

1 **Relevance of the synergy of surveillance and populational networks in understanding the**
2 **Usutu virus outbreak within common blackbirds (*Turdus merula*) in Metropolitan France,**
3 **2018**

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24

25 **Abstract**

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

29 killing mainly common blackbirds (*Turdus merula*) and to a lesser extent great grey owls (*Strix*
30 *nebulosa*), among other captive and non-captive wild bird species. Previous studies of USUV in
31 France have focused on reconstructing pathways of introduction, but not on structural aspects of
32 virus spread within the country. Data (RT-PCR of geolocated dead birds) Data on this 2018
33 outbreak were collected through both an event-based wildlife network named SAGIR~~network~~
34 ~~(SAGIR)~~ and the health surveillance of the French-speaking Association of Zoo Veterinarians
35 (AFVPZ). In addition, common blackbird populations could be monitored through another
36 network (REZOP). Statistical analysis (spatial, temporal, spatiotemporal and environmental
37 determinants) of the SAGIR and AFVPZ network data networks helped to highlight the early
38 appearance of separate large clusters of USUV cases in mid-July 2018, the subsequent diffusion
39 into smaller and secondary clusters at the end of August 2018, and a meanwhile enlargement of the
40 first clusters with an increase in the number of cases. High human density (top 10.5% densest areas
41 in France) and wetland concentration (top 19.3% most likely wetland areas) were significant factors
42 in USUV case locations~~The locations of USUV cases were also significantly associated with high~~
43 ~~human density and wetlands~~. Using generalised additive mixed models on REZOP data, we also
44 highlighted the decline in common blackbird population trends in areas with medium and even
45 more with high USUV pressure (areas defined based on SAGIR-AFVPZ data) following the 2018
46 outbreak (respectively -7.4% [-11.4; -3.9]_{95%} and -15.7% [-16.2; -9.1]_{95%}). A large area (radius ~150
47 km) in the centre and centre-west of France, and smaller areas in the south-east, north and north-
48 east of France (each with a radius ~ 50 km) were particularly affected. We conclude on the
49 importance ~~Our results highlighted the need~~ to work with synergistic networks to assess infection
50 spread in wild bird species, as well as the negative impact of an emerging arbovirus. The
51 responsiveness of such a network system could be improved by automating alerts.

52

53 **Introduction**

54 The Usutu virus (USUV), so called because of its isolation in *Culex neavei* mosquitoes in 1959
55 (McIntosh 1985) near the Usutu river in Swaziland (Eswatini since 2018) is an arbovirus of the
56 *Flaviviridae* family, *Orthoflavivirus* genus (Clé et al. 2019), belonging to the Japanese encephalitis virus
57 serocomplex, and phylogenetically close to Japanese Encephalitis Virus (JEV) and West Nile Virus
58 (WNV) (Calisher and Gould 2003). After South Africa, the virus was detected in other African
59 countries: Central African Republic, Senegal, Ivory Coast, Nigeria (Nikolay et al. 2011), Uganda
60 (Nikolay et al. 2011; Mossel et al. 2017), Burkina Faso (Nikolay et al. 2011), Mali, Madagascar
61 (Chevalier et al. 2020), Kenya (Ochieng et al. 2013), Tunisia (Ben Hassine et al. 2014; M'ghirbi et

62 al. 2023), Morocco (Durand et al. 2016), and Israel (Mannasse et al. 2017). The first known
63 occurrence in Europe dates back to 1996 in Italy (a retrospective finding in dead birds, mainly
64 blackbirds)_(Weissenböck et al. 2013). Since then, first detections of USUV followed in other
65 European countries: Austria (Weissenböck et al. 2002) in 2001, Czech Republic (Hubálek et al.
66 2008a)) in 2004, Hungary (Bakonyi et al. 2007) in 2005, Poland (Hubálek et al. 2008b), Spain
67 (Busquets et al. 2008) and Switzerland (Steinmetz et al. 2011) in 2006, Serbia in 2009 (Lupulovic et
68 al. 2011), Germany (Jöst et al. 2011), Greece (Chaintoutis et al. 2014) and Slovakia (Csank et al.
69 2018) in 2010, Croatia (Barbic et al. 2013) in 2011, Belgium (Garigliany et al. 2014) in 2012, France
70 (Lecollinet et al. 2016) in 2015, Netherlands (Rijks et al. 2016) in 2016 and United Kingdom (Folly
71 et al. 2020) in 2020 (Vilibic-Cavlek et al. 2020) .

72 The analysis of 92 complete USUV genomes, including 77 genomes from mosquito, bird and bat
73 species of Germany (2010-2014) highlighted that i) USUV can be classified in different lineages
74 (Engel et al. 2016), (eight in number – three African and five European (Cadar et al. 2017; Clé et
75 al. 2019)), ii) the most common ancestor emerged in Africa at the beginning of the 16th century, iii)
76 USUV was regularly introduced from Africa during the last ~~7550~~ years (a first introduction from
77 1950 through 1960's in Western Europe (Spain), a second one between 1970 and the 1980's in
78 Central Europe (Austria) and a third one around 1996 in Western Europe (Spain)) and iv) *in situ*
79 evolution could explain the genetic diversity of European lineages, while extensive gene flow drove
80 African ones. In addition, flyway networks of migratory birds were strongly consistent with spatial
81 movements observed through genetic data and suggested the possibility of USUV exportation from
82 Africa i) to Spain via the east Atlantic and/or Black Sea/Mediterranean flyways and ii) to Central
83 Europe through the Black Sea/Mediterranean flyway (Engel et al. 2016).

