Relevance of the synergy of surveillance and populational networks in understanding the Usutu virus outbreak within common blackbirds (*Turdus merula*) in Metropolitan France, 2018

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Abstract

Usutu virus (USUV) was first isolated in Africa in 1959 and has since spread to and through Europe with a typical enzootic mosquito-bird cycle. In France, it was first detected in birds in 2015, but in 2018 the spread of USUV was particularly significant throughout the country, killing mainly

common blackbirds (*Turdus merula*) and to a lesser extent great grey owls (*Strix nebulosa*), among other captive and non-captive wild bird species. Data on this 2018 outbreak were collected through both an event-based network (SAGIR) and the health surveillance of the French-speaking Association of Zoo Veterinarians (AFVPZ). In addition, common blackbird populations could be monitored through another network (REZOP). Statistical analysis (spatial, temporal, spatiotemporal and environmental determinants) of the SAGIR and AFVPZ networks helped to highlight the early appearance of separate large clusters of USUV cases in mid-July 2018, the subsequent diffusion into smaller and secondary clusters at the end of August 2018, and a meanwhile enlargement of the first clusters with an increase in the number of cases. The locations of USUV cases were also significantly associated with high human density and wetlands. Using generalised additive mixed models on REZOP data, we also highlighted the decline in common blackbird population trends in areas with high USUV pressure (areas defined based on SAGIR-AFVPZ data) following the 2018 outbreak. Our results highlighted the need to work with synergistic networks to assess infection spread in wild bird species, as well as the negative impact of an emerging arbovirus.

Introduction

The Usutu virus (USUV), so called because of its isolation in *Culex neavei* mosquitoes in 1959 (McIntosh 1985) near the Usutu river in Swaziland (Eswatini since 2018) is an arbovirus of the *Flaviviridae* family, *Orthoflavivirus* genus (Clé et al. 2019), belonging to the Japanese encephalitis virus serocomplex, and phylogenetically close to Japanese Encephalitis Virus (JEV) and West Nile Virus (WNV) (Calisher and Gould 2003). After South Africa, the virus was detected in other African countries: Central African Republic, Senegal, Ivory Coast, Nigeria (Nikolay et al. 2011), Uganda (Nikolay et al. 2011; Mossel et al. 2017), Burkina Faso (Nikolay et al. 2011), Mali, Madagascar (Chevalier et al. 2020), Kenya (Ochieng et al. 2013), Tunisia (Ben Hassine et al. 2014; M'ghirbi et al. 2023), Morocco (Durand et al. 2016), and Israel (Mannasse et al. 2017). The first known occurrence in Europe dates back to 1996 in Italy (a retrospective finding in dead birds, mainly blackbirds)(Weissenböck et al. 2013). Since then, first detections of USUV followed in other European countries: Austria (Weissenböck et al. 2002) in 2001, Czech Republic (Hubálek et al. 2008a)) in 2004, Hungary (Bakonyi et al. 2007) in 2005, Poland (Hubálek et al. 2008b), Spain (Busquets et al. 2008) and Switzerland (Steinmetz et al. 2011) in 2006, Serbia in 2009 (Lupulovic et al. 2011), Germany (Jöst et al. 2011), Greece (Chaintoutis et al. 2014) and Slovakia (Csank et al. 2018) in 2010, Croatia (Barbic et al. 2013) in 2011, Belgium (Garigliany et al. 2014) in 2012, France

(Lecollinet et al. 2016) in 2015, Netherlands (Rijks et al. 2016) in 2016 and United Kingdom (Folly et al. 2020) in 2020 (Vilibic-Cavlek et al. 2020) .

The analysis of 92 complete USUV genomes, including 77 genomes from mosquito, bird and bat species of Germany (2010-2014) highlighted that i) USUV can be classified in different lineages (Engel et al. 2016), (eight in number – three African and five European (Cadar et al. 2017; Clé et al. 2019)), ii) the most common ancestor emerged in Africa at the beginning of the 16th century, iii) USUV was regularly introduced from Africa during the last 50 years (a first introduction from 1950 through 1960's in Western Europe (Spain), a second one between 1970 and the 1980's in Central Europe (Austria) and a third one around 1996 in Western Europe (Spain)) and iv) *in situ* evolution could explain the genetic diversity of European lineages, while extensive gene flow drove African ones. In addition, flyway networks of migratory birds were strongly consistent with spatial movements observed through genetic data and suggested the possibility of USUV exportation from Africa i) to Spain via the east Atlantic and/or Black Sea/Mediterranean flyways and ii) to Central Europe through the Black Sea/Mediterranean flyway (Engel et al. 2016).

The natural life cycle of USUV involves passeriform and strigiform birds as amplifying hosts and ornithophilic species of mosquitoes as vectors (Clé et al. 2020). The virus has been detected in several Culicidae (*Culex*, *Aedes, Culiseta*, *Mansonia*) (Clé et al. 2019) but *Cx. pipiens* (experimentally shown to be USUV competent (Martinet et al. 2023)) is considered as the main vector in Europe (Becker et al. 2012; Martinet et al. 2023). To date, several incident or dead-end hosts (which certainly develop a low viremia, insufficient to re-infect mosquitoes (Martinet et al. 2023)) have been identified (Vilibic-Cavlek et al. 2020) : horses (Durand et al. 2016; Bażanów et al. 2018; Csank et al. 2018), dogs (Durand et al. 2016), rodents (Diagne et al. 2019), squirrels (Romeo et al. 2018), wild boar (Escribano-Romero et al. 2015; Bournez et al. 2019) and roe deer (Bournez et al. 2019). The virus has also been detected in bats, whose epidemiological role is as yet undetermined (Cadar et al. 2014). The virus has a zoonotic potential as humans (incident hosts) have been found infected with different clinical pictures: fever and rash in Africa (Ashraf et al. 2015), neuroinvasive infections (Gaibani and Rossini 2017) in Europe (meningoencephalitis (Cavrini et al. 2009; Pecorari et al. 2009); idiopathic facial paralysis (Simonin et al. 2018)). However, these manifestations remain anecdotal and the asymptomatic form appears to predominate, as demonstrated by incidental findings during screening of asymptomatic blood donors and seroprevalence studies (Angeloni et al. 2023).

In Africa, USUV seems not to be pathogenic for local bird populations, either because it is not a naturally virulent virus, or because of immunity or genetic resistance of birds, which have been in

contact with related flaviviruses for a long time (Bakonyi et al. 2004). In Europe, common blackbirds (*Turdus merula*) are particularly affected, and to a lesser extent some birds of prey (Giglia et al. 2021), such as the great grey owl (*Strix nebulosa*) (Clé et al. 2019). The lesion patterns of 160 common blackbirds in the Netherlands included hepatosplenomegaly (major symptom), coagulative necrosis, lymphoplasmacytic inflammation and vasculitis. Reported symptoms included non-specific ones (immobility, apathy and ruffled plumage) and neurological ones (depression, stiff neck, inability to fly and epileptic seizures) (Giglia et al. 2021).

In several European regions, epidemiological patterns increasingly suggest endemic profiles rather than repeated introductions from other endemic countries (Constant et al. 2022), without clear explanation of efficient overwintering and subsequent amplification (Clé et al. 2019). Moreover, co-circulation with WNV is frequent (Beck et al. 2013; Fros et al. 2015; Constant et al. 2020). These situations have led European countries such as Italy to institute annual surveillance (entomological, human and veterinary), rather than focusing on a specific season (Constant et al. 2022).

In France, the first virus detection (in August and September 2015) took place in the North-east (Haut-Rhin department) and Central-east of France (Rhône department), respectively in two and three dead common blackbirds (*Turdus merula*) collected through SAGIR, the French event-based surveillance network collecting and analysing dead free-ranging wildlife animals (see details in next paragraph). Phylogenetic analysis helped demonstrating proximity between isolates i) from Haut-Rhin and Germany and ii) from Rhône and Spain. In addition, isolates identified in the two French departments belonged to different USUV lineages (Europe 3 in Haut-Rhin, Africa 2 in Rhône). Those results suggested a different source of introduction for the two French departments (Lecollinet et al. 2016). Since 2015, outbreaks in French avifauna have been recorded yearly in 2016, 2017 and 2018, with new departments involved (Johnson et al. 2018). In 2018, the Usutu virus circulated earlier and more extensively than it had in preceding years (Épidémiosurveillance Santé animale (ESA) 2018). In Camargue (South of France), which is located at the crossroad of several bird migration routes (Vittecoq et al. 2013)) two lineages (Africa 2 and Africa 3) were found in 2016 among female *Cx. pipiens*, suggesting two independent introductions of the virus: i) one from West to East and probably explained by migratory birds following the East Atlantic flyways (Africa 2 lineage) and ii) one explained by migratory birds following a central Mediterranean flyway and spreading after to Northern Europe, or originating from Germany (Africa 3) (Eiden et al. 2018). The USUV detection, in the southern region of Camargue, of same lineage, in same region and for consecutive years (2015, 2018 and 2020) in mosquitoes highly suggests endemicity (Constant et al. 2022).

Until now, studies on USUV in France focused on emergences and genetic characterisation to reconstruct introduction paths (Vittecoq et al. 2013; Lecollinet et al. 2016; Eiden et al. 2018; Constant et al. 2022), but not on spatial, temporal, spatio-temporal and populational aspects of the virus diffusion within the country. The aim of our study was twofold: i) from an epidemiological perspective, we first analysed the USUV diffusion among related bird populations to understand the way the disease had spread throughout mainland France and thus to define an epidemiologic pattern of this 2018 bird outbreak and its relationships with environmental variables (in particular wetlands, supposed to be a proxy for the density of mosquitoes); ii) from an ecological perspective, we tried to understand how this diffusion could have impacted common blackbird populations.

For the epidemiological perspective (both infectious and spatial approach), we used data from the French event-based surveillance network named 'SAGIR' which is dedicated to epidemiological surveillance, through the collection of dead or moribund birds and mammals (opportunistic sampling method). This participatory surveillance program aims at detecting as early as possible abnormal mortality or morbidity signals (Millot et al. 2017; Decors et al. 2022). Following the emergence of USUV in Europe, the vigilance of the SAGIR network has been reinforced through several messages sent to its participants and which helped to the early detection of the virus in bird populations. Thus, data from SAGIR network offer an opportunity to study the epidemiology of USUV (date, location, and size of clusters), by identifying the timing and geography of reporting of dead birds positive to USUV in France. This dataset can be completed with the data from the French Association of Zoo Veterinarians (AFVPZ), as this association also carries out health surveillance on ill or dead zoo animals.

We expected that in clusters with high density of dead birds identified from this dataset, the high mortality would have an impact on the population dynamics of wild birds. Common blackbirds, in particular, are of interest as this species is the most affected species by USUV in France. The impact of USUV on common blackbird populations can be assessed thanks to the data collected by another French observation network named "REZOP" (Réseau Oiseaux de Passage, passing birds network), which is dedicated to the evaluation of bird population dynamics and focuses on non-captive wild Alaudidae, Columbidae, Turdidae, Phasianidae, Corvidae and Sturnidae. In this program, birds belonging to the focus species are counted every year, through a systematic sampling method and collected data are statistically analysed to define population trends (Villers et al. 2021).

