CI: 34.9–54.3 vs 32.3%; 95% CI: 26.7–38.2 without water) (Fig. 4).

None of the nine variables considered was excluded from the construction of the multivariable model (VIF < 5 for all variables, so collinearity with other predictor variables could be excluded). After the selection process based on the AICc (which excluded the use of insecticide on birds and in the

environment), the final model (AICc = 370.26) included seven variables, of which six were significantly associated with the bird's USUV status (p-value < 0.05, Table 4). Firstly, four significant variables were linked to the birds' individual characteristics: (i) its order, Strigiformes and Passeriformes being more frequently infected than the Falconiformes (OR = 8.16; 95% CI: 2.02–55.81 and OR = 23.11; 95% CI: 3.54-214.16 respectively), (ii) its body weight (OR = 1.28; 95% CI: 1.11–1.48), (iii) its year of birth, with birds born before 2015 more frequently infected than birds born after 2015 (OR = 2.67; 95% CI: 1.43–5.12), and (iv) its zoo of origin, zoos in the west having more birds infected by USUV than zoos in central France (OR = 7.82; 95% CI: 3.54–18.14). Two significant variables were then linked to the birds' housing conditions: (i) the use of mosquito nets, and (ii) past exposure to USUV. These variables were negatively associated with the birds' USUV status (with OR = 0.03 and 0.37, respectively and 95% CI: $1.6x10^{-3}$-0.21 and 0.17–0.76, respectively) (Table 4). The model's AUC was 83% (95%CI: 79%–87%), indicating its excellent discrimination. The average AUC of the simulated leave-one-out model (n = 1000) was also 83% (95%CI: 83%–83%), suggesting excellent reliability too.

Table 4
Odds ratios (OR) and their associated 95% confidence intervals (CI) (obtained from the logistic regression analysis of USUV infection among birds in French zoos between 2018 and 2020; *: p-value < 0.05).

Variable	**OR**	**95% CI**	**p-value**
Order			
Falconiformes	*Reference*	*Reference*	*Reference*
Accipitriformes	*0.85*	*(0.16–6.66)*	*0.09*
Cathartiformes	2.57	(0.32–27.26)	0.40
Galliformes	1.24	(0.05–18.02)	0.88
Passeriformes	23.11	(3.54-214.16)	0.002 (*)
Strigiformes	8.16	(2.02–55.81)	0.009 (*)
Struthioniformes	0.07	(0.00-2.34)	0.14
Body weight	1.28	(1.11–1.48)	$9.00*10^{-4}$ (*)
Year of birth			
After 2015	*Reference*	*Reference*	*Reference*
Before 2015	2.67	(1.43–5.12)	$2.49x10^{-4}$ (*)
Undetermined	0.58	(0.23–1.43)	0.24
Location of zoo of origin			
Central (C)	*Reference*	*Reference*	*Reference*
Eastern (E)	1.76	(0.83–3.80)	0.14
Western (W)	7.82	(3.54–18.14)	< 0.0001 (*)
Use of mosquito nets	0.03	($1.6x10^{-3}$-0.21)	0.002 (*)
Presence of water points	1.72	(0.87–3.45)	0.12
Past exposure to USUV	0.37	(0.17–0.76)	0.009 (*)

Temporal evolution of USUV immunity

We excluded 61 birds out of the 128 birds included in the temporal immunity study because we only had one year of sampling data for them. Out of the 67 remaining birds, four orders could be studied: Strigiformes (n = 42 birds, 11 species), Accipitriformes (n = 16 birds, 10 species), Cathartiformes (n = 3 birds, 2 species) and Passeriformes (n = 6 birds, 2 species).

Across the four orders studied, the mean and median values of USUV-IgY antibody titres remained above the immunity threshold, showing a generally stable trend over 3 years (Fig. 5). USUV antibody titres were generally high in Passeriformes, Cathartiformes, and most *Bubo* species (order Strigiformes), maintaining protective levels throughout the study period, with the exception of a single *Bubo bengalensis* individual. In contrast, Accipitriformes and smaller Strigiformes (*Strix* and *Tyto* species) exhibited lower mean and median titres, with some individuals occasionally dropping below the immunity threshold (*Gyps fulvus* in 2019, *Neophron percnopterus* in 2020, *Tyto alba* in 2019 and 2020), suggesting an earlier loss of immune protection (Fig. 5).

Discussion and conclusions

This three-year study (2018–2020) assessed USUV seroprevalence in French zoo birds, revealing an overall seroprevalence of 35%. Strigiformes and Passeriformes showed significantly higher exposure than Falconiformes, and western French zoos were more affected than central ones. Bird body weight, year of birth before 2015, use of mosquito nets, and past exposure to USUV were also identified as significant determinants of USUV exposure. USUV-IgY antibody titres remained stable and above neutralising thresholds throughout the follow-up period, with only 7% of birds (5/67) showing titres below the immunity threshold, indicating sustained protective immunity in most birds.

Serological analyses used a two-step approach combining cELISA screening with confirmatory VNT, a strategy widely employed in European avian orthoflavivirus studies [10, 42]. This combination provides high sensitivity and specificity to discriminate between closely related orthoflaviviruses while limiting cross-reactivity, enabling robust seroprevalence comparisons with European surveillance programmes.

Our overall seroprevalence (35%; 95% CI: 30%-40%) was considerably higher than previous European zoo studies: 8.75%, 5.3% and 6.59% in Vienna, Zurich and Basel respectively [25], and 14.6% in France in 2018 [10]. This difference may reflect the 2018 major infection wave, a particularly favourable year for *Culex*-transmitted arboviruses in Europe [29]. Struthioniformes showed the highest exposure (64%), consistent with previous French studies (46% in 2003–2019) [10], likely due to their increased attractiveness to *Culex* vectors through greater CO2 and volatile compound production. All Struthioniformes species were exposed (4/4) and nearly all Strigiformes species (12/15). While this could reflect larger sample sizes (11 and 157 birds respectively), this hypothesis is challenged by equally large numbers of Accipitriformes and Falconiformes (120 and 25 respectively), which showed less pronounced susceptibility (10/24 and 2/6 exposed species respectively) (Tables 2 and 3). Conversely, great grey owls showed low seroprevalence (8%), possibly reflecting the mass mortality event during summer 2018 in France [32]. This species appears particularly USUV-sensitive [3, 24, 43, 44], potentially due to lack of coevolution with the virus in northern latitudes where vectorial activity has been historically limited. Most infected individuals likely died during the outbreak, leaving primarily naive survivors. The absence of subsequent mortality events may be attributed to preventative measures implemented by participating institutions following the fatal 2018 outbreak and limited viral circulation during 2019–2023 (Lecollinet, personal communication).