84 The natural life cycle of USUV involves passeriform and strigiform birds as amplifying hosts and
85 ornithophilic species of mosquitoes as vectors (Clé et al. 2020). The virus has been detected in
86 several Culicidae (*Culex*, *Aedes*, *Culiseta*, *Mansonia*) (Clé et al. 2019) but *Cx. pipiens* (experimentally
87 shown to be USUV competent (Martinet et al. 2023)) is considered as the main vector in Europe
88 (Becker et al. 2012; Martinet et al. 2023). To date, several incident or dead-end hosts (which
89 certainly develop a low viremia, insufficient to re-infect mosquitoes (Martinet et al. 2023)) have
90 been identified (Vilibic-Cavlek et al. 2020) : horses (Durand et al. 2016; Bażanów et al. 2018; Csank
91 et al. 2018), dogs (Durand et al. 2016), rodents (Diagne et al. 2019), squirrels (Romeo et al. 2018),
92 wild boar (Escribano-Romero et al. 2015; Bournez et al. 2019) and roe deer (Bournez et al. 2019).
93 The virus has also been detected in bats, whose epidemiological role is as yet undetermined (Cadar
94 et al. 2014). The virus has a zoonotic potential as humans (incident hosts) have been found infected
95 with different clinical pictures: fever and rash in Africa (Ashraf et al. 2015), neuroinvasive infections

96 (Gaibani and Rossini 2017) in Europe (meningoencephalitis (Cavrini et al. 2009; Pecorari et al.
97 2009); idiopathic facial paralysis (Simonin et al. 2018)). However, these manifestations remain
98 anecdotal and the asymptomatic form appears to predominate, as demonstrated by incidental
99 findings during screening of asymptomatic blood donors and seroprevalence studies (Angeloni et
100 al. 2023).

101 In Africa, USUV seems not to be pathogenic for local bird populations, either because it is not a
102 naturally virulent virus, or because of immunity or genetic resistance of birds, which have been in
103 contact with related flaviviruses for a long time (Bakonyi et al. 2004). In Europe, common
104 blackbirds (~~*Turdus merula*~~) are particularly affected, and to a lesser extent some birds of prey (Giglia
105 et al. 2021), such as the great grey owl (~~*Strix nebulosa*~~) (Clé et al. 2019). The lesion patterns of 160
106 common blackbirds in the Netherlands included hepatosplenomegaly (major symptom),
107 coagulative necrosis, lymphoplasmacytic inflammation and vasculitis. Reported symptoms included
108 non-specific ones (immobility, apathy and ruffled plumage) and neurological ones (depression, stiff
109 neck, inability to fly and epileptic seizures) (Giglia et al. 2021).

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

116 In France, the first virus detection (in August and September 2015) took place in the North-east
117 (Haut-Rhin department) and Central-east of France (Rhône department), respectively in two and
118 three dead common blackbirds (~~*Turdus merula*~~) collected through SAGIR, the French event-based
119 surveillance network collecting and analysing dead free-ranging wildlife animals (see details in next
120 paragraph). Phylogenetic analysis helped demonstrating proximity between isolates i) from Haut-
121 Rhin and Germany and ii) from Rhône and Spain. In addition, isolates identified in the two French
122 departments belonged to different USUV lineages (Europe 3 in Haut-Rhin, Africa 2 in Rhône).
123 Those results suggested a different source of introduction for the two French departments
124 (Lecollinet et al. 2016). Since 2015, outbreaks in French avifauna have been recorded yearly in 2016,
125 2017 and 2018, with new departments involved (Johnson et al. 2018). In 2018, the Usutu virus
126 circulated earlier and more extensively than it had in preceding years (Épidémiosurveillance Santé
127 animale (ESA) 2018). In Camargue (South of France), which is located at the crossroad of several
128 bird migration routes (Vittecoq et al. 2013)) two lineages (Africa 2 and Africa 3) were found in

129 2016 among female *Cx. pipiens*, suggesting two independent introductions of the virus: i) one from
130 West to East and probably explained by migratory birds following the East Atlantic flyways (Africa
131 2 lineage) and ii) one explained by migratory birds following a central Mediterranean flyway and
132 spreading after to Northern Europe, or originating from Germany (Africa 3) (Eiden et al. 2018).
133 The USUV detection, in the southern region of Camargue, of same lineage, in same region and for
134 consecutive years (2015, 2018 and 2020) in mosquitoes highly suggests endemicity (Constant et al.
135 2022).

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

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

157 Moreover, in 2018, abnormal mortalities due to USUV have also been observed on captive birds
158 in zoos and were reported in the database of the French Association of Zoo Veterinarians
159 (AFVPZ). The French Agency for Food, Environmental and Occupational Health & Safety
160 (Anses) combined SAGIR and AFVPZ data. This dataset offered an opportunity to study the

161 epidemiology of USUV (date, location, and size of clusters), by identifying the timing and
162 geography of reporting of dead birds positive to USUV in France.

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

173 We hypothesised that USUV circulation in 2018 resulted in decreased common blackbird
174 populations in the years following the 2018 epizootics, based on a previous study on blackbirds
175 dead following USUV infection in Germany between 2011 and 2015 (Lühken et al. 2017). Although
176 the approach of this German study was slightly different, as we will discuss in the dedicated part
177 of this paper, we also expected that USUV circulation in birds would be associated with some
178 environmental variables and would have a negative impact on common blackbird population
179 trends.