We hypothesised that USUV circulation in 2018 resulted in decreased common blackbird populations in the years following the 2018 epizootics, based on a previous study on blackbirds

dead following USUV infection in Germany between 2011 and 2015 (Lühken et al. 2017). Although the approach of this German study was slightly different, as we will discuss in the dedicated part of this paper, we also expected that USUV circulation in birds would be associated with some environmental variables and would have a negative impact on common blackbird population trends.

Materials and methods

USUV diffusion process

Epidemiological surveillance network and provided data

Data were provided i) mainly by the event-based surveillance network named 'SAGIR', in which the investigation of death or pathology aetiology requires a cross-disciplinary approach, involving field clues, post-mortem examination and scientific expertise (Millot et al. 2017); and ii) by the AFVPZ surveillance of dead animals in zoos (with systematic necropsy and analysis of additional samples depending on observed lesions and ante-mortem clinical picture).

SAGIR is based on a collaboration between the French Office of Biodiversity (OFB), who administrates the network, hunters, local and national federations of hunters and the French Association of directors and executives of public veterinary analysis laboratories (Decors et al. 2022). In each *department* (a department being a French administrative district covering in average 5,700 km² [SD = 2,500 km²]), two departmental technical contacts (one belonging to the departmental service of OFB, the other belonging to the departmental federation of hunters) (Decors et al. 2022) coordinate a network of volunteers (professionals, as well as hunters, naturalists and farmers) who can report abnormal events (mortality or morbidity) that they accidentally find in the field. The sampling of these events is therefore opportunistic. Note that the reported events can also punctually include dead animals of local rescue centres (facility operated by non-profit organizations, designed to provide aid, care, and shelter to injured, sick, or orphan wild animals found in the field). For traceability purposes, an individual form is filled for each mortality event collected, reporting epidemiological, agricultural and ecological circumstances surrounding the discovery of carcasses. Where available, clinical signs are also provided (Millot et al. 2017), deduced from observation of the carcass and signs left in the environment. Sometimes animals are seen alive before they die (e.g. 12% of common blackbirds in the database from 2014 to 2023, regardless of cause of death) (A. Decors, personal communication). The digitisation of forms and the standardisation of data associated with the reported animals was implemented in 2014 (Decors et

193 al. 2022). Moribund animals can also be filmed to get veterinary expertise. Carcasses (fresh, chilled
194 or frozen) accompanied by their form are addressed to the local administrative laboratory of
195 veterinarian analyses where necropsies are realized. Following a gross pathologic examination,
196 relevant aetiology tests are conducted: parasitology, bacteriology, virology, mycology, toxicology
197 and/or histology (Millot et al. 2017).

198 Following the emergence of USUV in Europe, the vigilance of the SAGIR network has been
199 reinforced: a first alert message was sent to local coordinators on the 2nd of May 2012 warning them
200 of the presence of the virus in Germany and the need to be vigilant in case of abnormal common
201 blackbird mortality (to consider USUV analysis in such cases). On 21 September 2015, the first
202 detection of USUV in common blackbirds in France was shared through another alert message
203 and recommended the inclusion of USUV in the differential diagnosis in cases of abnormal
204 mortality or nervous symptoms in owls, blackbirds, tits, sparrows, robins and starlings. Early
205 detection of the 2018 USUV outbreak was allowed by a well-informed network. Hence, on the 3rd
206 of August 2018, an alert message informed the network that abnormal mortality had been observed
207 in common blackbird populations in several departments for the past fortnight and that USUV
208 had already been confirmed in one of them. Recommendations instigated in 2015 were therefore
209 reiterated.

210 In AFVPZ network, dead zoo animals are systematically necropsied within a maximum of 20 hours
211 (usually less than six hours after death). The zoo veterinarian performs the necropsy and takes
212 biological samples when lesions are observed, for analysis (bacteriological, mycological,
213 parasitological, or virological if relevant). The samples are sent to the nearest departmental
214 veterinary laboratory or, in some cases, to a laboratory specialised in the species concerned.
215 Histological analysis is also carried out almost systematically. In 2018, abnormal mortalities have
216 been observed in zoos and as a consequence, the French Agency for Food, Environmental and
217 Occupational Health & Safety (Anses), which had access to the two sets of data, was able to
218 combine them and then linked AFVPZ and SAGIR surveillance networks. As the two networks
219 coordinators interact regularly, the data collected by AFVPZ were transferred to the SAGIR
220 database (note that the data were pooled for the purposes of the study, but not integrated into the
221 SAGIR database, since the AFVPZ data concerned captive fauna). These were the first cases in
222 captive wildlife in France (as opposed to wild birds) and as soon as they were suspected, the zoo
223 veterinarians concerned contacted the SAGIR network.

224 Thus, birds in our study were collected through the SAGIR network and zoological gardens from
225 July 15th to August 31st, 2018 (after this latter date, a message was sent to the SAGIR network

226 asking to rationalise collections and analyse only one sample per department, because of the
227 additional cost of analyses linked to the increased epizootic outbreak; data were thus defined at the
228 department level from September onwards). Before August 31st, the most precise geographical
229 information for each reported case was the municipality (a municipality being the smallest French
230 administrative subdivision, corresponding to town and village areas, with a median area of 9 km^2).
231 After necropsy examination, each bird collected within SAGIR was tested by the National
232 Reference Laboratory (NRL, Anses) for USUV and WNV through Real-Time Polymerase Chain
233 Reaction (hereafter RT-PCR) detection on liver, spleen and brain (sometimes kidney and heart).
234 Tissues samples were stored at -80°C and before RT-PCR analyses, were grinded in Dulbecco
235 modified Eagle's minimal essential medium (DMEM) with ceramic beads (MP Biomedicals,
236 Illkirch, France) and FastPrep ribolyzer in BSL3 facilities. Automated total RNA extraction was
237 performed with the QIAamp Viral RNA kit (Qiagen, Hilden, Germany) or the MagVet™ Universal
238 Isolation kit (ThermoFisher Scientific, Lissieu, France) on extraction robots QIAcube and
239 Kingfisher respectively. USUV genome was amplified and detected with primers UV_F and R and
240 probe UV_P, as described in (Moutailler et al. 2019) using the AgPath-ID™ One-Step RT-PCR
241 Reagents (Thermofisher Scientific). Primers and probes were used at a concentration of 0.4µM and
242 0.2µM, respectively. The samples were maintained at 45°C for 10 min and at 95°C for 10 min and
243 then subjected to 40 cycles consisting of incubations at 95°C for 15s and at 60°C for 60s. Birds
244 collected from zoos through the AFVPZ network were also tested by the NRL, using the same
245 protocol, if i) their ante-mortem clinical picture was suggestive of an USUV infection (e.g.
246 neurological symptoms) and/or ii) the epidemiologic context was consistent with a USUV
247 suspicion (close mortality among sensitive species, and especially great grey owls) and/or iii) lesions
248 at post-mortem examination were suggestive of an USUV infection (liver, spleen, cerebral
249 necrosis). Spleen, liver, brain, kidney and lung samples were then sent to the NRL.

250 In our study, we focused only on birds reported USUV infected, and we discarded other reported
251 birds. We found it easier to analyse the space and time structure with point patterns (availability of
252 a large variety of methods to deal with such data) so that we transformed our dataset into a point
253 pattern. Thus, for each reported bird, we randomly attributed a precise point location by randomly
254 selecting a point within the municipality where it was collected. Hereafter, the terms 'points' or
255 'cases' will refer to these birds. All USUV-infected birds were considered, whatever their species
256 or origin (captive versus non-captive).

257 We analysed the temporal, spatial and spatio-temporal distribution of reported cases (see below),
258 as well as the relationship with spatial clusters of cases with wetlands and with human density.
259 Wetlands and human density were supposed to be a proxy for the density of *Culex pipiens*

mosquitoes (Vogels et al. 2016). Human density was also supposed to be a proxy for common blackbirds sampling pressure, as those birds are highly commensal with humans and easy to detect in gardens. A map of potential wetlands was derived from a raster map of continental France built by INRAE (Orléans) (http://geowww.agrocampus-ouest.fr/web/?p=1538): the INRAE map estimates the probability that each 1km x 1km pixel of the map harbours a wetland and then discretize this probability in three classes (0 = no wetlands, 1 = rather strong probability, 2 = strong probability, or 3 = very strong probability). This map also identifies lakes and foreshores in France. We downloaded this map, and redefined pixels with lakes and foreshores as "confirmed wetlands" (new class 4). Then, we smoothed this map using a sliding window, by calculating for each pixel the sum of this ordered factor (0 to 4) for the focus pixel as well as the pixels immediately on the left, the right, the top and the bottom of the focus pixel (leading to a score comprised between 0 and 20 giving a smoothed probability of wetland in a given place).

A map of human density was calculated by finding the municipality in which the centre of each 1km x 1km pixel was located and attributing the 2005 human density to it (number of inhabitants divided by municipality area). We then log-transformed this map (transformation: $x \rightarrow log(x+1)$ where x was the number of inhabitants per km^2). The 2005 human density was provided by Institut Geographique National (France) (IGN) (https://geoservices.ign.fr).

We then carried out a statistical analysis of the SAGIR and AFVPZ data aiming at identifying spatial and spatio-temporal patterns (see below). All our analyses were carried out with the R software (R Core Team 2022). We have programmed an R package named usutuFrance, available at https://github.com/ClementCalenge/usutuFrance, containing all the code and data used to fit the model. It can be installed in R with the package devtools, using the function `devtools::install_github("ClementCalenge/usutuFrance", ref="main")`. This package includes a vignette describing how the user can reproduce the calculations carried out in this paper (vignette available with the command vignette("usutuFrance") once the package has been installed and contains supplementary analyses that helped to understand the structure of our data. This vignette serves as the supplementary material of our paper.

Spatial analysis

We first used a Ripley's K function to identify the scales at which clusters of points could be identified (Diggle 2013). This function allows the characterisation of second order point pattern properties: it is proportional to the mean number of other cases expected in a x km radius around a typical case of this point pattern (so that the larger it is, and the more there are cases in the neighbourhood of a case in average – in other words, clusters can be observed at this scale). The

K function was computed for all distances *t* comprised between 0 and 250 km on the observed point pattern ($\hat{K}(t)$)); the theoretical value of this function was also calculated for the same range of distances under the hypothesis of complete spatial randomness (CSR) ($K_{CSR}(t)$). Thus, under the hypothesis that the cases are randomly distributed in space, the difference $\hat{L}(t)$ between $\hat{K}(t)$ and $K_{CSR}(t)$ should be equal to 0 (Baddeley et al. 2014), so that positive deviations of $\hat{L}(t)$ from zero reflect clustering in the data (and conversely, negative values reflect repulsion mechanisms). We also built confidence envelopes around simulated $L_r(t)$ functions expected under CSR: we repeated n=100 simulations of the CSR (by randomly distributing *N* cases in France, where *N* is the observed number of cases in our study), and for each simulation *r*, we estimated the function $L_r(t)$ for all values of t from 1 km to 250 km. We plotted the observed value $\hat{L}(t)$ and the distribution of values expected under the CSR hypothesis on the same graph to compare them ($\hat{L}(t) = L_r(t)$: complete random reporting of cases; $\hat{L}(t) > L_r(t)$: more points within a *t* radius around a typical case than under CSR hypothesis ; $\hat{L}(t) < L_r(t)$: less points within a t radius around a typical case than under CSR hypothesis i.e. avoidance process).