Although our study provides robust assessment of USUV epidemiology in captive birds, several limitations should be noted. The studied population may not fully represent the entire captive bird population, as avian orders were not sampled comparably. Sampling efforts primarily targeted species known to be highly susceptible to USUV or easily accessible, explaining the predominance of Strigiformes (43% of individuals) and underrepresentation of Passeriformes (4%). Some orders like Charadriiformes were not included despite evidence of USUV sensitivity in *Larus marinus* [45]. Additionally, sampling was conducted over a limited period (post-summer) and geographical range, potentially not reflecting epidemic patterns in other regions or seasons. Results at the avian order level cannot be directly compared with wild bird data, as zoo species differ substantially from local wildlife. Despite these limitations and potential sampling bias inherent to non-random recruitment [46], our seroprevalence estimates provide useful baseline values for future investigations, particularly for studies requiring sampling size calculations and probability-based methods.

Participating zoos were mainly located in northern mainland France, consistent with higher USUV-related mortality in the same region during the 2018 epizootic [32]. However, our spatial pattern differed from 2018 findings: central France showed lower seroprevalence than western and eastern regions, whereas the 2018 study reported higher wild bird mortality in central and central-western areas [32]. This discrepancy may reflect study design differences, as our study focused on zoo birds that survived USUV infection, and differing sampling periods (October-January 2018–2020 versus mid-July to late August 2018), which likely influenced observed spatial patterns.

Significant differences between central and western or eastern zoos may reflect heterogeneous USUV exposure along major avian migratory routes. Migratory birds likely introduced USUV from Africa into Europe via East Atlantic and Black Sea–Mediterranean flyways [47], with past introductions possibly causing earlier, silent circulation in western coastal areas [33]. Migratory passerines like song thrushes (*Turdus philomelos*) have been hypothesized to spatially disseminate USUV, notably in Spain [48]. Seroprevalence differences between eastern and central zoos may reflect France's historical USUV spread pattern, characterized by recent viral introductions in the northeast from countries east of the French border [18, 49]. Lower positive bird numbers in most affected areas could also result from protective measures zoos implemented during 2018 epizootics.

Infection odds varied geographically, likely reflecting different exposure levels based on weather conditions (affecting vector activity), environment, surrounding wildlife, and housing conditions. Higher infection odds in older birds (born before 2015) versus younger ones (born after 2015) may result from increased cumulative exposure and larger body surface area for vector bites compared to juveniles. Co-infections with *Haemoproteus*, a common haemosporidian in Strigiformes red blood cells [50], represent another potential USUV infection determinant documented in previous studies [51–53]. Although *Haemoproteus* infections are common among owls in participating institutions, blood smears from included Strigiformes were not thoroughly evaluated. Further studies are needed to assess this potential infection determinant.

The significant environmental determinants identified (mosquito nets and previous USUV exposure) highlight the benefits of implementing protective measures in zoos to prevent mortality in susceptible species. The authors recommend temporarily housing susceptible Strigiformes indoors during viral circulation periods (with appropriate antifungal prophylaxis), and using mosquito traps and environmental larvicides. However, insecticides applied directly to birds did not protect against USUV exposure in our study, and raise concerns about repeated animal handling and environmental health effects [54, 55]. Biological larvicides such as *Bacillus thuringiensis* subsp. *israelensis* (Derraik 2005; Virgillito et al. 2022) and physical measures disrupting mosquito breeding cycles, eliminating artificial containers and natural water collection features [56, 57], may also be beneficial, though water points were not identified as significant determinants in our model.

Our results suggest that birds acquired USUV immunity, though no infectious challenge confirmed this hypothesis and USUV circulation was limited in France during 2019–2023. The absence of mortality in 2019–2020 could reflect either lack of viral exposure or acquired immunity. Limited viral circulation may indicate collective immunity in local wildlife following 2018 epizootics, consistent with reported 3-year USUV cyclical patterns in Austria [58]. The stability of immune responses over three seasons (2018–2020) supports sustained population-level immunity establishment. However, results for Cathartiformes and Passeriformes require cautious interpretation due to small sample sizes. Possible herd immunity has been observed in common blackbirds, where USUV-related deaths declined over time while birds with low viral loads increased [59]. Comparison with European studies shows consistent USUV antibodies in Falconiformes and Strigiformes, though unlike our findings, Passeriformes showed no immunity in Hungarian, Austrian and Swiss zoos (2006–2007) [25]. For long-term management, monitoring blood IgY levels could identify individuals with acquired immunity, potentially reducing protective measures and targeting immunocompetency in captive populations.

Natural USUV exposure and acquired immunity demonstrate vaccination's preventive potential. Cross-protective efficacy of WNV vaccination against USUV has been shown in mice [60] and sparrow models, where prior WNV infection provided protection during subsequent USUV challenge [61]. German zoo trials revealed that WNV vaccines enhanced protection in birds against USUV [62]. Given USUV's cyclical outbreak pattern (Meister et al. 2008), future waves are likely, emphasizing the need for proactive immunization in captive avian populations. From a One Health perspective, these findings inform best practices for mitigating ornithophilic and zoonotic vector-borne viruses like USUV.

Declarations

Ethics approval and consent to participate

This study involved captive birds housed in participating zoological institutions.