180

181 **Materials and methods**

182 Study design

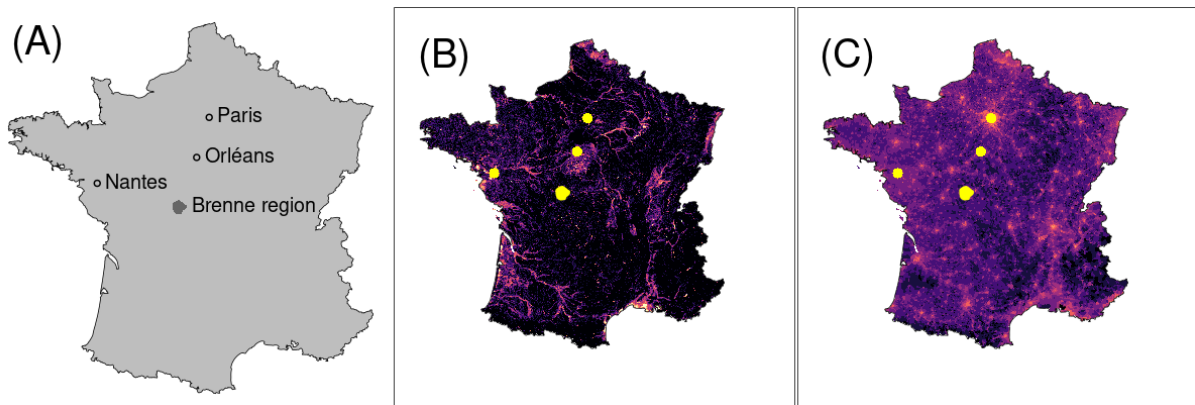
183 This study employed an observational ecological design, using data from existing surveillance
184 networks to assess the spatial and temporal distribution of USUV infections among bird
185 populations in France. Data were collected opportunistically from mortality events reported
186 through the SAGIR and AFVPZ surveillance networks, encompassing wild and captive birds. The
187 study design allowed for the examination of environmental and ecological variables influencing
188 USUV distribution, such as proximity to wetlands and human population density, which serve as
189 proxies for mosquito habitats and bird sampling pressures respectively. We also identified spatial
190 patterns (i.e., clusters) and temporal trends of USUV outbreaks at the population level. We used
191 REZOP data to assess the correlation between estimated USUV infection levels and observed
192 trends in blackbird populations across various geographic areas.

193 Data collection and surveillance networks

194 USUV diffusion process

195 The birds in our study were collected through the SAGIR network and zoological gardens across
196 mainland France (Fig. 1A) from July 15th to August 31st, 2018 (after this latter date, a message was
197 sent to the local coordinators of the SAGIR network, asking them to limit data collection to only
198 one sample per department, thus avoiding the additional cost of analyses linked to the increased
199 epizootic outbreak; data collected by the network were thus defined at the department level from
200 September onwards). Before August 31st, the most precise geographical information for each
201 reported case was the municipality (a municipality being the smallest French administrative
202 subdivision, corresponding to town and village areas, with a median area of 9 km²).

203



204

205 **Fig. 1.** Study area (grey) (A) with regions and towns mentioned in the study, wetlands (B) and
206 human population densities (C) (B: from black to pink: from the least likely to the most likely
207 wetlands; C: from black to pink: from the least to the most densely populated areas; B and C:
208 yellow surrounded by a yellow line : main towns and regions mentioned in the study)

209

210 Epidemiological surveillance network and provided data

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

216 SAGIR is based on a collaboration between the French Office of Biodiversity (OFB), who
217 administrates the network, hunters, local and national federations of hunters and the French
218 Association of directors and executives of public veterinary analysis laboratories (Decors et al.
219 2022). In each *department* (a department being a French administrative district covering in average
220 5,700 km² [SD = 2,500 km²]), two departmental technical contacts (one belonging to the
221 departmental service of OFB, the other belonging to the departmental federation of hunters)
222 (Decors et al. 2022) coordinate a network of volunteers (professionals, as well as hunters, naturalists
223 and farmers) who can report abnormal events (mortality or morbidity) that they accidentally find
224 in the field. ~~The reported sampling of these events is therefore opportunistic. Note that the~~
225 ~~reported~~ events can also ~~punctually~~ include dead animals of local rescue centres on rare occasions.
226 ~~The sampling of these events is therefore opportunistic (facility operated by non profit~~
227 ~~organizations, designed to provide aid, care, and shelter to injured, sick, or orphan wild animals~~
228 ~~found in the field).~~ For traceability purposes, a standardised individual digital an individual form is
229 filled for each mortality event collected, reporting epidemiological, agricultural and ecological
230 circumstances surrounding the discovery of carcasses. ~~When~~ Where available, clinical signs are also
231 provided (Millot et al. 2017), deduced from observation of the carcass and signs left in the
232 environment. Sometimes animals are seen alive before they die (e.g. 12% of common blackbirds in
233 the database from 2014 to 2023, regardless of cause of death) (A. Decors, personal
234 communication). ~~The digitisation of forms and the standardisation of data associated with the~~
235 ~~reported animals was implemented in 2014 (Decors et al. 2022). Moribund animals can also be~~
236 ~~filmed to get veterinary expertise. Carcasses (fresh, chilled or frozen) accompanied by their form~~
237 ~~are addressed to the local administrative laboratory of veterinarian analyses where necropsies are~~
238 ~~realized. Following a gross pathologic examination, relevant aetiology tests are conducted:~~
239 ~~parasitology, bacteriology, virology, mycology, toxicology and/or histology (Millot et al. 2017).~~

240 Laboratory procedures

241 In SAGIR, carcasses (fresh, chilled or frozen) accompanied by their form are addressed to the local
242 administrative laboratory of veterinarian analyses where necropsies are realized. Following a gross
243 pathologic examination, relevant aetiology tests are conducted: parasitology, bacteriology, virology,
244 mycology, toxicology and/or histology (Millot et al. 2017).