To identify areas of high density of cases in the point pattern, we carried out a kernel smoothing of the point pattern, using a smoothing parameter of 125 km (Wand and Jones 1995), to visualize them on a map. The results (see below) suggested that the point pattern could be adequately described by a Thomas process (Diggle 2013), i.e. a process in which cases are clustered in the following way (Baddeley et al. 2014): i) a random number of "parents" is generated from a Poisson distribution parameterized by a parameter κ controlling the density of 'parent' cases per km². These parents are randomly placed in the study area. ii) For each "parent", a random number of "children" (corresponding to the cases in our study) is generated from a Poisson distribution parameterized by a parameter λ controlling the mean number of "children" per "parent", and iii) for each "parent", the children locations are randomly distributed around the parent's location. More precisely, the locations of the children are supposed to be located at a distance from the parent randomly drawn from a semi-normal distribution with a standard deviation σ, with an angle from west direction drawn from a uniform distribution bounded by π and -π. We fitted this process to our dataset using the method of minimum contrasts based on the function *K*. Estimated parameters of the fitted model helped defining the point pattern typical structure and the product of λ and κ provided the cases density per km². Note that our spatial analysis revealed a clustering of the dataset at very small scale (see results), possibly due to the tendency of observers to look for other cases in a municipality where a first case was identified. Consequently, and to improve model fit, we decided to thin the point pattern prior to the fit to remove this artefactual structure,

keeping only the first bird found when two birds were collected at a distance lower than 5 km (see Supplementary material for more details).

Environmental determinants

Then, we used this fitted model to test, using a randomization approach, whether the mean value of the two environmental variables (wetlands and population density) were greater in the places where the dead birds infected by USUV were found than expected by chance (Manly 1991): i) we first calculated the means of the density of wetlands and the mean of the log of human population density within the observed points pattern, ii) we simulated the fitted Thomas process 999 times over France, iii) we calculated for each simulation the mean of the density of wetlands and the mean of the log of human population density (simulated distribution), and iv) we compared the observed values (means) with the simulated distribution (means and standard errors) and determined p-values with the help of a bivariate randomization test.

Temporal analysis

To define the temporal evolution of the number of cases throughout the study period, we computed the mean number of cases per day during this period and smoothed the curve using moving average approach (Diggle 1990): for each *d* day of the study period, we calculated the mean number of cases per day, over a week centred on *d*. We plotted the obtained values on a graph to visualize temporal fluctuations.

Spatio-temporal analysis

We computed a space-time K function ($\hat{K}(u,v)$) (Gabriel et al. 2013) giving the expected number of cases within a distance of u km around a randomly sampled case of the observed point pattern (for all values of *u* comprised between 0 and 250 km), and which occurred at most v days after this case (for all values of *v* comprised between 0 and 10 days). To test whether the spatio-temporal patterns identified by this function could have been obtained by chance, we also used a randomization approach. More precisely, for each one of the 1,000 simulations of this approach, we randomly permuted the dates associated to the reported birds (i.e. the cases order was randomised), thus conserving the spatial and temporal point pattern structures but randomising the association between space and time. For each simulation, we calculated the related $K(u,v)$ on the randomized dataset. We then compared, for each distance *u* and each duration *v*, the observed value of $\hat{K}(u,v)$ to the distribution of simulated values $K(u,v)$. Probabilities that a simulation could lead to $K(u,v) > \hat{K}(u,v)$ were plotted, with duration on the x-axis, distances on the y-axis (probabilities near zero indicating clustering) and a contour limit identifying the set of

distance/duration pairs for which the proportion of simulation with $K(u,v) > \hat{K}(u,v)$ was lower than 5%. This allowed to identify the space-time clusters in our dataset.

Impact on common blackbird population densities

Bird survey and data collection

The abundance of common blackbirds was monitored in France over the 1996-2022 using a large-scale survey designed to assess population trends of common game species (*ACT Survey* (Boutin et al. 2001, 2003)). The spatial coverage of this survey is based on 1,070 grid cells measuring 28 x 20 km (1/50 000e IGN map; see Supplementary material). Each cell includes a sampling route (~4 km in length) randomly selected outside highly urbanized areas, with five points counts spaced by ~1km. Each year, these points are surveyed twice during the breeding season by hundreds of observers (affiliated to OFB or departmental federation of hunters): from April 1st to 30th and from May 15th to June 15th. The census protocol adopts the point count methodology: each point is surveyed for ten minutes in early morning. During this period, observers record the number of different singing males within a 500 m radius circle around the point (see Villers et al. 2021 for more details). Over the 1996-2022 period, each route was surveyed on average for 24.6 years (SD +/- 3.8 years; range: 1-27) and each year, the number of surveyed route was on average 977.1 routes (SD +/- 69.5; range 750-1038).

Population trends

For a given route and survey date, the number of singing birds was summed across the five points. We ensured that for each observation, all the following conditions were met: i) time of the count was available, ii) the start time of the survey was between -60 mn and +180 mn relative to sunrise time and iii) the date of the observation was posterior to the 31st of March and anterior to the 30th of June. We used these date and time intervals as presumed windows that minimize biases related to the detection rate of singing males. Our final dataset included 1,070 different routes (see Supplementary material), totalizing 50,454 counts over the study period.

We used the framework of Generalized Additive Mixed Models (GAMM) implemented in the R package mgcv (Wood 2006) to calculate annual population indices and underlying population trends. The number of singing males per route and per date (*Counts*) was modelled as a function of a smoothed function (using thin plate regression splines) of the counting *Date* (expressed as a Julian

date, i.e. the number of days elapsed since the beginning of the year), a smoothed function of *Time-of-day* (expressed in minutes relative to local sunrise thanks to the R package suncalc (Thieurmel and Elmarhraoui 2022), and a random effect associated to *Route ID* (a 1,070-levels factor). We used *Date* and *Time-of-day* as smooth terms to capture some components of the detection process (Knape 2016), including plausible change in singing rates in relation to breeding phenology and/or singing behaviour during the morning. In order to straightforwardly compare trends in the abundance index according to different levels of USUV incidence, we also created a factor *Usutu.Area*, indicative of the severity of the 2018 USUV episode in space. For each point of the *ACT Survey* we extracted the value of the density of USUV cases estimated by the kernel smoothing carried out previously (cf. supra, section USUV diffusion process, Spatial analysis). These values were averaged for each *route* (mean value of the five sampling points), and converted into a 3-levels factor, according to the 50th and 75th percentiles of the distribution of USUV cases density: *routes* displaying kernel values below the median were coded as *low*, over the 75th percentile as *high,* and *medium* otherwise. In order to model the temporal variation of the abundance index in each area, we included the *Usutu.Area* as a fixed effect, a smooth function of years (therefore considered as continuous covariate, *Year,* with a thin plate regression spline), and an interaction term allowing to fit a temporal trend to each level of the *Usutu.Area* factor (with independent smoothing parameters (Pedersen et al. 2019)). This interaction allowed to identify different population trends corresponding to different levels of USUV cases density. Finally, we also included in the model a random effect associated to the year considered as a 27-levels factor (*Year.as.Factor*). This statistical approach allowed separating short-term fluctuations (from one year to the next) from longer-term (over a decade) population changes, through the inclusion of temporal random effects in simulation of smooth trends (Knape 2016). The number of degrees of freedom of the basis function of the smoother of the year effect was set equal to k=10.

Models were fitted with a Tweedie distribution. Tweedie distributions, a special cases of exponential dispersion models, are probability distributions encompassing the continuous (normal, gamma and inverse Gaussian), discrete (Poisson) and compound (Poisson-gamma) distributions. In the R package mgcv, the value of the power parameter p of the Tweedie distributions (with $1 < p < 2$) can be estimated simultaneously with the estimation of other parameters, offering flexibility in the type of response that can be modelled.

To compute the trends and their associated confidence intervals from the fitted model, we adopted a posterior simulation *sensu* (Wood 2017) (chapter 6.10, p. 293), in order to obtain the distribution of predicted values of abundance for each level of the *Usutu.Area* factor. A similar approach is

implemented in the R package poptrend (Knape 2016), but it does not offer the possibility to compute trends for specific models, e.g. aiming at comparing trends for different levels of a grouping variable. The general idea is to combine prediction matrices and simulation (replicate) from the statistical distribution of the parameters β of the model, in order to obtain distribution estimates (i.e. including uncertainty of parameter estimate) of any quantity of interest, in our case the relative abundance index of common blackbirds for an average route and year, hereafter referred to as the variable *Index*. Details of the computations can be found in the supplementary material.

Trends were expressed as:

$$\%change = 100 \times \frac{\left(Index_{endsurvey} - Index_{startsurvey}\right)}{Index_{startsurvey}} \quad Eq.(1)$$

To estimate population trends accounting for the variability of the abundance index, we computed, for each simulated replicate, the mean abundance index for the four years before (2015-2018) and after (2019-2022) the USUV episode of the 2018 summer. We then plotted for each level of the *Usutu.Area* factor the value of the abundance index for the two periods (2015-2018 and 2019-2022) and compared the population trends for each area thanks to Eq. (1).

Results

USUV diffusion process

Epidemiological surveillance network and provided data

The dataset (60 % SAGIR, 34% AFVPZ and 6% rescue centres) included 60 birds tested for USUV of which 50 were detected infected in 29 French departments between July 15th and August 31st, 2018. Wild common blackbirds (*Turdus merula*) (26 from SAGIR, three from rescue centres and one from AFVPZ) and captive great grey owls (*Strix nebulosa*) (12 from AFVPZ) were particularly affected even if several other species were concerned (among wild birds from SAGIR: two blue tits (*Cyanistes caeruleus*), one robin (*Erithacus rubecula*) and one song thrush (*Turdus philomelos*); among captive birds from AFVPZ: two snowy owls (*Bubo scandiacus*), one *Strigidae* (unspecified species) and one western capercaillie (*Tetrao urogallus*)).

Spatial analysis

The comparison of observed $\hat{L}(t)$ and the distribution of simulated $L(t)$ (n=100) allowed to identify a clustering of the point pattern at 5 km, 125 km and 200 km (**Fig. 1**A).