The blood samples analysed in this study were collected as part of the avian influenza serological follow-up, i.e. the health monitoring program of the birds, in accordance with applicable national guidelines and institutional veterinary procedures. No specimens were collected specifically for the

purposes of this study, and no experimental procedures were performed on the animals as part of the study. The samples were subsequently used for monitoring USUV exposure among captive birds, with the agreement of the participating zoological institutions. Each participating zoological institution provided written, signed and dated authorization to participate in the study and to use and publish the data and samples collected as part of the project. Accordingly, no animal ethics committee approval was required for the use of samples collected during routine veterinary procedures.

A short questionnaire was also administered to staff members of the participating zoological institutions, to collect information on housing practices, insecticide treatments and environmental conditions of the aviaries. No personal, identifying or health-related data on the respondents were collected. Participation was voluntary, and respondents were informed of the study's purpose and agreed to take part before completing the questionnaire. Under French regulation, this non-interventional survey does not fall within the scope of the "loi Jardé" (Law No. 2012-300, published in the Official Journal on 6 March 2012) and therefore did not require approval by a "Comité de Protection des Personnes" (CPP) or another institutional review board. The study was conducted in accordance with the relevant ethical guidelines, including the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing Interests

The authors declare no competing interests.

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Authors' contributions

SL, MM and MBZ designed the study. VF, AL, BQ, AM, JL, KL, CB, RAM, GG and SL contributed to data acquisition and analysis. MBZ, MM, AL, BQ and SL contributed to data interpretation. AL, BQ, MBZ, MM and SL drafted the manuscript. All authors critically revised the manuscript and approved the final version.

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References

1. Ashraf U, Ye J, Ruan X, et al. Usutu virus: an emerging flavivirus in Europe. Viruses. 2015;7:219–38. https://doi.org/10.3390/v7010219.
2. Vilibic-Cavlek T, Petrovic T, Savic V, et al. Epidemiology of Usutu Virus: The European Scenario. Pathogens. 2020;9:699. https://doi.org/10.3390/pathogens9090699.
3. Weissenböck H, Kolodziejek J, Url A, et al. Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe. Emerg Infect Dis. 2002;8:652–6. https://doi.org/10.3201/eid0807.020094.
4. Weissenböck H, Bakonyi T, Rossi G, et al. Usutu virus, Italy, 1996. Emerg Infect Dis. 2013;19:274–7. https://doi.org/10.3201/eid1902.121191.
5. Kvapil P, Račnik J, Kastelic M, et al. A Sentinel Serological Study in Selected Zoo Animals to Assess Early Detection of West Nile and Usutu Virus Circulation in Slovenia. Viruses. 2021;13:626. https://doi.org/10.3390/v13040626.
6. Prioteasa FL, Dinu S, Tiron GV, et al. First Detection and Molecular Characterization of Usutu Virus in *Culex pipiens* Mosquitoes Collected in Romania. Microorganisms. 2023;11:684. https://doi.org/10.3390/microorganisms11030684.
7. Fontoura-Gonçalves C, Llorente F, Pérez-Ramírez E, et al. Dynamics of Bagaza, West Nile, and Usutu Viruses in Red-Legged Partridges, Portugal, 2018–2022. Emerg Infect Dis. 2025;31:824–8. https://doi.org/10.3201/eid3104.241293.
8. Clé M, Beck C, Salinas S, et al. Usutu virus: A new threat? Epidemiol Infect. 2019;147:e232. https://doi.org/10.1017/S0950268819001213.
9. Klobucar A, Savic V, Curman Posavec M, et al. Screening of Mosquitoes for West Nile Virus and Usutu Virus in Croatia, 2015–2020. Trop Med Infect Disease. 2021;6:45. https://doi.org/10.3390/tropicalmed6020045.
10. Constant O, Bollore K, Clé M, et al. Evidence of Exposure to USUV and WNV in Zoo Animals in France. Pathogens. 2020;9:1005. https://doi.org/10.3390/pathogens9121005.
11. Fros JJ, Miesen P, Vogels CB, et al. Comparative Usutu and West Nile virus transmission potential by local *Culex pipiens* mosquitoes in north-western Europe. One Health. 2015;1:31–6.

https://doi.org/10.1016/j.onehlt.2015.08.002.

12. Weissenböck H, Kolodziejek J, Fragner K, et al. Usutu virus activity in Austria, 2001–2002. Microbes Infect. 2003;5:1132–6. https://doi.org/10.1016/S1286-4579(03)00204-1.
13. Michel F, Fischer D, Eiden M, et al. West Nile Virus and Usutu Virus Monitoring of Wild Birds in Germany. Int J Environ Res Public Health. 2018;15:171. https://doi.org/10.3390/ijerph15010171.
14. Simonin Y. Circulation of West Nile Virus and Usutu Virus in Europe: Overview and Challenges. Viruses. 2024;16:599. https://doi.org/10.3390/v16040599.
15. Becker N, Jöst H, Ziegler U, et al. Epizootic emergence of Usutu virus in wild and captive birds in Germany. PLoS ONE. 2012;7:e32604. https://doi.org/10.1371/journal.pone.0032604.
16. Horton DL, Lawson B, Egbetade A, et al. Targeted surveillance for Usutu virus in British birds (2005–2011). Vet Rec. 2013;172:17–17. https://doi.org/10.1136/vr.101275.
17. Dziadek K, Niczyporuk JS, Styś-Fijoł N, et al. Usutu virus continues to spread across Europe: first report of multiple molecular detections of the USUV Africa 2 and Africa 3 lineages in free-living and captive birds in Poland, July–November 2023. Vet Res. 2025;56:43. https://doi.org/10.1186/s13567-025-01460-9.
18. Lecollinet S, Blanchard Y, Manson C, et al. Dual Emergence of Usutu Virus in Common Blackbirds, Eastern France, 2015. Emerg Infect Dis. 2016;22:2225. https://doi.org/10.3201/eid2212.161272.
19. Giglia G, Agliani G, Munnink BBO, et al. Pathology and Pathogenesis of Eurasian Blackbirds (*Turdus merula*) Naturally Infected with Usutu Virus. Viruses. 2021;13:1481. https://doi.org/10.3390/v13081481.
20. Nikolay B. A review of West Nile and Usutu virus co-circulation in Europe: how much do transmission cycles overlap? Trans R Soc Trop Med Hyg. 2015;109:609–18. https://doi.org/10.1093/trstmh/trv066.
21. Angeloni G, Bertola M, Lazzaro E, et al. Epidemiology, surveillance and diagnosis of Usutu virus infection in the EU/EEA, 2012 to 2021. Eurosurveillance. 2023;28:2200929. https://doi.org/10.2807/1560-7917.ES.2023.28.33.2200929.
22. Cadar D, Simonin Y. (2023) Human Usutu Virus Infections in Europe: A New Risk on Horizon? Viruses 15:77. https://doi.org/10.3390/v15010077
23. Szabó BG, Nagy A, Nagy O, et al. First documented case of a fatal autochthonous Usutu virus infection in an immunocompromised patient in Hungary: a clinical-virological report and implications from the literature. Virol J. 2025;22:261. https://doi.org/10.1186/s12985-025-02890-9.
24. Rijks JM, Kik ML, Slaterus R, et al. Widespread Usutu virus outbreak in birds in the Netherlands, 2016. Eurosurveillance. 2016;21:30391. https://doi.org/10.2807/1560-7917.ES.2016.21.45.30391.
25. Buchebner N, Zenker W, Wenker C, et al. Low Usutu virus seroprevalence in four zoological gardens in central Europe. BMC Vet Res. 2013;9:153. https://doi.org/10.1186/1746-6148-9-153.
26. Bergmann F, Schmoock-Wellhausen M, Fast C, et al. Longitudinal Study of the Occurrence of Usutu Virus and West Nile Virus Infections in Birds in a Zoological Garden in Northern Germany.