245 ~~Following the emergence of USUV in Europe, the vigilance of the SAGIR network has been~~
246 ~~reinforced: a first alert message was sent to local coordinators on the 2nd of May 2012 warning them~~
247 ~~of the presence of the virus in Germany and the need to be vigilant in case of abnormal common~~
248 ~~blackbird mortality (to consider USUV analysis in such cases). On 21 September 2015, the first~~

249 ~~detection of USUV in common blackbirds in France was shared through another alert message~~
250 ~~and recommended the inclusion of USUV in the differential diagnosis in cases of abnormal~~
251 ~~mortality or nervous symptoms in owls, blackbirds, tits, sparrows, robins and starlings. Early~~
252 ~~detection of the 2018 USUV outbreak was allowed by a well-informed network. Hence, on the 3rd~~
253 ~~of August 2018, an alert message informed the network that abnormal mortality had been observed~~
254 ~~in common blackbird populations in several departments for the past fortnight and that USUV~~
255 ~~had already been confirmed in one of them. Recommendations instigated in 2015 were therefore~~
256 ~~reiterated.~~

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

271 Birds collected from both networks were also tested by the National Reference Laboratory (NRL,
272 Anses), if i) their ante-mortem clinical picture was suggestive of an USUV infection (e.g.
273 neurological symptoms) and/or ii) the epidemiologic context was consistent with a USUV
274 suspicion (close mortality among sensitive species, and especially great grey owls) and/or iii) lesions
275 at post-mortem examination were suggestive of an USUV infection (liver, spleen, cerebral
276 necrosis). Spleen, liver, brain and lung (sometimes kidney and heart) samples were then sent to the
277 NRL and tested for USUV and WNV through Real-Time Polymerase Chain Reaction (hereafter
278 RT-PCR). Tissues samples were stored at -80°C before RT-PCR analyses and were grinded in
279 Dulbecco modified Eagle's minimal essential medium (DMEM) (Lecollinet et al. 2016) with
280 ceramic beads (MP Biomedicals, Illkirch, France) and FastPrep ribolyzer in BSL3 facilities.
281 Automated total RNA extraction, amplification and detection of USUV genome were carried out
282 as described in (Moutailler et al. 2019).

283 Thus, birds in our study were collected through the SAGIR network and zoological gardens from
284 July 15th to August 31st, 2018 (after this latter date, a message was sent to the SAGIR network
285 asking to rationalise collections and analyse only one sample per department, because of the
286 additional cost of analyses linked to the increased epizootic outbreak; data were thus defined at the
287 department level from September onwards). Before August 31st, the most precise geographical
288 information for each reported case was the municipality (a municipality being the smallest French
289 administrative subdivision, corresponding to town and village areas, with a median area of 9 km²).
290 After necropsy examination, each bird collected within SAGIR was tested by the National
291 Reference Laboratory (NRL, Anses) for USUV and WNV through Real-Time Polymerase Chain
292 Reaction (hereafter RT-PCR) detection on liver, spleen and brain (sometimes kidney and heart).
293 Tissues samples were stored at -80°C and before RT-PCR analyses, were grinded in Dulbecco
294 modified Eagle's minimal essential medium (DMEM) with ceramic beads (MP Biomedicals,
295 Illkirch, France) and FastPrep ribolyzer in BSL3 facilities. Automated total RNA extraction was
296 performed with the QIAamp Viral RNA kit (Qiagen, Hilden, Germany) or the MagVet™ Universal
297 Isolation kit (ThermoFisher Scientific, Lissieu, France) on extraction robots QIAcube and
298 Kingfisher respectively. USUV genome was amplified and detected with primers UV_F and R and
299 probe UV_P, as described in (Moutailler et al. 2019) using the AgPath-ID™ One-Step RT-PCR
300 Reagents (ThermoFisher Scientific). Primers and probes were used at a concentration of 0.4µM and
301 0.2µM, respectively. The samples were maintained at 45°C for 10 min and at 95°C for 10 min and
302 then subjected to 40 cycles consisting of incubations at 95°C for 15s and at 60°C for 60s. Birds
303 collected from zoos through the AFVPZ network were also tested by the NRL, using the same
304 protocol, if i) their ante mortem clinical picture was suggestive of an USUV infection (e.g.
305 neurological symptoms) and/or ii) the epidemiologic context was consistent with a USUV
306 suspicion (close mortality among sensitive species, and especially great grey owls) and/or iii) lesions
307 at post mortem examination were suggestive of an USUV infection (liver, spleen, cerebral
308 necrosis). Spleen, liver, brain, kidney and lung samples were then sent to the NRL.