The kernel smoothing carried out with a smoothing parameter equal to 125 km showed a high density of reported cases in the Brenne area (largest cluster located at the southwest of Paris on Fig. 1B – Paris being the northernmost cluster delimited by contour lines on the figure). Other high cases density areas could be identified, particularly in Paris, towards Orléans (south of Paris) and Nantes (west of France; **Fig. 1**B). The distance between Paris and Orléans high USUV cases density areas is around 200-250 km, as well as between those of Brenne and Nantes. This last result was consistent with the third peak observed in **Fig. 1**A (clustering at 200 km).

The fitting of the Thomas process model helped determining the density of clusters ("parents") per 10,000 km^2 (κ parameter: 0.17) and the mean cluster size (λ parameter: 4.36, mean number of "children" per "parent"). Thus, the mean density of detected cases was estimated to be 0.74 cases per 10,000 km^2. The standard deviation of the distribution of distances between the cases and the centroid of the cluster (the parent case) was of 77 km. This last result was consistent with the K function's second peak, which indicated the presence of clusters at a scale of about 130 km. Indeed, this standard deviation measures the typical distance between cases and the centroid of the cluster, while the K function is based on the distance between two cases. By simulation, we checked that the mean distance between points sampled in a bivariate Gaussian distribution with a standard deviation of 77 km was close to 130 km (see Supplementary material).

Environmental determinants

The observed mean of wetland probability score in USUV places was equal to 4.81, while the one expected under the absence of effect of the environmental variables (for n=999 simulations of the fitted Thomas process) was of 2.69 (SD=0.65). The difference was significant (p-value=0.007), indicating that USUV cases were more often reported in or close to wetland than expected by chance. Similarly, the observed mean of human log-density population in USUV places was of 5.02, while the one expected under the hypothesis of absence of effect of the environmental variables was of 3.50 (SD=0.25). The difference was also significant (p-value=0.001), indicating that places where dead birds positive to USUV were reported were more often located in places with high human density.

Temporal analysis

There was an initial increase in the number of cases between July 15th and 20th 2018 (**Fig. 1**C). Then from July 20th to August 15th the number of cases reported per day stagnated at about one per day. From August 15th onwards, there was a sharp increase, with an average of more than two cases reported per day towards the end of the month.

Spatio-temporal analysis

The plot of probabilities that a simulation could lead to $K(u,v) > \hat{K}(u,v)$ showed a strong clustering at small distances of about 10 to 20 km as soon as two or three days after a case, expanding to 50-60 km five days after a case. But we also observed a clustering on larger distances (150 km) starting as soon as two or three days after a case, which increased to reach a large range of distances after a week (90 to 150 km) (**Fig. 1**D). The large distances clustering was consistent with the first phase of the diffusion (between July 15th and 20th) (**Fig. 1**C), with the emergence of the main clusters of Brenne, Paris, Orléans and Nantes (**Fig. 1**B). The small distance of clustering was consistent with the emergence of secondary clusters and the expansion of preexisting main clusters (**Fig. 1**B) during the third phase of the diffusion (from August 15th) ((**Fig. 1**C).

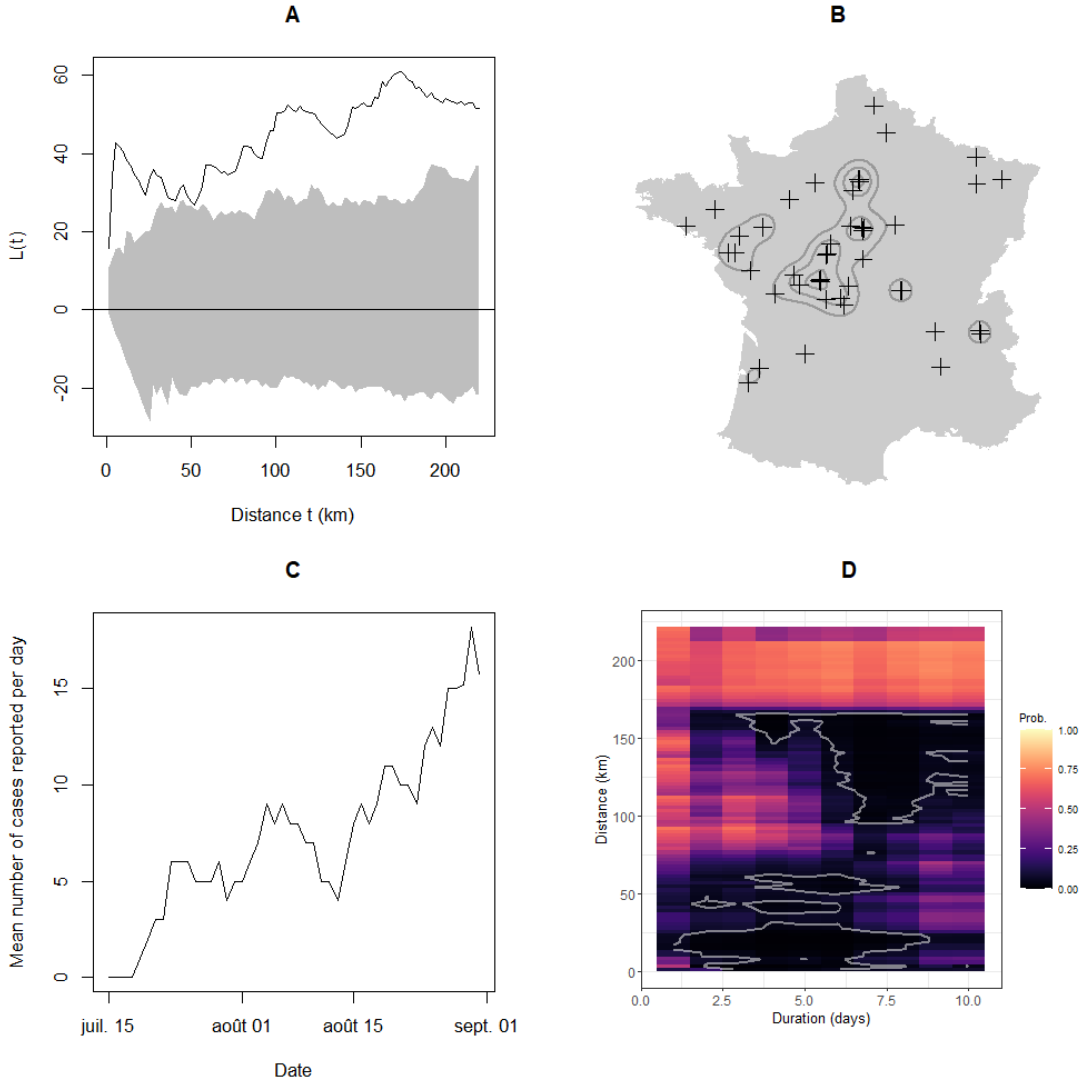

Fig. 1. Spatial (**A**, **B**), temporal (**C**) and spatio-temporal (**D**) characteristics of the point pattern of birds detected infected to USUV during the study period (**A**: Comparison between $\hat{L}(t)$ (observed, grey line) and $L_r(t)$ (envelope of simulated point pattern under CSR hypothesis, grey area); **B**: Kernel smoothing of the point pattern (grey lines) and birds locations (black crosses) ; **C**: Mean number of cases (mobile average approach) reported per day and depending on date; **D**: Probabilities that spatio-temporal K function $K(u,v)$ simulated under the hypothesis of independence between spatial and temporal patterns, was higher than observed spatio-temporal K function $\hat{K}(u,v)$, depending on duration and distance (white line: contour limit of the set of distance/duration pairs for which the proportion of simulation with $K(u,v) > \hat{K}(u,v)$ was lower than 5%)

<u>Impact on common blackbird population densities</u>

509 The means and confidence intervals depicting cells of respectively low, medium and high USUV
510 cases density have been reported in Tab.1, the distribution of those cases in Fig. 2A and their spatial
511 distribution in Fig. 2B.

512 The estimation of common blackbird population trends estimated through variations of the mean
513 population indexes for 2015-2018 and 2019-2022 periods was not statistically different from 0 for
514 the low USUV area, but presented a significant decline for both medium and high USUV areas,
515 being more pronounced for the second than for the former (Tab.1). Two main results were
516 highlighted: i) a decreasing trend for common blackbird populations that have been *a priori* exposed
517 to USUV since the 2018 USUV outbreak and ii) the greater the pressure of infection was, the
518 greater the negative effect on common blackbird populations was (Fig. 2C).

519

520 **Tab. 1.** Trends computed between 2015-2018 and 2019-2022 for the three different levels of
521 density of USUV common blackbird cases (CI: Confidence Interval).

USUV area	Mean trend	2.5%CI	97.5%CI
low	+0.8%	-0.4%	+2.1%
medium	-7.4%	-11.0%	-3.9%
high	-12.7%	-16.2%	-9.1%