Pathogens. 2023;12:753. https://doi.org/10.3390/pathogens12060753.

27. Lu L, Zhang F, Munnink BBO, et al. West Nile virus spread in Europe: Phylogeographic pattern analysis and key drivers. PLoS Pathog. 2024;20:e1011880. https://doi.org/10.1371/journal.ppat.1011880.
28. Ziegler U, Lühken R, Keller M, et al. West Nile virus epizootic in Germany, 2018. Antiviral Res. 2019;162:39–43. https://doi.org/10.1016/j.antiviral.2018.12.005.
29. Camp JV, Nowotny N. The knowns and unknowns of West Nile virus in Europe: what did we learn from the 2018 outbreak? Expert Rev Anti-infective Therapy. 2020;18:145–54. https://doi.org/10.1080/14787210.2020.1713751.
30. Krol L, Blom R, Dellar M, et al. Interactive effects of climate, land use and soil type on *Culex pipiens/torrentium* abundance. One Health. 2023;17:100589. https://doi.org/10.1016/j.onehlt.2023.100589.
31. Tuten HC. Habitat Characteristics of Larval Mosquitoes in Zoos of South Carolina. USA moco. 2011;27:111–9. https://doi.org/10.2987/10-6061.1.
32. Bouchez-Zacria M, Calenge C, Villers A, et al. Relevance of the synergy of surveillance and populational networks in understanding the Usutu virus outbreak within common blackbirds (*Turdus merula*) in Metropolitan France, 2018. Peer Community J. 2025;5. https://doi.org/10.24072/pcjournal.513.
33. Bournez L, Umhang G, Faure E, et al. Exposure of Wild Ungulates to the Usutu and Tick-Borne Encephalitis Viruses in France in 2009–2014: Evidence of Undetected Flavivirus Circulation a Decade Ago. Viruses. 2019;12:10. https://doi.org/10.3390/v12010010.
34. WOAH. (2024) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 13th ed. Paris, France.
35. Beck C, Desprès P, Paulous S et al. (2015) A High-Performance Multiplex Immunoassay for Serodiagnosis of Flavivirus-Associated Neurological Diseases in Horses. BioMed Research International 2015:678084. https://doi.org/10.1155/2015/678084
36. Brown LD, Cai TT, DasGupta A. Interval Estimation for a Binomial Proportion. Stat Sci. 2001;16:101–33. https://doi.org/10.1214/ss/1009213286.
37. Dohoo I, Martin W, Stryhn H. (2009) Veterinary epidemiologic research, Second edition. VER Inc., Charlottetown, Prince Edward Island, Canada.
38. McCullagh P, Nelder JA. Generalized Linear Models. 2nd ed. London: Chapman & Hall/CRC; 1989.
39. Venables W. BD Ripley (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.
40. Hurvich CM, Tsai C-L. Model selection for extended quasi-likelihood models in small samples. Biometrics. 1995;51:1077–84. https://doi.org/10.2307/2533006.
41. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2025.