309 Epidemiological data analysis

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

317 We analysed the temporal, spatial and spatio-temporal distribution of reported cases ~~(see below)~~,
318 as well as the relationship with spatial clusters of cases with wetlands and with human density (Fig.
319 1B and C).—Wetlands and human density were supposed to be a proxy for the density of *Culex*
320 *pipiens* mosquitoes (BeckerVogels et al. 2010; Haba and McBride 20222016). Human density was
321 also supposed to be a proxy for common blackbirds sampling pressure, as those birds are highly
322 commensal with humans and easy to detect in gardens. A map of potential wetlands was derived
323 from a raster map of continental France built by INRAE (Orléans) ([http://geowww.agrocampus-](http://geowww.agrocampus-ouest.fr/web/?p=1538)
324 [ouest.fr/web/?p=1538](http://geowww.agrocampus-ouest.fr/web/?p=1538)): the INRAE map estimates the probability that each 1km x 1km pixel of
325 the map harbours a wetland and then discretize this probability in three classes (0 = no wetlands,
326 1 = rather strong probability, 2 = strong probability, or 3 = very strong probability). This map also
327 identifies lakes and foreshores in France. We downloaded this map, and redefined pixels with lakes
328 and foreshores as "confirmed wetlands" (new class 4). Then, we smoothed this map using a sliding
329 window, by calculating for each pixel the sum of this ordered factor (0 to 4) for the focus pixel as
330 well as the pixels immediately on the left, the right, the top and the bottom of the focus pixel
331 (leading to a score comprised between 0 and 20 giving a smoothed probability of wetland in a given
332 place; see Fig. 1B).

333 A map of human density was calculated by finding the municipality in which the centre of each
334 1km x 1km pixel was located and attributing the 2005 human density to it (number of inhabitants
335 divided by municipality area; see Fig. 1C). We then log-transformed this map (transformation: $x \rightarrow$
336 $\log(x+1)$ where x was the number of inhabitants per km²). The 2005 human density was provided
337 by Institut Geographique National (France) (IGN) (<https://geoservices.ign.fr>).

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

348 *Spatial analysis*

349 We first used a Ripley's K function to identify the scales at which clusters of points could be
350 identified (Diggle 2013). This function allows the characterisation of second order point pattern
351 properties: it is proportional to the mean number of other cases expected in a x km radius around
352 a typical case of this point pattern (so that the larger it is, and the more there are cases in the
353 neighbourhood of a case in average – in other words, clusters can be observed at this scale). The
354 K function was computed for all distances t comprised between 0 and 250 km on the observed
355 point pattern ($\hat{K}(t)$); the theoretical value of this function was also calculated for the same range
356 of distances under the hypothesis of complete spatial randomness (CSR) ($K_{CSR}(t)$). Thus, under
357 the hypothesis that the cases are randomly distributed in space, the difference $\hat{L}(t)$ between $\hat{K}(t)$
358 and $K_{CSR}(t)$ should be equal to 0 (Baddeley et al. 2014), so that positive deviations of $\hat{L}(t)$ from
359 zero reflect clustering in the data (and conversely, negative values reflect repulsion mechanisms).
360 We also built confidence envelopes around simulated $L_r(t)$ functions expected under CSR: we
361 repeated $n=100$ simulations of the CSR (by randomly distributing N cases in France, where N is
362 the observed number of cases in our study), and for each simulation r , we estimated the function
363 $L_r(t)$ for all values of t from 1 km to 250 km. We plotted the observed value $\hat{L}(t)$ and the
364 distribution of values expected under the CSR hypothesis on the same graph to compare them
365 ($\hat{L}(t) = L_r(t)$: complete random reporting of cases; $\hat{L}(t) > L_r(t)$: more points within a t radius
366 around a typical case than under CSR hypothesis ; $\hat{L}(t) < L_r(t)$: less points within a t radius around
367 a typical case than under CSR hypothesis i.e. avoidance process).

368 To identify areas of high density of cases in the point pattern, we carried out a kernel smoothing
369 of the point pattern, using a smoothing parameter of 125 km (Wand and Jones 1995), to visualize
370 them on a map. The results (see [Results section below](#)) suggested that the point pattern could be
371 adequately described by a Thomas process (Diggle 2013), i.e. a process in which cases are clustered
372 in the following way (Baddeley et al. 2014): i) a random number of “parents” is generated from a
373 Poisson distribution parameterized by a parameter κ controlling the density of ‘parent’ cases per
374 km^2 . These parents are randomly placed in the study area. ii) For each “parent”, a random number
375 of “children” (corresponding to the cases in our study) is generated from a Poisson distribution
376 parameterized by a parameter λ controlling the mean number of “children” per “parent”, and iii)
377 for each “parent”, the children locations are randomly distributed around the parent’s location.
378 More precisely, the locations of the children are supposed to be located at a distance from the
379 parent randomly drawn from a semi-normal distribution with a standard deviation σ , with an angle
380 from west direction drawn from a uniform distribution bounded by π and $-\pi$. We fitted this process
381 to our dataset using the method of minimum contrasts based on the function K . Estimated

382 parameters of the fitted model helped defining the point pattern typical structure and the product
383 of λ and κ provided the cases density per km². Note that our spatial analysis revealed a clustering
384 of the dataset at very small scale (see results), possibly due to the tendency of observers to look for
385 other cases in a municipality where a first case was identified. Consequently, and to improve model
386 fit, we decided to thin the point pattern prior to the fit to remove this artefactual structure, keeping
387 only the first bird found when two birds were collected at a distance lower than 5 km (see
388 Supplementary material for more details).