522

523

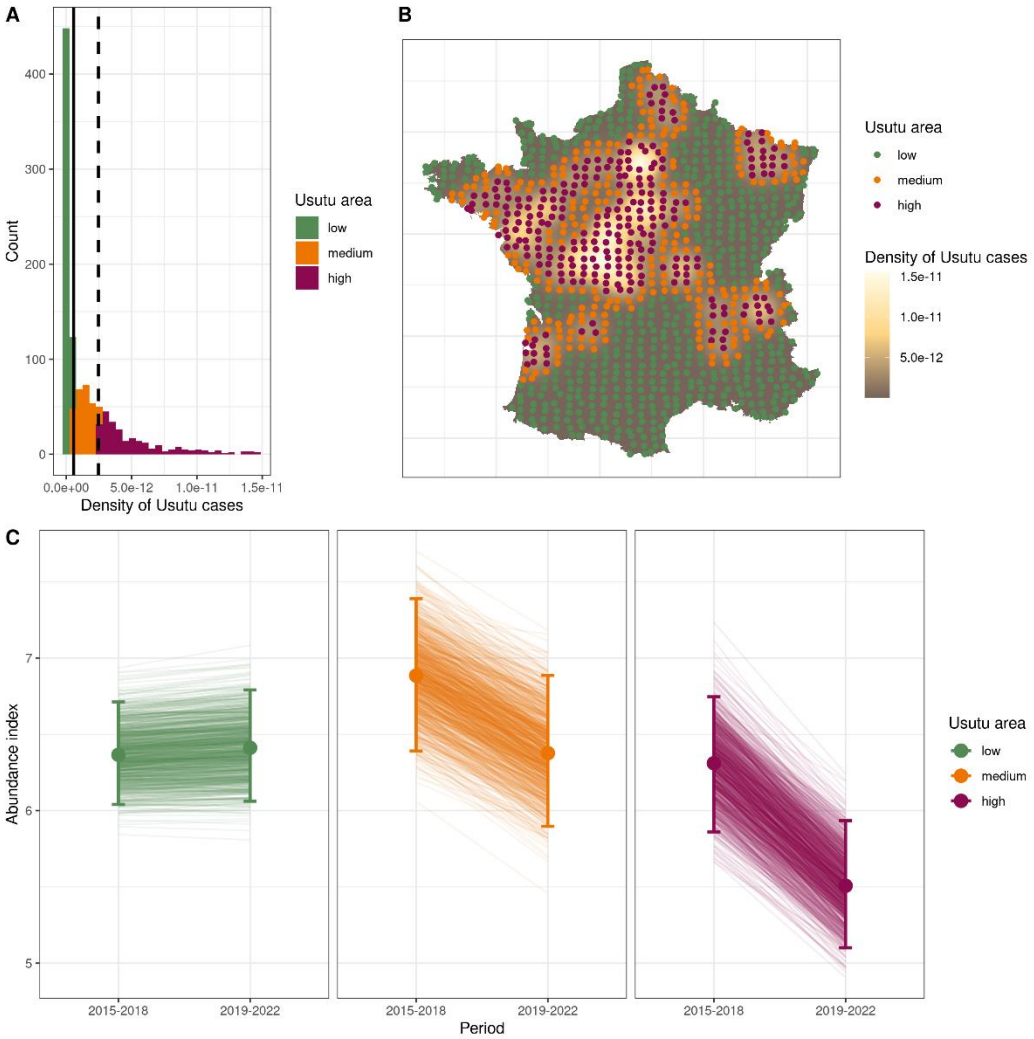

Fig. 2. Common blackbird population trends in response to the USUV episode (**A**: Histogram of USUV cases density, from a kernel smoothing, for the routes where common blackbird were monitored (solid line: median of the distribution, dashed line: 75% percentile of the distribution); **B**: Maps of the kernel smoothing of USUV cases and locations of the centroids of REZOP road (pale green: low, orange: medium and dark purple: high densities of USUV cases as estimated by the kernel method); **C**: Predicted abundance index (mean values and their 95% confidence intervals) for the three classes of USUV cases density (pale green: low, orange: medium and dark purple: high densities) before (2015-2018) and after (2019-2022) the 2018 USUV outbreak. Each solid line represents changes in abundance value for a given simulation (1,000 simulated values were generated).

Discussion

Birds affected by USUV between July 15th and August 31st, 2018, in France were mainly common blackbirds and to a lesser extent captive great grey owls. Cases were clustered at i) 5 km, ii) 125 km (which was the average distance between two typical cases which we highlighted with a Thomas process model) and iii) 200 km (which was the distance between main big clusters which appeared simultaneously at the beginning of the outbreak and expanded thereafter). Three periods of USUV diffusion have been featured: i) a first increase of USUV cases between July 15th and 20th (with clustering at large distances of about 150 km between two and three days after a case and between 90-150 km a week after a case, corresponding to the emergence of main clusters – Brenne, Paris, Orléans and Nantes), ii) a stagnation between July 20th and August 15th, and iii) an important increase from August 15th (with strong clustering at small distances of about 10-20 km, between two and three days after a case and expanding to 50-60 km five days after a case, corresponding to the emergence of secondary clusters and the expansion of preexisting main clusters). Detected case locations were statistically associated with wetlands and high human log-density population areas. Common blackbird population trends estimated suggested an impact of the USUV infection on them, with a decrease of the mean population index over 2015-2018 compared to 2019-2022. Areas of *a priori* greater USUV pressure presented more pronounced negative trends than the two other areas.

Species involved in the 2018 outbreak in the present study were those also found in other European countries such as Germany (Becker et al. 2012; Lühken et al. 2017). Several hypotheses might be put forward regarding the overrepresentation of common blackbirds: i) they are not trans-Saharan migratory species and as a consequence probably deprived of an immunity they could have acquired in Africa, ii) they might be more genetically susceptible than other birds species, iii) African strains introduced in Europe might have adapted to common blackbirds and become more virulent for this host (Bakonyi et al. 2004), iv) co-infections with *Plasmodium* might exist with a possible interplay of the two agents, as highlighted in the Netherlands (Agliani et al. 2023), v) a feeding preference has been identified for them (when they were sufficiently abundant) by *Cx. pipiens* (Rizzoli et al. 2015) and vi) this species is abundant, ubiquitous, human commensal (Lühken et al. 2017) and thus easy to detect by the SAGIR network.

Any pattern identified by the analysis of data collected by a participatory program relying on non-controlled sampling pressure can be caused by the opportunistic observation process (characterized by a very heterogenous sampling pressure) as well as by the ecological or epidemiological process. In particular, the data collection process differs across departments (differences in detectability, in probability to report a carcass to the network, in probability to collect this carcass by the local contact, mode of transport, mode of storage, etc.). Thus, when a pattern was identified in the data

572 (e.g. a cluster of cases), we could not decide between (i) the fact that this pattern was caused by the
573 observation process (e.g. higher local sampling pressure due to higher human density and thereby
574 detectability) and (ii) a biological cause (e.g. local factors favouring the diffusion of USUV in this
575 place). Our approach was exploratory, and our results could only help formulating hypotheses, and
576 could not allow confirming them. Keeping in mind this limit, we must stress that our results pointed
577 out that such a network and statistical analysis of admittedly imperfect data could however allow
578 the understanding of diffusion pattern and epidemiological process. Indeed, the network was
579 alerted to the possible presence of USUV cases in France and the level of attention given to
580 surveillance was greater for this disease than for other diseases. We considered the possibility that
581 any pattern identified in our data could be caused either by the observation process (e.g. the
582 heterogenous sampling pressure) or by ecological/epidemiological processes.

583 The short distance clustering (at 5 km) identified by our analysis could be explained by local
584 sampling effort. We could not exclude that observers would have been more proactive in detecting
585 dead birds around first detections. However, this short distance of 5 km is also close to flight
586 distances of *Cx. pipiens* estimated in a study carried out in Chicago, Illinois, on *Culex* female
587 mosquitoes. Indeed the mean dispersal distance was 1.15 km, and 90% of individuals stayed within
588 3 km from their larval habitat (Hamer et al. 2014). Another explanation for the 5 km clusters we
589 observed would be a USUV diffusion mainly due to mosquitoes. Sufficient amplification of the
590 virus in its various hosts would also have allowed a large number of mosquitoes to be infected.

591 The occurrence of different clusters simultaneously at the beginning of this 2018 USUV outbreak,
592 far apart from each other, is in favour of USUV endemicity throughout the country. The endemicity
593 has already been proven in Southern (Constant et al. 2022) and North-Eastern France (Johnson et
594 al. 2018), but the 2018 outbreak we studied and the diffusion pattern we showed raised the
595 hypothesis of a national endemicity. Previous outbreaks, even of lesser magnitude, might have
596 enabled certain lineages to settle. Indeed, in France, USUV Europe 3 strain has been identified in
597 common blackbirds in two north-eastern departments between 2015 and 2017. USUV Africa 2
598 strain has been isolated in common blackbirds, mosquitoes and humans in three south-eastern
599 departments and in Rhone between 2015 and 2016, while USUV Africa 3 strains have been
600 identified in mosquitoes and birds in two central and four south-eastern French departments
601 between 2015 and 2017 (Johnson et al. 2018, NRL data). Favourable conditions (e.g. sizes, naive
602 immune status and/or turnover of bird populations, temperature, rainfall, human population
603 movements) could then lead to new outbreaks thereafter. The early detection of this 2018 outbreak
604 was made possible by the well-informed SAGIR network, which vigilance has been reinforced
605 since the 2015 outbreak for USUV relevant species. Throughout Europe, endemic circulation and

spread of USUV infections have been reported, e.g. Northern Italy, Hungary or Austria (Constant et al. 2022). The study of lineages involved within this 2018 outbreak could help validate endemicity.

The different phases of the 2018 USUV diffusion we observed remained difficult to explain: a first increase of cases, with clusters far from each other, a stagnation and then an important increase of cases, with secondary clusters and the enlargement of the first ones. If endemicity might explain the cases pattern of the first phase, the origins of the third phase remain unclear. Meteorological variables, as mean temperature (Hamer et al. 2014), could explain this result. Indeed, the duration of *Cx. pipiens* eggs development depends on temperature (hatching after only one day at 30°C, three days at 20°C, ten days at 10°C and incomplete embryonic development below 7°C (Becker et al. 2010)). Temperatures were particularly high in July and August 2018 in France (respectively +2.18°C and +1.1°C higher than the 1991-2020 baseline average (https://www.meteocontact.fr/climatologie/france/bilans-climatiques)). *Cx. pipiens* density might have increased in August due to these favourable conditions and promoted an increased possibility of contact between common blackbirds and the arthropod. Moreover, laboratory experiments have shown that higher temperatures resulted in higher infection rate of *Cx. pipiens* (Fros et al. 2015)). These conditions could have allowed an important development of *Cx. pipiens* and the third phase of USUV diffusion we could observe. Further studies collecting local relevant meteorological data would be useful to assess this hypothesis. Nevertheless, we could not exclude that the diffusion of information and knowledge of the presence of USUV in France would have also led to better sampling effort from August 15th.

In addition to the above-mentioned hypothesis related to the population dynamics of the main vector of the disease, other non-mutually exclusive hypotheses could explain the appearance of USUV clusters in late summer (after August 15th). This period coincides with the initiation of movements in common blackbirds, particularly juveniles that disperse outside their parents' territories (Snow 1958), and to a lesser extent, with the first mentions of post-breeding migrants (Toulotte et al. 2022). Similar movements have been shown as the main drivers of seasonal epizootics of the avian influenza in mallards (van Dijk et al. 2014), and the same processes might be at play in the USUV episode reported here. USUV naïve birds such as juveniles, or individuals whose original populations have not yet been exposed to USUV, might be more prone to develop and spread the virus, easing the apparition of clusters.

In resident blackbirds, this period also overlaps with resource-demanding activities including breeding for some late breeders (Sauvage 2016), post-breeding (complete) moult in adults (Snow

1969; Morrison et al. 2015) and partial moult in juveniles (Cramp 1988). Previous works have shown that such activities may induce trade-offs with immunity in birds (e.g. Moreno et al. 1999; Sanz et al. 2004; Martin II 2005; Moreno rueda 2010). Accordingly, late summer may coincide with a period of greater susceptibility to infections in blackbirds. Unfortunately, the lack of available data on age, sex, or moulting status, both for the recorded USUV cases, but also for individuals in the exposed populations, did not allow us to confirm these assumptions. This constitutes a relevant area of investigation for future studies and highlights the need for a deeper description of specimens found dead.

The fitted Thomas process was a simple model, with the assumption that all clusters were of the same size, that the mean number of cases per clusters did not vary from one cluster to another and that the density of "parents" generating secondary cases was uniform across the study area. However, we could not highlight any lack of fit of this model to our dataset, and by simulating this process we were able to simulate a realistic point process and thereby test the effect of environmental variables on the occurrence of USUV. This test showed that the density of USUV cases varied as a function of wetlands and human density, two variables that we supposed to reflect mainly mosquito abundance.