42. Kamiš J, Grymová V, Suvorov P, et al. First report of Usutu virus fatal infections in Chilean tinamous (*Nothoprocta perdicaria*), brahminy starlings (*Sturnia pagodarum*), and multiple other bird species in zoological gardens and wildlife in the Czech Republic. One Health Outlook. 2026;8:4. https://doi.org/10.1186/s42522-025-00191-3.
43. Lumeij JT. Usutu virus. In: Speer BL, editor. Current therapy in avian medicine and surgery. St. Louis, MO: Elsevier; 2016. pp. 98–106.
44. Störk T, de le Roi M, Haverkamp A-K, et al. Analysis of avian Usutu virus infections in Germany from 2011 to 2018 with focus on dsRNA detection to demonstrate viral infections. Sci Rep. 2021;11:24191. https://doi.org/10.1038/s41598-021-03638-5.
45. Sohier C, Breman FC, Vervaeke M, De Regge N. West Nile Virus Monitoring in Flanders (Belgium) During 2022–2023 Reveals Endemic Usutu Virus Circulation in Birds. Transbound Emerg Dis. 2024;2024:4146156. https://doi.org/10.1155/tbed/4146156.
46. Thrusfield M. (2013) Veterinary epidemiology, Third edition. John Wiley & Sons.
47. Engel D, Jöst H, Wink M, et al. Reconstruction of the Evolutionary History and Dispersal of Usutu Virus, a Neglected Emerging Arbovirus in Europe and Africa. mBio. 2016;7. 10.1128/mbio.01938-15.
48. Höfle U, Gamino V, de Mera IGF, et al. Usutu Virus in Migratory Song Thrushes, Spain. Emerg Infect Dis. 2013;19. https://doi.org/10.3201/eid1907.130199.
49. Cadar D, Maier P, Müller S, et al. Blood donor screening for West Nile virus (WNV) revealed acute Usutu virus (USUV) infection, Germany, September 2016. Euro Surveill. 2017;22:30501. https://doi.org/10.2807/1560-7917.ES.2017.22.14.30501.
50. Giorgiadis M, Guillot J, Duval L, et al. Haemosporidian parasites from captive Strigiformes in France. Parasitol Res. 2020;119:2975–81. https://doi.org/10.1007/s00436-020-06801-5.
51. Rouffaer LO, Steensels M, Verlinden M, et al. Usutu Virus Epizootic and Plasmodium Coinfection in Eurasian Blackbirds (*Turdus merula*) in Flanders, Belgium. J Wildl Dis. 2018;54:859–62. https://doi.org/10.7589/2017-07-163.
52. Agliani G, Giglia G, de Bruin E, et al. The pathology of co-infection with Usutu virus and *Plasmodium* spp. in naturally infected Eurasian blackbirds (*Turdus merula*). One Health. 2023;16:100534. https://doi.org/10.1016/j.onehlt.2023.100534.
53. Hattendorf C, Cadar D, Bosch S, et al. Weak association of Usutu virus and haemosporidian infection in birds collected in Germany. One Health. 2024;19:100868. https://doi.org/10.1016/j.onehlt.2024.100868.
54. Perkins R, Whitehead M, Civil W, Goulson D. Potential role of veterinary flea products in widespread pesticide contamination of English rivers. Sci Total Environ. 2021;755:143560. https://doi.org/10.1016/j.scitotenv.2020.143560.
55. Tassin de Montaigu C, Glauser G, Guinchard S, Goulson D. High prevalence of veterinary drugs in bird's nests. Sci Total Environ. 2025;964:178439. https://doi.org/10.1016/j.scitotenv.2025.178439.
56. Derraik J. Recommendations for mosquito control in zoological parks to reduce disease transmission risk. Weta. 2005;29:16–20.

57. Virgillito C, Manica M, Marini G, et al. Evaluation of *Bacillus thuringiensis* Subsp. *Israelensis* and *Bacillus sphaericus* Combination Against *Culex pipiens*. Highly Vegetated Ditches J Am Mosq Control Association. 2022;38:40–5. https://doi.org/10.2987/21-7024.
58. Meister T, Lussy H, Bakonyi T, et al. Serological evidence of continuing high Usutu virus (Flaviviridae) activity and establishment of herd immunity in wild birds in Austria. Vet Microbiol. 2008;127:237–48. https://doi.org/10.1016/j.vetmic.2007.08.023.
59. Chvala S, Bakonyi T, Bukovsky C, et al. Monitoring of Usutu virus activity and spread by using dead bird surveillance in Austria, 2003–2005. Vet Microbiol. 2007;122:237–45. https://doi.org/10.1016/j.vetmic.2007.01.029.
60. Salgado R, Hawks SA, Frere F, et al. West Nile Virus Vaccination Protects against Usutu Virus Disease in Mice. Viruses. 2021;13:2352. https://doi.org/10.3390/v13122352.
61. Bosco-Lauth AM, Kooi K, Hawks SA, Duggal NK. Cross-Protection between West Nile Virus and Emerging Flaviviruses in Wild Birds. Am J Trop Med Hyg. 2025;112:657–62. https://doi.org/10.4269/ajtmh.24-0363.
62. Bergmann F, Fischer D, Fischer L, et al. Vaccination of Zoo Birds against West Nile Virus—A Field Study. Vaccines. 2023;11:652. https://doi.org/10.3390/vaccines11030652.