389 *Environmental determinants*

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

399 *Temporal analysis*

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

405 *Spatio-temporal analysis*

406 We computed a space-time K function ($\hat{K}(u, v)$) (Gabriel et al. 2013) giving the expected number
407 of cases within a distance of u km around a randomly sampled case of the observed point pattern
408 (for all values of u comprised between 0 and 250 km), and which occurred at most v days after this
409 case (for all values of v comprised between 0 and 10 days). To test whether the spatio-temporal
410 patterns identified by this function could have been obtained by chance, we also used a
411 randomization approach. More precisely, for each one of the 1,000 simulations of this approach,
412 we randomly permuted the dates associated to the reported birds (i.e. the cases order was
413 randomised), thus conserving the spatial and temporal point pattern structures but randomising

414 the association between space and time. For each simulation, we calculated the related $K(u, v)$ on
415 the randomized dataset. We then compared, for each distance u and each duration v , the observed
416 value of $\hat{K}(u, v)$ to the distribution of simulated values $K(u, v)$. Probabilities that a simulation could
417 lead to $K(u, v) > \hat{K}(u, v)$ were plotted, with duration on the x-axis, distances on the y-axis
418 (probabilities near zero indicating clustering) and a contour limit identifying the set of
419 distance/duration pairs for which the proportion of simulation with $K(u, v) > \hat{K}(u, v)$ was lower
420 than 5%. This allowed to identify the space-time clusters in our dataset.

421

422 Impact on common blackbird population densities

423 *Bird survey and data collection*

424 The abundance of common blackbirds was monitored in France over the 1996-2022 using a large-
425 scale survey designed to assess population trends of common game species (*ACT Survey* (Boutin et
426 al. 2001, 2003)). The spatial coverage of this survey is based on 1,070 grid cells measuring 28 x 20
427 km (1/50 000e IGN map; see Supplementary material). Each cell includes a sampling route (~4
428 km in length) randomly selected outside highly urbanized areas, with five points counts spaced by
429 ~1km. Each year, these points are surveyed twice during the breeding season by hundreds of
430 observers (affiliated to OFB or departmental federation of hunters): from April 1st to 30th and from
431 May 15th to June 15th. The census protocol adopts the point count methodology: each point is
432 surveyed for ten minutes in early morning. During this period, observers record the number of
433 different singing males within a 500 m radius circle around the point (see Villers et al. 2021 for
434 more details). Over the 1996-2022 period, each route was surveyed on average for 24.6 years (SD
435 +/- 3.8 years; range: 1-27) and each year, the number of surveyed route was on average 977.1
436 routes (SD +/- 69.5; range 750-1038).

437

438 *Population trends*

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

446 We used the framework of Generalized Additive Mixed Models (GAMM) implemented in the R
447 package *mgcv* (Wood 2006) to calculate annual population indices and underlying population
448 trends. The number of singing males per route and per date (*Counts*) was modelled as a function of
449 a smoothed function (using thin plate regression splines) of the counting *Date* (expressed as a Julian
450 date, i.e. the number of days elapsed since the beginning of the year), a smoothed function of *Time-*
451 *of-day* (expressed in minutes relative to local sunrise thanks to the R package *suncalc* (Thieurmel
452 and Elmarhraoui 2022), and a random effect associated to *Route ID* (a 1,070-levels factor). We used
453 *Date* and *Time-of-day* as smooth terms to capture some components of the detection process (Knappe
454 2016), including plausible change in singing rates in relation to breeding phenology and/or singing
455 behaviour during the morning. In order to straightforwardly compare trends in the abundance
456 index according to different levels of USUV incidence, we also created a factor *Usutu.Area*,
457 indicative of the severity of the 2018 USUV episode in space. For each point of the *ACT Survey* we
458 extracted the value of the density of USUV cases estimated by the kernel smoothing carried out
459 previously (cf. supra, section USUV diffusion process, Spatial analysis). These values were averaged
460 for each *route* (mean value of the five sampling points), and converted into a 3-levels factor,
461 according to the 50th and 75th percentiles of the distribution of USUV cases density: *routes* displaying
462 kernel values below the median were coded as *low*, over the 75th percentile as *high*, and *medium*
463 otherwise. In order to model the temporal variation of the abundance index in each area, we
464 included the *Usutu.Area* as a fixed effect, a smooth function of years (therefore considered as
465 continuous covariate, *Year*, with a thin plate regression spline), and an interaction term allowing to
466 fit a temporal trend to each level of the *Usutu.Area* factor (with independent smoothing parameters
467 (Pedersen et al. 2019)). This interaction allowed to identify different population trends
468 corresponding to different levels of USUV cases density. Finally, we also included in the model a
469 random effect associated to the year considered as a 27-levels factor (*Year.as.Factor*). This statistical
470 approach allowed separating short-term fluctuations (from one year to the next) from longer-term
471 (over a decade) population changes, through the inclusion of temporal random effects in simulation
472 of smooth trends (Knappe 2016). The number of degrees of freedom of the basis function of the
473 smoother of the year effect was set equal to $k=10$.

474 Models were fitted with a Tweedie distribution. Tweedie distributions, a special cases of
475 exponential dispersion models, are probability distributions encompassing the continuous (normal,
476 gamma and inverse Gaussian), discrete (Poisson) and compound (Poisson-gamma) distributions.
477 In the R package *mgcv*, the value of the power parameter p of the Tweedie distributions (with $1 <$
478 $p < 2$) can be estimated simultaneously with the estimation of other parameters, offering flexibility
479 in the type of response that can be modelled.

480 To compute the trends and their associated confidence intervals from the fitted model, we adopted
481 a posterior simulation *sensu* (Wood 2017) (chapter 6.10, p. 293), in order to obtain the distribution
482 of predicted values of abundance for each level of the *Usutu.Area* factor. A similar approach is
483 implemented in the R package *poptrend* (Knappe 2016), but it does not offer the possibility to
484 compute trends for specific models, e.g. aiming at comparing trends for different levels of a
485 grouping variable. The general idea is to combine prediction matrices and simulation (replicate)
486 from the statistical distribution of the parameters β of the model, in order to obtain distribution
487 estimates (i.e. including uncertainty of parameter estimate) of any quantity of interest, in our case
488 the relative abundance index of common blackbirds for an average route and year, hereafter
489 referred to as the variable *Index*. Details of the computations can be found in the supplementary
490 material.