USUV is assumed to be mainly transmitted among birds by mosquitoes during their blood meal (Lühken et al. 2017), but a transmission from bird to bird could not be excluded in the diffusion process and the endemicity. Oral route transmission was suggested by damage of cells of the gastrointestinal tract observed as part of necropsies of infected common blackbirds and possible vertical transmission could not be excluded with antigen detection in the genital system (Giglia et al. 2021). An oral transmission could explain distances between cases higher than maximum flight distances of mosquitoes, while vertical transmission could be involved in endemicity mechanisms and perhaps even in the resurgence of cases after the reproduction period (from August 15th).

The association between wetlands and USUV was consistent with the life cycle of USUV, with mosquitoes as vectors and wetlands known as suitable habitat for *Cx. pipiens* (Vogels et al. 2016). The association with high human population densities could be more surprising at first sight. In an event-based network, the number of observers could partly explain such a result. Indeed, the greater the number of people able to collect birds affected by the disease would be, the greater the potential for the disease to be detected in densely populated areas would be. In addition, common blackbirds are familiar to urbanised areas (Partecke and Gwinner 2007), and their size and colour (especially in males) are striking (Lühken et al. 2017): collecting USUV dead common blackbirds would be easier in such areas. We therefore could not exclude that the association between human

high-density areas and USUV places could be explained by a sampling bias. The contribution of AFVPZ data could be particularly interesting from this point of view, making it possible to multiply the sources and to reduce the bias. However, given the small number of birds from rescue centres (n=3), the only observation pressure hypothesis alone cannot explain the association with areas of high human density. Another part of the explanation could be *Cx. pipiens*, which have also been shown to be associated with urban (Haba and McBride 2022) or peri-urban (Vogels et al. 2016) environments, where breeding grounds favourable to mosquitoes could be found (e.g. rainwater collection containers or ponds in gardens) (Becker et al. 2010). This habitat preference might also explain the association we observed between cases of this vectorial disease and high human densities.

In our study, data from the SAGIR surveillance network were used to determine USUV pressure areas on common blackbird population sizes, which have been estimated using GAMM in each class of USUV pressure. The combination of data from these two networks helped assessing the impact of USUV on common blackbird populations. The negative impact of USUV on common blackbird populations that we highlighted is consistent with what has been showed in Germany, where a difference of about 15.7% has been highlighted between the means of populations indices in 2016 in common blackbird populations (lower population index compared to the baseline year) and statistically significant lower population index in the USUV-suitable areas compared with the USUV-unsuitable areas (Lühken et al. 2017). Even if in our study the cut in three classes of USUV pressure helped us to highlight gradual effects on common blackbird populations tendencies (the greater was the infection pressure, the greater was the negative effect on common blackbird populations), our results and the German one converge in the conclusion that common blackbird populations are affected by the USUV circulation. This result points out the impact of USUV circulation on the balance of ecosystems including common blackbirds and the services they provide (e.g. a role in seed dispersal) (Whelan et al. 2008).

The presence of USUV infection in zoological gardens also raised the question of conservation of foreign species. As previously said, SAGIR network is a precious tool for early detection. But in case of endemicity, control measures must also be considered (e.g. elimination of artificial container habitats, reduction of shade sources over aquatic habitats (Tuten 2011), use of biological larvicides such as *Bacillus thuringiensis* subsp. *israelensis* (Virgillito et al. 2022), indoor caging during periods of high density of mosquitoes, mosquito nets). For the first time in 2018, the AFVPZ and SAGIR networks were really coordinated to face the USUV outbreak. Data collected and related to both captive and non-captive wild birds allowed the description and the characterization of the outbreak within mainland France (time of onset and duration). Non-captive wild common blackbirds could

706 be seen in this context as USUV sentinel for captive wild birds and would be of great importance
707 for species with conservation issues. This role could also be extended to non-captive other sensitive
708 species (e.g. capercaillie or eagle-owl), and more specifically Passeriformes, as the birds collected in
709 rescue centres have highlighted.

710 Another issue of the diffusion of USUV in France in wild birds as common blackbirds, living close
711 to human populations, and in zoological species is contact with humans, who are known as USUV
712 incident hosts (the asymptomatic form being predominant and fever illness, or neurological
713 symptoms as encephalitis and meningitis (Vilibic-Cavlek et al. 2014) being rare (Angeloni et al.
714 2023)). Zoological parks are particularly favourable to the circulation of mosquitoes which can feed
715 on both humans and animals (Martínez-de la Puente et al. 2020). In addition, screening of
716 asymptomatic blood donors and seroprevalence studies have also revealed incidental findings of
717 the virus in asymptomatic human populations (Cadar et al. 2017; Bakonyi et al. 2017; Zaaijer et al.
718 2019; Angeloni et al. 2023). Their link to epizootic outbreaks has raised the question of the need
719 to strengthen USUV detection in blood donors, particularly during such periods (Cadar et al. 2017).
720 Long-term epidemiological studies would be necessary to provide a better understanding of viral
721 dynamics in potential wild reservoirs (Vittecoq et al. 2013). The USUV antigen detection in feather
722 (follicle shafts and bulbs) of infected birds raised the possibility for live bird testing (Giglia et al.
723 2021). Risk mapping of competent vectors for USUV (*Cx. pipiens* and *Ae. albopictus*) could also help
724 developing appropriate prevention and control measures (Martinet et al. 2023).

725 In a context of climate change (Walther et al. 2002; Parmesan and Yohe 2003), the impact on
726 arboviruses is complex to define (Franklinos et al. 2019), but the perspective of a shorter wintering
727 period and a longer period of activity cannot be ruled out. Such changes would not be without
728 effect on the distribution patterns of arboviruses such as USUV and recent research has shown the
729 role of climate change in the increase of the risk associated with West Nile Virus circulation through
730 Europe (Erazo et al. 2024). Moreover, the analysis of the origins and the within-France spatial
731 distribution of common blackbirds have highlighted that i) they mainly came from Western and
732 Central Europe and ii) were strongly segregated in autumn and winter in France (Lahournat et al.
733 2021). The coming seasonal disturbances in a context of climate change therefore raise the question
734 of the impact of USUV in France on its epidemiology on a European scale. The contributions of
735 networks such as SAGIR and REZOP are therefore more necessary than ever.

736 Following this analysis of surveillance data, one could question the potential impact of the reactivity
737 of a network such as SAGIR, coordinated with the other networks mentioned above, and what
738 strategy should be adopted in the event of a future outbreak. Indeed, we highlighted here the

relevance of combining data collected by different networks primarily dedicated to different purposes (e.g., SAGIR and REZOP). For example, an automatization of alerts could enhance the reactivity of the entire system of networks. The results of spatial analysis could be quickly accessible and help the definition of areas of risk, in near real time, and where surveillance (and if possible, control) efforts could be concentrated. Such an automatization could also be interesting in a financial perspective: as reported above, the number of cases had to be limited to one per department after August 31st, because of too high analysis costs associated with the growing outbreak. This drawback could be circumvented with better reactivity and if necessary, control of the virus diffusion. However, additional data (i.e. additional funding to analyse more samples even during the peak of the outbreak) would make it possible to reconstruct the spread of the virus over time by following different lineages more closely.

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Compliance with ethical standards

In their publications, the beneficiary and associated partners specify that the work was carried out under the terms of an order derogating from the strict protection of species.

Conflict of interest

The authors declare that they comply with the PCI rule of having no financial conflict of interest in relation to the content of the article.

Author contributions

CC performed the statistical analysis on USUV data. AV performed the populational statistical analysis on common blackbird populations. AD, BQ, AL and SL supervised USUV data collection. AV and CE supervised REZOP data collection. CC and AD interpreted results on USUV analysis. AV, CE, CC and AD interpreted both USUV and populational results. MBZ executed and reviewed the R code related to USUV diffusion and drafted the original manuscript. CC, AV, CE, SL and AD critically revised the manuscript. All authors contributed to the manuscript revision and approved the present version.

Data, Scripts, code, and supplementary information availability

All the data, script, code and supplementary information have been packaged in an R package named usutuFrance, available on Github at https://github.com/ClementCalenge/usutuFrance. We have also stored this package on Zenodo (https://doi.org/10.5281/zenodo.10992191; (Calenge et al. 2024)). The raw dataset used in this paper has also been stored as a text file on Zenodo (https://doi.org/10.5281/zenodo.10992555;(Bouchez-Zacria et al. 2024)).

References

Adams TF, Wongchai C, Chaidee A, Pfeiffer W (2016) "Singing in the Tube"—audiovisual assay of plant oil repellent activity against mosquitoes (*Culex pipiens*). Parasitol Res 115:225–239. https://doi.org/10.1007/s00436-015-4739-x

Agliani G, Giglia G, de Bruin E, et al (2023) The pathology of co-infection with Usutu virus and *Plasmodium* spp. in naturally infected Eurasian blackbirds (*Turdus merula*). One Health 16:100534. https://doi.org/10.1016/j.onehlt.2023.100534

Angeloni G, Bertola M, Lazzaro E, et al (2023) Epidemiology, surveillance and diagnosis of Usutu virus infection in the EU/EEA, 2012 to 2021. Eurosurveillance 28:2200929. https://doi.org/10.2807/1560-7917.ES.2023.28.33.2200929

Ashraf U, Ye J, Ruan X, et al (2015) Usutu virus: an emerging flavivirus in Europe. Viruses 7:219–238. https://doi.org/10.3390/v7010219

Baddeley A, Diggle PJ, Hardegen A, et al (2014) On tests of spatial pattern based on simulation envelopes. Ecological Monographs 84:477–489. https://doi.org/10.1890/13-2042.1

Bakonyi T, Erdélyi K, Ursu K, et al (2007) Emergence of Usutu virus in Hungary. J Clin Microbiol 45:3870–3874. https://doi.org/10.1128/JCM.01390-07

Bakonyi T, Gould EA, Kolodziejek J, et al (2004) Complete genome analysis and molecular characterization of Usutu virus that emerged in Austria in 2001: Comparison with the South African Strain SAAR-1776 and other flaviviruses. Virology 328:301–310. https://doi.org/10.1016/j.virol.2004.08.005

Bakonyi T, Jungbauer C, Aberle SW, et al (2017) Usutu virus infections among blood donors, Austria, July and August 2017 - Raising awareness for diagnostic challenges. Euro Surveill 22:17–00644. https://doi.org/10.2807/1560-7917.ES.2017.22.41.17-00644

Barbic L, Vilibic-Cavlek T, Listes E, et al (2013) Demonstration of Usutu virus antibodies in horses, Croatia. Vector Borne Zoonotic Dis 13:772–774. https://doi.org/10.1089/vbz.2012.1236

Bażanów B, Jansen van Vuren P, Szymański P, et al (2018) A Survey on West Nile and Usutu Viruses in Horses and Birds in Poland. Viruses 10:87. https://doi.org/10.3390/v10020087

Beck C, Jimenez-Clavero MA, Leblond A, et al (2013) Flaviviruses in Europe: Complex Circulation Patterns and Their Consequences for the Diagnosis and Control of West Nile Disease. Int J Environ Res Public Health 10:6049–6083. https://doi.org/10.3390/ijerph10116049

Becker N, Jöst H, Ziegler U, et al (2012) Epizootic emergence of Usutu virus in wild and captive birds in Germany. PLoS One 7:e32604. https://doi.org/10.1371/journal.pone.0032604