Figures

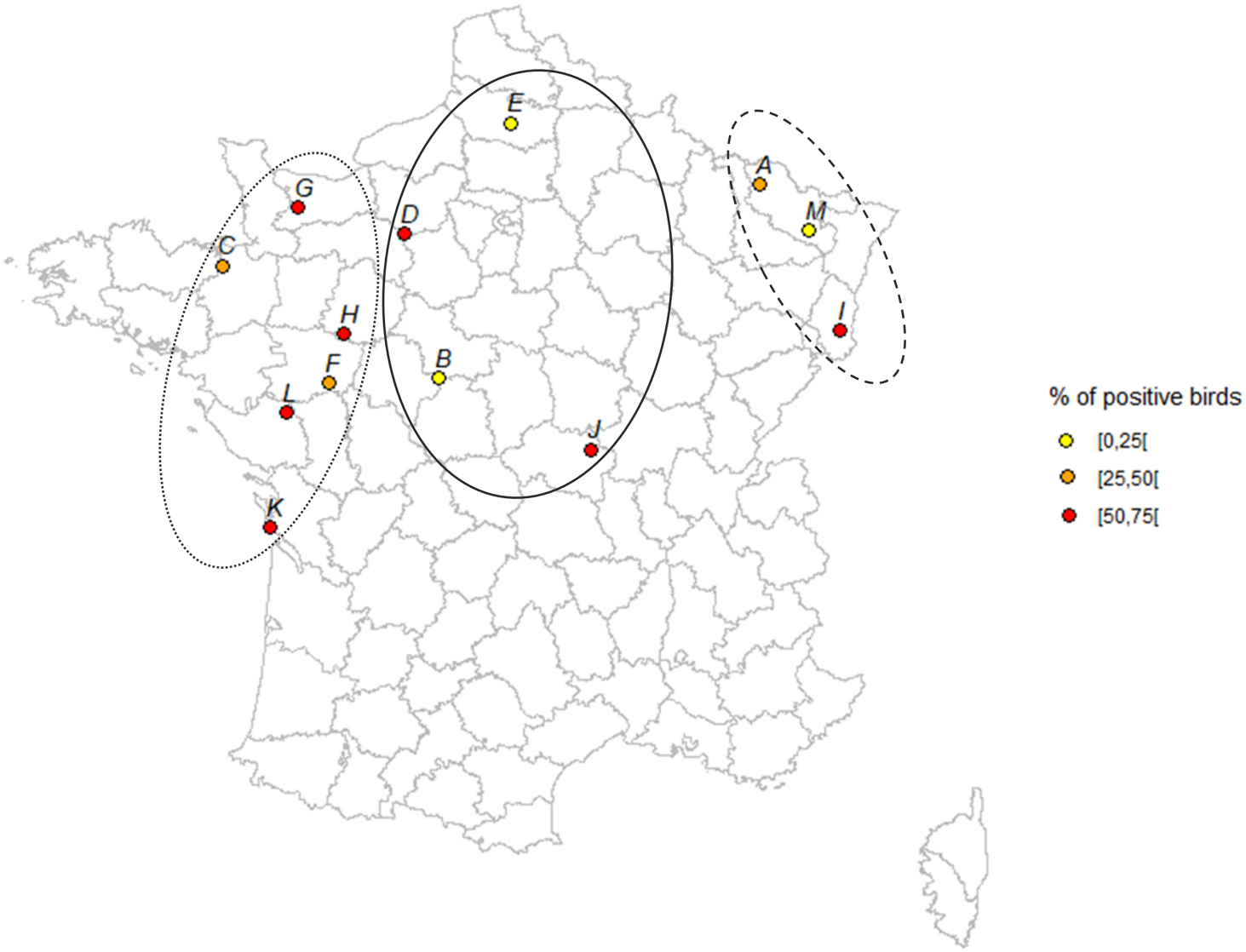


Figure 1

Proportion of USUV-positive birds per zoo in France (letters refer to the zoo; dotted line ellipse: western zoos, solid line ellipse: central zoos, broken line ellipse: eastern zoos; a positive bird was one that was cELISA-positive at least once between 2018 and 2020, with USUV prior exposure confirmed by the USUV VNT).

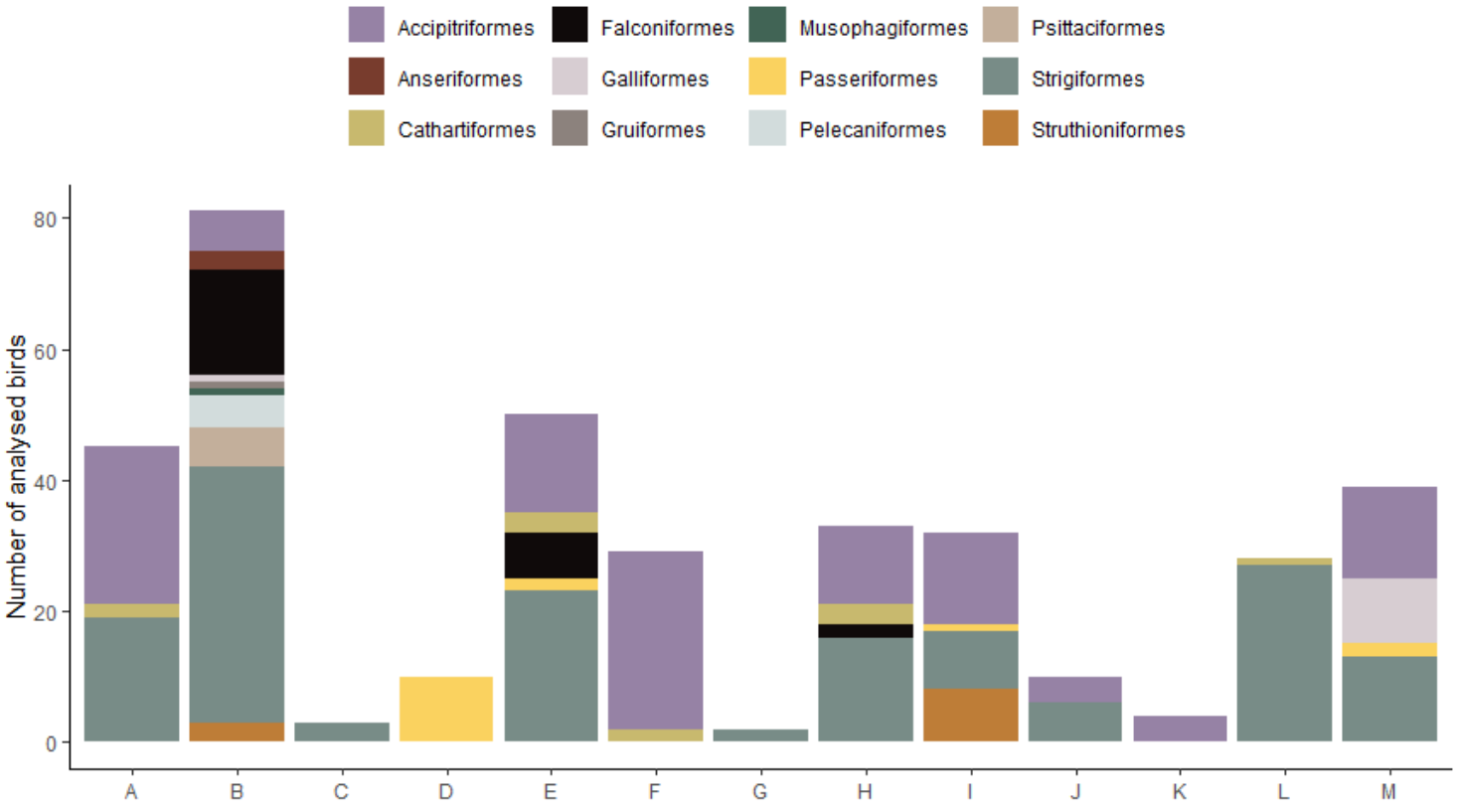


Figure 2

Distribution of analysed birds per zoo and order in France between 2018 and 2020.