491 Trends were expressed as:

$$492 \quad \%change = 100 \times \frac{(Index_{endsurvey} - Index_{startsurvey})}{Index_{startsurvey}}$$

Eq. (1)

493

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

499

500 **Results**

501 USUV diffusion process

502 *Epidemiological surveillance network and provided data*

503 The dataset (60 % SAGIR, 34% AFVPZ and 6% rescue centres) included 60 birds tested for USUV
504 of which 50 were detected infected in 29 French departments between July 15th and August 31st,
505 2018. Wild common blackbirds ~~and (*Turdus merula*) (26 from SAGIR, three from rescue centres~~
506 ~~and one from AFVPZ) and~~ captive great grey owls (*Strix nebulosa*) (12 from AFVPZ) were
507 particularly affected -even if several other species were concerned (**Tab. 1** among wild birds from

508 ~~SAGIR: two blue tits (*Cyanistes caeruleus*), one robin (*Erithacus rubecula*) and one song thrush (*Turdus*~~
 509 ~~*philomelos*); among captive birds from AFVPZ: two snowy owls (*Bubo scandiacus*), one *Strigidae*~~
 510 ~~(unspecified species) and one western capercaillie (*Tetrao urogallus*)).~~

511 **Tab. 1.** Distribution of birds collected and detected infected by USUV within the two
 512 epidemiological surveillance networks of the study between July 15th and August 31st, 2018 (data
 513 from rescue centres were stored in the SAGIR database).

Species	Origin		
	SAGIR	AFVPZ	Rescue centres
Wild birds			
<u>Common blackbird (<i>Turdus merula</i>)</u>	<u>26</u>	<u>1</u>	<u>3</u>
<u>Blue tit (<i>Cyanistes caeruleus</i>)</u>	<u>2</u>	<u>0</u>	<u>0</u>
<u>Robin (<i>Erithacus rubecula</i>)</u>	<u>1</u>	<u>0</u>	<u>0</u>
<u>Song thrush (<i>Turdus philomelos</i>)</u>	<u>1</u>	<u>0</u>	<u>0</u>
Captive birds			
<u>Great grey owl (<i>Strix nebulosa</i>)</u>	<u>0</u>	<u>12</u>	<u>0</u>
<u>Snowy owl (<i>Bubo scandiacus</i>)</u>	<u>0</u>	<u>2</u>	<u>0</u>
<u><i>Strigidae</i> (unspecified species)</u>	<u>0</u>	<u>1</u>	<u>0</u>
<u>Western capercaillie (<i>Tetrao urogallus</i>)</u>	<u>0</u>	<u>1</u>	<u>0</u>

514

515 *Spatial analysis*

516 The comparison of observed $\hat{L}(t)$ and the distribution of simulated $L(t)$ (n=100) showed that
 517 infected birds were not randomly distributed and were concentrated in specific geographic areas:
 518 it allowed to identify a clustering of the point pattern at 5 km, 125 km and 200 km (**Fig. 2A**).

519 The kernel smoothing carried out with a smoothing parameter equal to 125 km showed a high
 520 density of reported cases in the Brenne area (largest cluster located at the southwest of Paris on
 521 Fig. ~~2B4B~~ – Paris being the northernmost cluster delimited by contour lines on the figure (see Fig.
 522 1A for the names of towns and regions). Other high cases density areas could be identified,
 523 particularly in Paris, towards Orléans (south of Paris) and Nantes (west of France; **Fig. 1A**, **Fig.**
 524 **2B**). The distance between Paris and Orléans high USUV cases density areas is around 200-250
 525 km, as well as between those of Brenne and Nantes. This last result was consistent with the third
 526 peak observed in **Fig. 2A** (clustering at 200 km).

527 The fitting of the Thomas process model helped determining the density of clusters (“parents”)
 528 per 10,000 km² (κ parameter: 0.17) and the mean cluster size (λ parameter: 4.36, mean number of
 529 “children” per “parent”). Thus, the mean density of detected cases was estimated to be 0.74 cases
 530 per 10,000 km². The standard deviation of the distribution of distances between the cases and the

531 centroid of the cluster (the parent case) was of 77 km. This last result was consistent with the K
532 function's second peak, which indicated the presence of clusters at a scale of about 130 km. Indeed,
533 this standard deviation measures the typical distance between cases and the centroid of the cluster,
534 while the K function is based on the distance between two cases. By simulation, we checked that
535 the mean distance between points sampled in a bivariate Gaussian distribution with a standard
536 deviation of 77 km was close to 130 km (see Supplementary material).

537 *Environmental determinants*

538 The observed mean of wetland probability score in USUV places was equal to 4.81, while the one
539 expected under the absence of effect of the environmental variables (for n=999 simulations of the
540 fitted Thomas process) was of 2.69 (SD=0.65). The difference was significant (p-value=0.007),
541 indicating that USUV cases were more often reported in or close to wetland than expected by
542 chance. This mean wetland probability score in USUV places corresponded to the top 19.3% most
543 likely wetland areas. Similarly, the observed mean of human log-density population in USUV places
544 was of 5.02, while the one expected under the hypothesis of absence of effect of the environmental
545 variables was of 3.50 (SD=0.25). The difference was also significant (p-value=0.001), indicating
546 that places where dead birds positive to USUV were reported were more often located in places
547 with high human density. This mean log-density population in USUV places corresponded to the
548 top 10.5% densest areas in France.