Becker N, Petrić D, Zgomba M, et al (2010) Mosquitoes and their control, 2nd edition. Springer Science & Business Media, Heidelberg, Dordrecht, New York

Ben Hassine T, De Massis F, Calistri P, et al (2014) First detection of co-circulation of West Nile and Usutu viruses in equids in the south-west of Tunisia. Transbound Emerg Dis 61:385–389. https://doi.org/10.1111/tbed.12259

Bouchez-Zacria M, Calenge C, Villers A, et al (2024) Data used by Bouchez-Zacria et al. in “Analysis of the Usutu episode of summer 2018 in birds in France.”. https://doi.org/10.5281/zenodo.10992555

Bournez L, Umhang G, Faure E, et al (2019) 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 12:10. https://doi.org/10.3390/v12010010

Boutin J, Barbier L, Roux D (2001) Suivi des effectifs nicheurs d’alaudidés, colombidés et turdidés en France: le programme ACT. In: Actes du 25čme Colloque francophone d’ornithologie, 18 et 19 mars 2000 ŕ Caen. S.E.O.F. Alauda, Caen, pp 53–61

Boutin J, Roux D, Eraud C (2003) Breeding bird monitoring in France: the ACT survey. Ornis Hungarica 12:1–2. http://zeus.nye.hu/~szept/EBCC/25-Boutin_Eraud.pdf

Buckley A, Dawson A, Moss SR, et al (2003) Serological evidence of West Nile virus, Usutu virus and Sindbis virus infection of birds in the UK. Journal of General Virology 84:2807–2817. https://doi.org/10.1099/vir.0.19341-0

Busquets N, Alba A, Allepuz A, et al (2008) Usutu virus sequences in *Culex pipiens* (Diptera: *Culicidae*), Spain. Emerg Infect Dis 14:861–863. https://doi.org/10.3201/eid1405.071577

Cadar D, Becker N, Campos R de M, et al (2014) Usutu virus in bats, Germany, 2013. Emerg Infect Dis 20:1771–1773. https://doi.org/10.3201/eid2010.140909

Cadar D, Maier P, Müller S, et al (2017) Blood donor screening for West Nile virus (WNV) revealed acute Usutu virus (USUV) infection, Germany, September 2016. Euro Surveill 22:30501. https://doi.org/10.2807/1560-7917.ES.2017.22.14.30501

Calenge C, Villers A, Bouchez-Zacria M (2024) R package usutuFrance describing the data and analysis of the Usutu episode in France in summer 2018. https://doi.org/10.5281/zenodo.10992191

Calisher CH, Gould EA (2003) Taxonomy of the virus family *Flaviviridae*. Adv Virus Res 59:1–19. https://doi.org/10.1016/s0065-3527(03)59001-7

Cavrini F, Gaibani P, Longo G, et al (2009) Usutu virus infection in a patient who underwent orthotropic liver transplantation, Italy, August-September 2009. Eurosurveillance 14:19448. https://doi.org/10.2807/ese.14.50.19448-en

Chaintoutis SC, Dovas CI, Papanastassopoulou M, et al (2014) Evaluation of a West Nile virus surveillance and early warning system in Greece, based on domestic pigeons. Comp Immunol Microbiol Infect Dis 37:131–141. https://doi.org/10.1016/j.cimid.2014.01.004

Chevalier V, Marsot M, Molia S, et al (2020) Serological Evidence of West Nile and Usutu Viruses Circulation in Domestic and Wild Birds in Wetlands of Mali and Madagascar in 2008. International Journal of Environmental Research and Public Health 17:1998. https://doi.org/10.3390/ijerph17061998

Clé M, Barthelemy J, Desmetz C, et al (2020) Study of Usutu virus neuropathogenicity in mice and human cellular models. PLoS Negl Trop Dis 14:e0008223. https://doi.org/10.1371/journal.pntd.0008223

Clé M, Beck C, Salinas S, et al (2019) Usutu virus: A new threat? Epidemiol Infect 147:e232. https://doi.org/10.1017/S0950268819001213

Constant O, Bollore K, Clé M, et al (2020) Evidence of Exposure to USUV and WNV in Zoo Animals in France. Pathogens 9:1005. https://doi.org/10.3390/pathogens9121005

Constant O, Gil P, Barthelemy J, et al (2022) One Health surveillance of West Nile and Usutu viruses: a repeated cross-sectional study exploring seroprevalence and endemicity in Southern France, 2016 to 2020. Euro Surveill 27:2200068. https://doi.org/10.2807/1560-7917.ES.2022.27.25.2200068

Cramp S (1988) Handbook of the birds of Europe, the Middle East and North Africa : the birds of the Western Paleartic. Vol.5, Tyrant flycatchers to thrushes

Csank T, Drzewnioková P, Korytár Ľ, et al (2018) A Serosurvey of Flavivirus Infection in Horses and Birds in Slovakia. Vector Borne Zoonotic Dis 18:206–213. https://doi.org/10.1089/vbz.2017.2216

Culbert NJ, Balestrino F, Dor A, et al (2018) A rapid quality control test to foster the development of genetic control in mosquitoes. Sci Rep 8:16179. https://doi.org/10.1038/s41598-018-34469-6

Decors A, Desvaux S, Payne A, et al (2022) Le réseau SAGIR, la surveillance au carrefour des enjeux. BullEpidSanté AnimAlim 95:1–9

Diagne MM, Ndione MHD, Di Paola N, et al (2019) Usutu Virus Isolated from Rodents in Senegal. Viruses 11:181. https://doi.org/10.3390/v11020181

Diggle P (1990) Time series. A biostatistical introduction., Oxford University Press, Oxford

Diggle PJ (2013) Statistical Analysis of Spatial and Spatio-Temporal Point Patterns, Third Edition. CRC Press

Durand B, Haskouri H, Lowenski S, et al (2016) Seroprevalence of West Nile and Usutu viruses in military working horses and dogs, Morocco, 2012: dog as an alternative WNV sentinel species? Epidemiol Infect 144:1857–1864. https://doi.org/10.1017/S095026881600011X

Eiden M, Gil P, Ziegler U, et al (2018) Emergence of two Usutu virus lineages in *Culex pipiens* mosquitoes in the Camargue, France, 2015. Infect Genet Evol 61:151–154. https://doi.org/10.1016/j.meegid.2018.03.020

Engel D, Jöst H, Wink M, et al (2016) Reconstruction of the Evolutionary History and Dispersal of Usutu Virus, a Neglected Emerging Arbovirus in Europe and Africa. mBio 7:10.1128/mbio.01938-15. https://doi.org/10.1128/mbio.01938-15

Épidémiosurveillance Santé animale (ESA) (2018) Bilan de la circulation virus Usutu en France au 27 août 2018

Erazo D, Grant L, Ghisbain G, et al (2024) Contribution of climate change to the spatial expansion of West Nile virus in Europe. Nat Commun 15:1196. https://doi.org/10.1038/s41467-024-45290-3

Escribano-Romero E, Lupulović D, Merino-Ramos T, et al (2015) West Nile virus serosurveillance in pigs, wild boars, and roe deer in Serbia. Vet Microbiol 176:365–369. https://doi.org/10.1016/j.vetmic.2015.02.005

Folly AJ, Lawson B, Lean FZ, et al (2020) Detection of Usutu virus infection in wild birds in the United Kingdom, 2020. Euro Surveill 25:2001732. https://doi.org/10.2807/1560-7917.ES.2020.25.41.2001732

Franklinos LHV, Jones KE, Redding DW, Abubakar I (2019) The effect of global change on mosquito-borne disease. The Lancet Infectious Diseases 19:e302–e312. https://doi.org/10.1016/S1473-3099(19)30161-6

Fros JJ, Miesen P, Vogels CB, et al (2015) Comparative Usutu and West Nile virus transmission potential by local Culex pipiens mosquitoes in north-western Europe. One Health 1:31–36. https://doi.org/10.1016/j.onehlt.2015.08.002

Gabriel E, Rowlingson BS, Diggle PJ (2013) stpp: An R Package for Plotting, Simulating and Analyzing Spatio-Temporal Point Patterns. Journal of Statistical Software 53:1–29. https://doi.org/10.18637/jss.v053.i02

Gaibani P, Rossini G (2017) An overview of Usutu virus. Microbes and Infection 19:382–387. https://doi.org/10.1016/j.micinf.2017.05.003

Garigliany M-M, Marlier D, Tenner-Racz K, et al (2014) Detection of Usutu virus in a bullfinch (*Pyrrhula pyrrhula*) and a great spotted woodpecker (Dendrocopos major) in north-west Europe. Vet J 199:191–193. https://doi.org/10.1016/j.tvjl.2013.10.017

Giglia G, Agliani G, Munnink BBO, et al (2021) Pathology and Pathogenesis of Eurasian Blackbirds (*Turdus merula*) Naturally Infected with Usutu Virus. Viruses 13:1481. https://doi.org/10.3390/v13081481

Haba Y, McBride L (2022) Origin and status of *Culex pipiens* mosquito ecotypes. Current Biology 32:R237–R246. https://doi.org/10.1016/j.cub.2022.01.062

Hamer GL, Anderson TK, Donovan DJ, et al (2014) Dispersal of Adult Culex Mosquitoes in an Urban West Nile Virus Hotspot: A Mark-Capture Study Incorporating Stable Isotope Enrichment of Natural Larval Habitats. PLOS Neglected Tropical Diseases 8:e2768. https://doi.org/10.1371/journal.pntd.0002768

Hubálek Z, Halouzka J, Juřicová Z, et al (2008a) Serologic Survey of Birds for West Nile Flavivirus in Southern Moravia (Czech Republic). Vector-Borne and Zoonotic Diseases 8:659–666. https://doi.org/10.1089/vbz.2007.0283

Hubálek Z, Wegner E, Halouzka J, et al (2008b) Serologic Survey of Potential Vertebrate Hosts for West Nile Virus in Poland. Viral Immunology 21:247–254. https://doi.org/10.1089/vim.2007.0111

Johnson N, Fernández de Marco M, Giovannini A, et al (2018) Emerging Mosquito-Borne Threats and the Response from European and Eastern Mediterranean Countries. International Journal of Environmental Research and Public Health 15:2775. https://doi.org/10.3390/ijerph15122775

Jöst H, Bialonski A, Maus D, et al (2011) Isolation of usutu virus in Germany. Am J Trop Med Hyg 85:551–553. https://doi.org/10.4269/ajtmh.2011.11-0248

Knape J (2016) Decomposing trends in Swedish bird populations using generalized additive mixed models. Journal of Applied Ecology 53:1852–1861. https://doi.org/10.1111/1365-2664.12720

Lahournat M, Jiguet F, Villers A, et al (2021) Where do thrushes migrating to France come from? Within-France distribution and temporal changes over 70 years. Eur J Wildl Res 67:95. https://doi.org/10.1007/s10344-021-01525-y