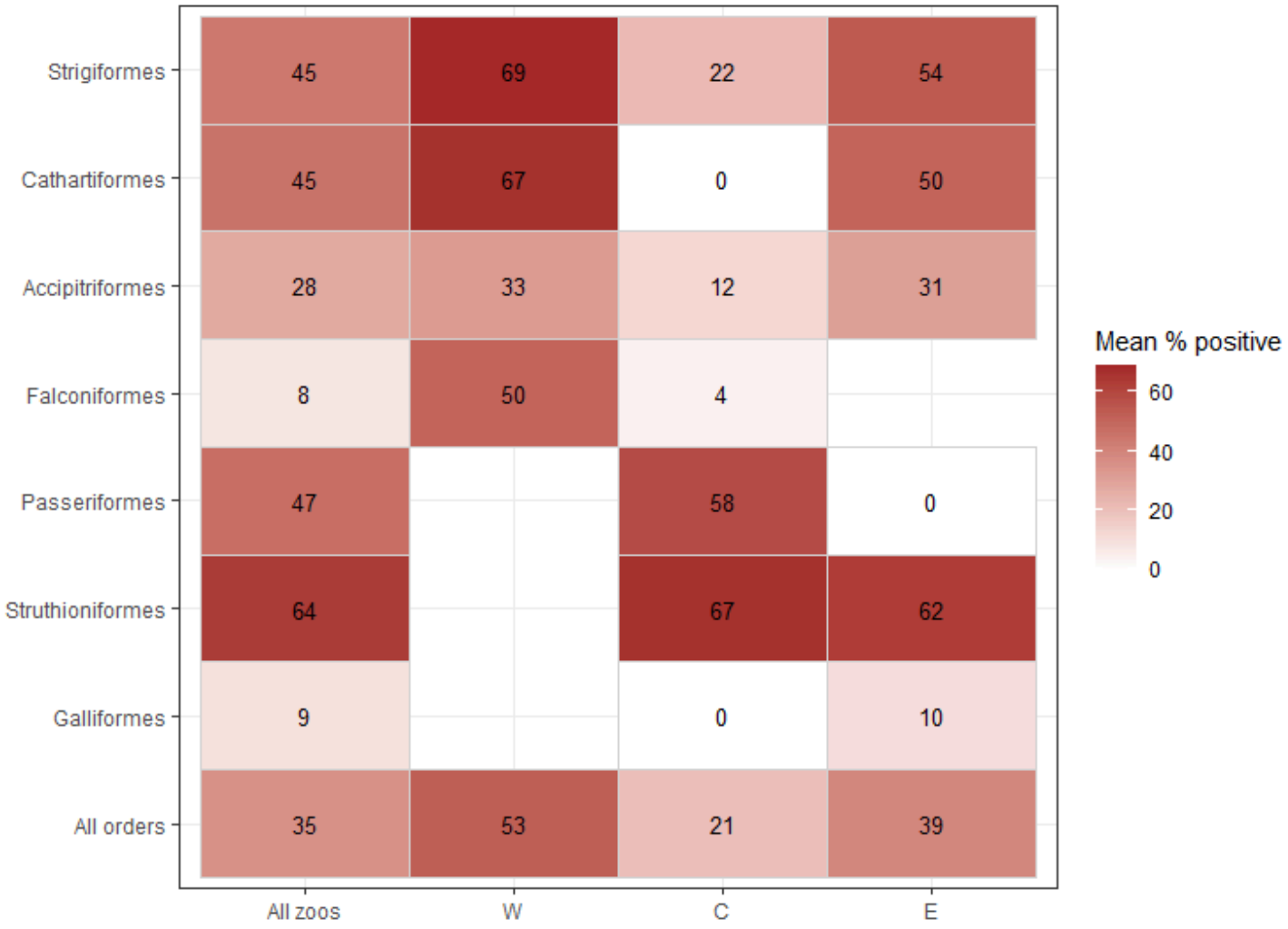


Figure 3

Proportion of USUV-positive birds per zoo and order in France (only data considered in the multivariate logistic regression model are shown; a positive bird was one that was cELISA-positive at least once between 2018 and 2020, with USUV prior exposure confirmed by the USUV VNT).

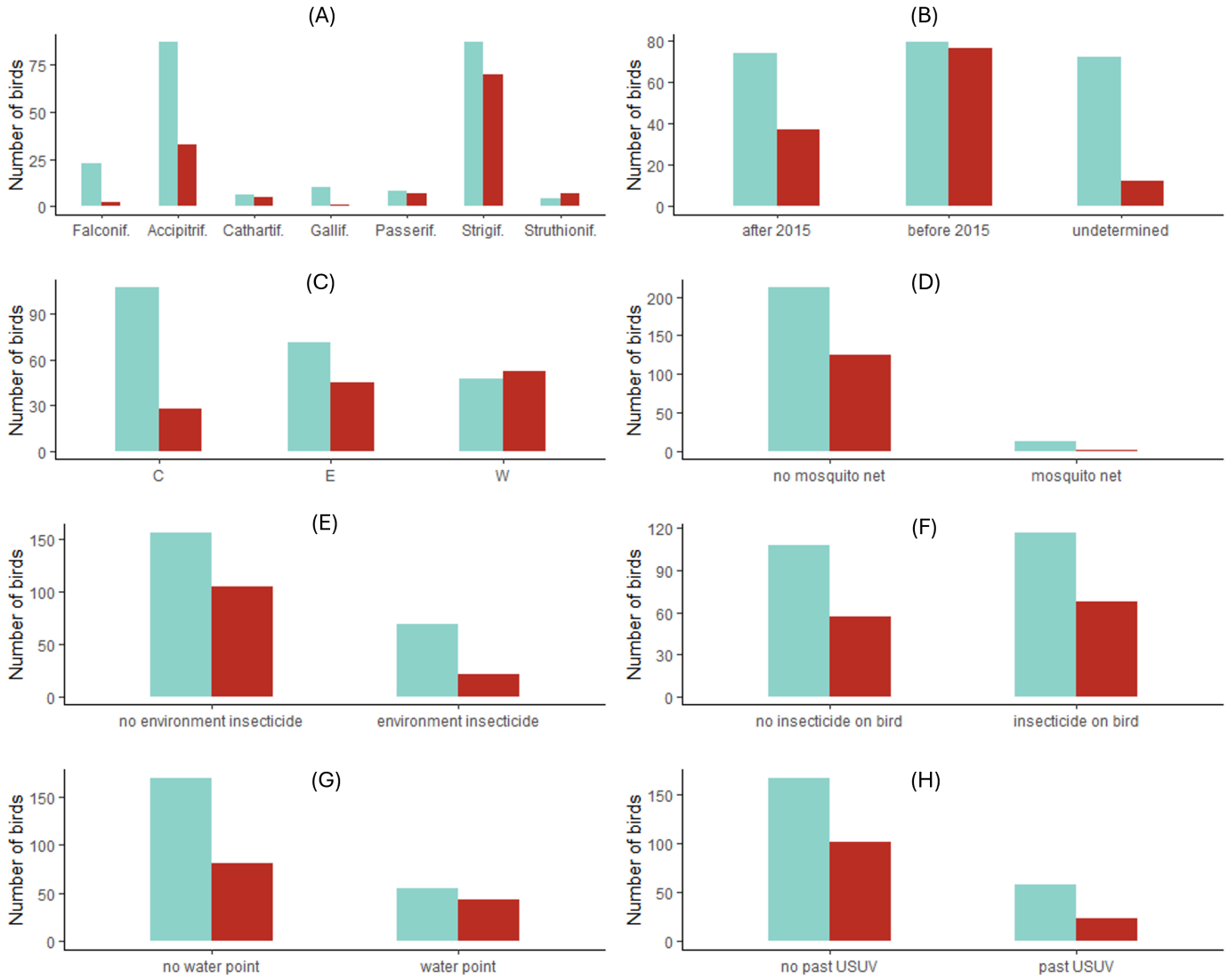


Figure 4

Distribution of positive and negative birds for USUV depending on variables included in the model (positive birds: red bars; negative birds: light blue bars; A: order, B: year of birth and C: zoo location, D : the presence or absence of mosquito nets , E: environment insecticide , F: bird insecticide, G: water point , H: past infection with USUV; only data considered in the multivariate logistic regression model are shown; a positive bird was one that was cELISA-positive at least once between 2018 and 2020, with USUV exposure confirmed by the USUV VNT; birds were considered negative if they showed no evidence of USUV exposure (cELISA-negative) at any time between 2018 and 2020).

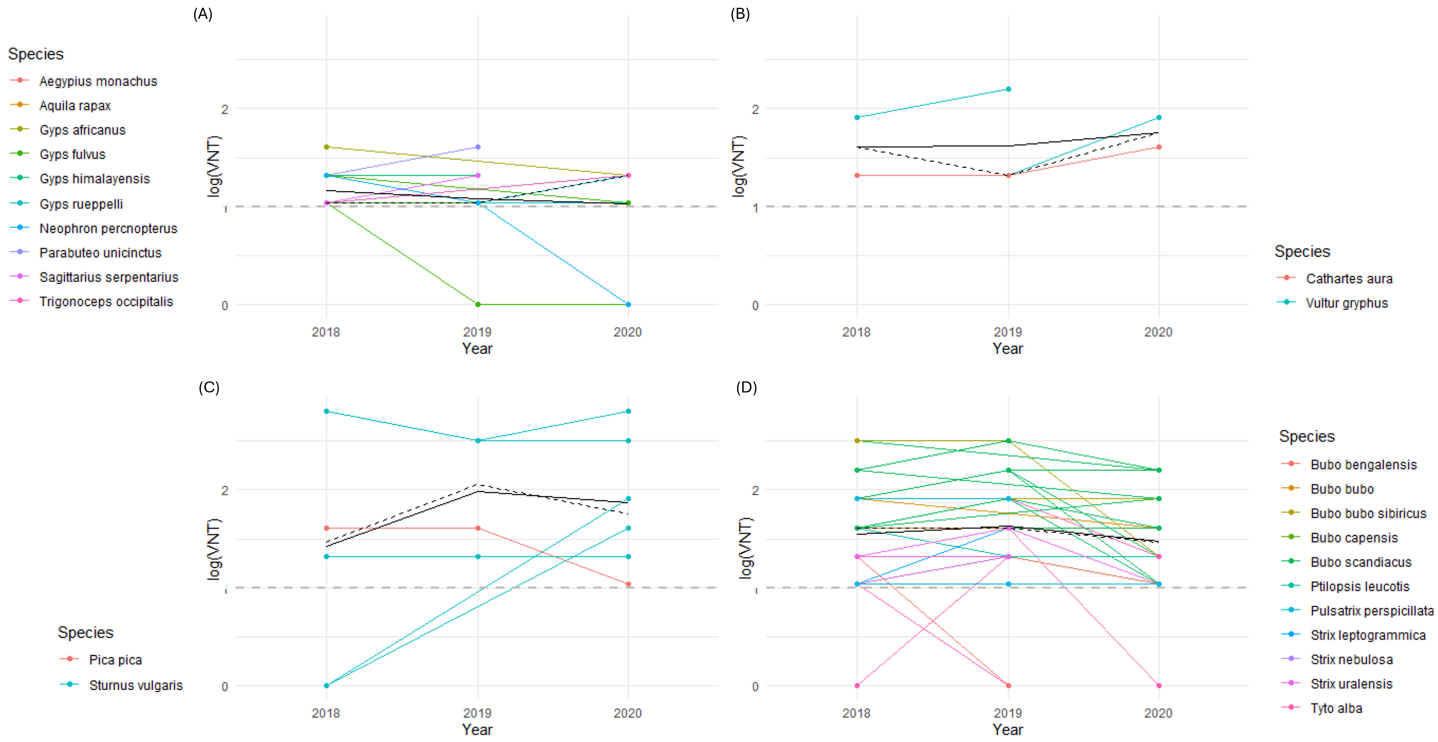


Figure 5

Individual kinetics of USUV immune response per species between 2018 and 2020 (A: Accipitriformes, B: Cathartiformes, C: Passeriformes, D: Strigiformes, grey dashed line: antibody threshold below which immunity is considered non-protective, dark dashed line: median antibody titre value, dark solid line: mean antibody titre value; titre values were measured by the virus neutralisation test (VNT)).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- additionalfile1.csv
- additionalfile2.csv
- additionalfile3.docx