Lecollinet S, Blanchard Y, Manson C, et al (2016) Dual Emergence of Usutu Virus in Common Blackbirds, Eastern France, 2015. Emerg Infect Dis 22:2225. https://doi.org/10.3201/eid2212.161272

Lühken R, Jöst H, Cadar D, et al (2017) Distribution of Usutu Virus in Germany and Its Effect on Breeding Bird Populations. Emerg Infect Dis 23:1994–2001. https://doi.org/10.3201/eid2312.171257

Lupulovic D, Martín-Acebes MA, Lazic S, et al (2011) First serological evidence of West Nile virus activity in horses in Serbia. Vector Borne Zoonotic Dis 11:1303–1305. https://doi.org/10.1089/vbz.2010.0249

Manly BFJ (1991) Randomization and Monte-Carlo Methods in Biology, Springer US

Mannasse B, Mendelson E, Orshan L, et al (2017) Usutu Virus RNA in Mosquitoes, Israel, 2014-2015. Emerg Infect Dis 23:1699–1702. https://doi.org/10.3201/eid2310.171017

Martin II LB (2005) Trade-offs between molt and immune activity in two populations of house sparrows (*Passer domesticus*). Can J Zool 83:780–787. https://doi.org/10.1139/z05-062

Martinet J-P, Bohers C, Vazeille M, et al (2023) Assessing vector competence of mosquitoes from northeastern France to West Nile virus and Usutu virus. PLOS Neglected Tropical Diseases 17:e0011144. https://doi.org/10.1371/journal.pntd.0011144

Martínez-de la Puente J, Soriguer R, Senar JC, et al (2020) Mosquitoes in an Urban Zoo: Identification of Blood Meals, Flight Distances of Engorged Females, and Avian Malaria Infections. Frontiers in Veterinary Science 7:

McIntosh B (1985) Usutu (SAAr 1776) : nouvel arbovirus du groupe B. Int Cat Arboviruses 1059–1060

M'ghirbi Y, Mousson L, Moutailler S, et al (2023) West Nile, Sindbis and Usutu Viruses: Evidence of Circulation in Mosquitoes and Horses in Tunisia. Pathogens 12:360. https://doi.org/10.3390/pathogens12030360

Millot F, Decors A, Mastain O, et al (2017) Field evidence of bird poisonings by imidacloprid-treated seeds: a review of incidents reported by the French SAGIR network from 1995 to 2014. Environ Sci Pollut Res 24:5469–5485. https://doi.org/10.1007/s11356-016-8272-y

Moreno J, Sanz JJ, Arriero E (1999) Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers *Ficedula hypoleuca*. Proceedings of the Royal Society of London Series B: Biological Sciences 266:1105–1109. https://doi.org/10.1098/rspb.1999.0750

Moreno-rueda G (2010) Experimental test of a trade-off between moult and immune response in house sparrows *Passer domesticus*. Journal of Evolutionary Biology 23:2229–2237. https://doi.org/10.1111/j.1420-9101.2010.02090.x

Morrison CA, Baillie SR, Clark JA, et al (2015) Flexibility in the timing of post-breeding moult in passerines in the UK. Ibis 157:340–350. https://doi.org/10.1111/ibi.12234

Mossel EC, Crabtree MB, Mutebi J-P, et al (2017) Arboviruses Isolated From Mosquitoes Collected in Uganda, 2008–2012. Journal of Medical Entomology 54:1403–1409. https://doi.org/10.1093/jme/tjx120

Moutailler S, Yousfi L, Mousson L, et al (2019) A New High-Throughput Tool to Screen Mosquito-Borne Viruses in Zika Virus Endemic/Epidemic Areas. Viruses 11:904. https://doi.org/10.3390/v11100904

Nikolay B, Diallo M, Boye CSB, Sall AA (2011) Usutu virus in Africa. Vector Borne Zoonotic Dis 11:1417–1423. https://doi.org/10.1089/vbz.2011.0631

Ochieng C, Lutomiah J, Makio A, et al (2013) Mosquito-borne arbovirus surveillance at selected sites in diverse ecological zones of Kenya; 2007 – 2012. Virology Journal 10:140. https://doi.org/10.1186/1743-422X-10-140

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42. https://doi.org/10.1038/nature01286

Partecke J, Gwinner E (2007) Increased Sedentariness in European Blackbirds Following Urbanization: A Consequence of Local Adaptation? Ecology 88:882–890. https://doi.org/10.1890/06-1105

Pecorari M, Longo G, Gennari W, et al (2009) First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. Euro Surveill 14:19446

Pedersen EJ, Miller DL, Simpson GL, Ross N (2019) Hierarchical generalized additive models in ecology: an introduction with mgcv. PeerJ 7:e6876. https://doi.org/10.7717/peerj.6876

Rijks JM, Kik ML, Slaterus R, et al (2016) Widespread Usutu virus outbreak in birds in the Netherlands, 2016. Euro Surveill 21:30391. https://doi.org/10.2807/1560-7917.ES.2016.21.45.30391

Rizzoli A, Bolzoni L, Chadwick EA, et al (2015) Understanding West Nile virus ecology in Europe: Culex pipiens host feeding preference in a hotspot of virus emergence. Parasites & Vectors 8:213. https://doi.org/10.1186/s13071-015-0831-4

Romeo C, Lecollinet S, Caballero J, et al (2018) Are tree squirrels involved in the circulation of flaviviruses in Italy? Transbound Emerg Dis 65:1372–1376. https://doi.org/10.1111/tbed.12874

Sanz JJ, Moreno J, Merino S, Tomás G (2004) A trade-off between two resource-demanding functions: post-nuptial moult and immunity during reproduction in male pied flycatchers. Journal of Animal Ecology 73:441–447. https://doi.org/10.1111/j.0021-8790.2004.00815.x

Sauvage A (2016) Merle noir *Turdus merula*, in LPO Champagne-Ardenne. Nidification, migration, hivernage. Ouvrage collectif des ornithologues champardennais. Delachaux et Niestlé, Paris, pp: 360-362

Simonin Y, Sillam O, Carles MJ, et al (2018) Human Usutu Virus Infection with Atypical Neurologic Presentation, Montpellier, France, 2016. Emerg Infect Dis 24:875–878. https://doi.org/10.3201/eid2405.171122

Snow DW (1958) The Breeding of the Blackbird *Turdus merula* at Oxford. Ibis 100:1–30. https://doi.org/10.1111/j.1474-919X.1958.tb00362.x

Snow DW (1969) The moult of British thrushes and chats. Bird Study 16:115–129. https://doi.org/10.1080/00063656909476230

Steinmetz HW, Bakonyi T, Weissenböck H, et al (2011) Emergence and establishment of Usutu virus infection in wild and captive avian species in and around Zurich, Switzerland--genomic and pathologic comparison to other central European outbreaks. Vet Microbiol 148:207–212. https://doi.org/10.1016/j.vetmic.2010.09.018

Thieurmel B, Elmarhraoui A (2022) suncalc: Compute Sun Position, Sunlight Phases, Moon Position and Lunar Phase. R package version 0.5.1, https://CRAN.R-project.org/package=suncalc

Toulotte F, Dupuy J, Eraud C, Sallé L (2022) Merle noir *Turdus merula*, in Dupuy J. & Sallé, L. 2022, Atlas des oiseaux migrateurs de France. LPO, Rochefort; Biotope editions, Mèze; Muséum national d'histoire naturelle, Paris. pp 897-900.

Tuten HC (2011) Habitat Characteristics of Larval Mosquitoes in Zoos of South Carolina, USA. moco 27:111–119. https://doi.org/10.2987/10-6061.1

Utarini A, Indriani C, Ahmad RA, et al (2021) Efficacy of Wolbachia-Infected Mosquito Deployments for the Control of Dengue. N Engl J Med 384:2177–2186. https://doi.org/10.1056/NEJMoa2030243

van Dijk JGB, Hoye BJ, Verhagen JH, et al (2014) Juveniles and migrants as drivers for seasonal epizootics of avian influenza virus. Journal of Animal Ecology 83:266–275. https://doi.org/10.1111/1365-2656.12131

Vilibic-Cavlek T, Kaic B, Barbic L, et al (2014) First evidence of simultaneous occurrence of West Nile virus and Usutu virus neuroinvasive disease in humans in Croatia during the 2013 outbreak. Infection 42:689–695. https://doi.org/10.1007/s15010-014-0625-1

Vilibic-Cavlek T, Petrovic T, Savic V, et al (2020) Epidemiology of Usutu Virus: The European Scenario. Pathogens 9:699. https://doi.org/10.3390/pathogens9090699

Villers A, Prudhomme O, Eraud C (2021) Suivi des populations nicheuses (1996-2019) et hivernantes (2000-2019). Réseau national d'observation "Oiseaux de passage", OFB-FNC-FDC, Rapport interne OFB, version 2021-07-06, 22 pages

Virgillito C, Manica M, Marini G, et al (2022) Evaluation of *Bacillus thuringiensis* Subsp. *Israelensis* and *Bacillus sphaericus* Combination Against *Culex pipiens* in Highly Vegetated Ditches. Journal of the American Mosquito Control Association 38:40–45. https://doi.org/10.2987/21-7024

Vittecoq M, Lecollinet S, Jourdain E, et al (2013) Recent circulation of West Nile virus and potentially other closely related flaviviruses in Southern France. Vector Borne Zoonotic Dis 13:610–613. https://doi.org/10.1089/vbz.2012.1166

Vogels CBF, Möhlmann TWR, Melsen D, et al (2016) Latitudinal Diversity of *Culex pipiens* Biotypes and Hybrids in Farm, Peri-Urban, and Wetland Habitats in Europe. PLOS ONE 11:e0166959. https://doi.org/10.1371/journal.pone.0166959

Walther G-R, Post E, Convey P, et al (2002) Ecological responses to recent climate change. Nature 416:389–395. https://doi.org/10.1038/416389a

Wand MC, Jones M (1995) Kernel Smoothing. Chapman and Hall/CRC, New York

Weissenböck H, Bakonyi T, Rossi G, et al (2013) Usutu virus, Italy, 1996. Emerg Infect Dis 19:274–277. https://doi.org/10.3201/eid1902.121191

Weissenböck H, Kolodziejek J, Url A, et al (2002) Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe. Emerg Infect Dis 8:652–656. https://doi.org/10.3201/eid0807.020094

Whelan CJ, Wenny DG, Marquis RJ (2008) Ecosystem Services Provided by Birds. Annals of the New York Academy of Sciences 1134:25–60. https://doi.org/10.1196/annals.1439.003

Wood S (2006) Generalized Additive Models: An Introduction With R., CRC Press, Boca Raton, FL. CRC Press, Boca Raton, FL, USA

Wood SN (2017) Generalized Additive Models: An Introduction with R, 2nd Edition. Chapman & Hall/CRC, New York

Zaaijer HL, Slot E, Molier M, et al (2019) Usutu virus infection in Dutch blood donors. Transfusion 59:2931–2937. https://doi.org/10.1111/trf.15444