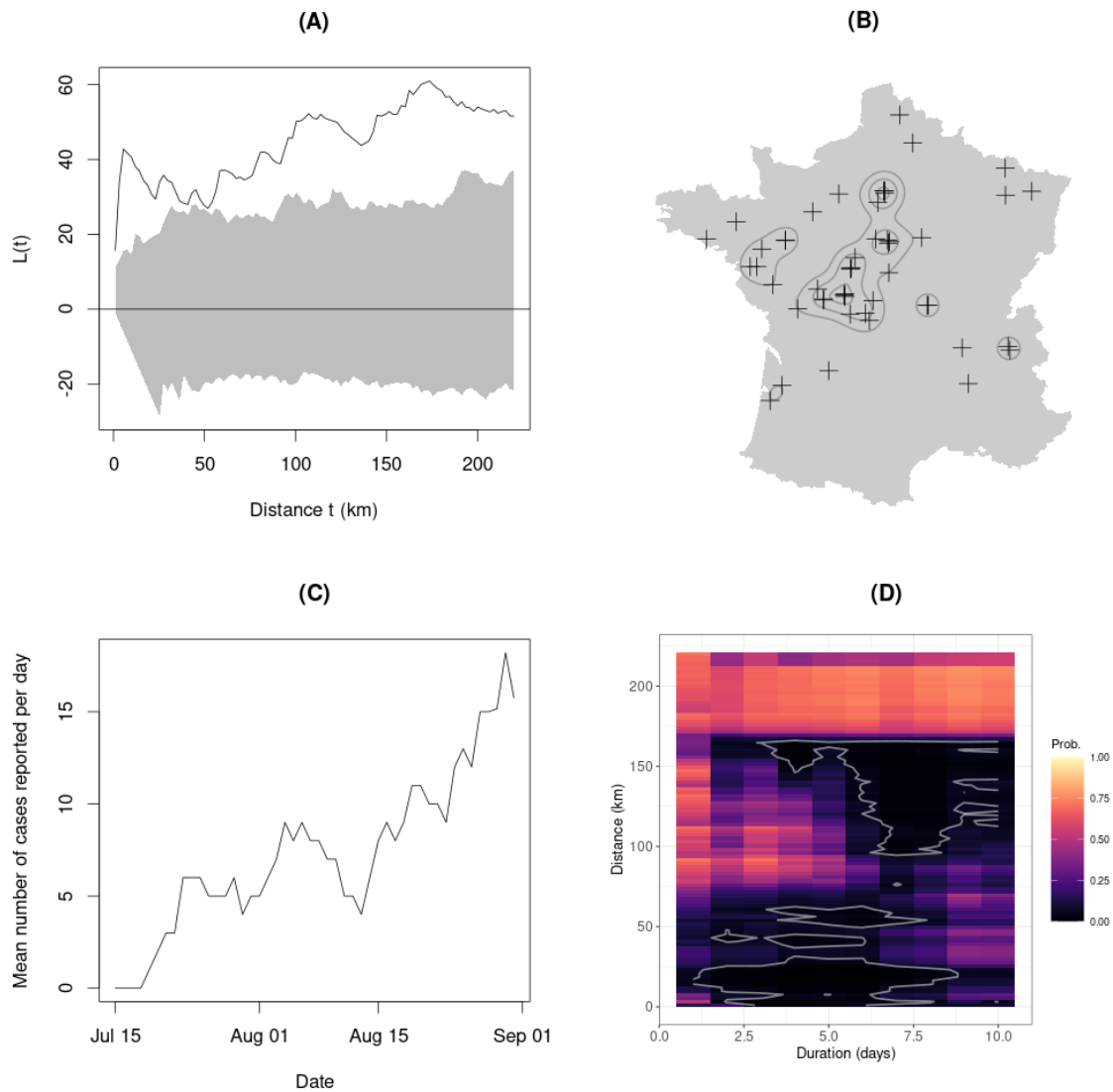
549 *Temporal analysis*

550 There was an initial increase in the number of cases between July 15th and 20th 2018 (**Fig. 2C**).
551 Then from July 20th to August 15th the number of cases reported per day stagnated at about one
552 per day. From August 15th onwards, there was a sharp increase, with an average of more than two
553 cases reported per day towards the end of the month.

554 *Spatio-temporal analysis*

555 The plot of probabilities that a simulation could lead to $K(u, v) > \hat{K}(u, v)$ showed a strong
556 clustering at small distances of about 10 to 20 km as soon as two or three days after a case,
557 expanding to 50-60 km five days after a case. But we also observed a clustering on larger distances
558 (150 km) starting as soon as two or three days after a case, which increased to reach a large range
559 of distances after a week (90 to 150 km) (**Fig. 2D**). The large distances clustering was consistent
560 with the first phase of the diffusion (between July 15th and 20th) (**Fig. 2C**), with the emergence of
561 the main clusters of Brenne, Paris, Orléans and Nantes (**Fig. 2B**). The small distance of clustering

562 was consistent with the emergence of secondary clusters and the expansion of preexisting main
 563 clusters (**Fig. 2B**) during the third phase of the diffusion (from August 15th) (**Fig. 2C**).
 564



565

566 **Fig. 2.** Spatial (**A, B**), temporal (**C**) and spatio-temporal (**D**) characteristics of the point pattern of
 567 birds detected infected to USUV during the study period (**A**: Comparison between $\hat{L}(t)$ (observed,
 568 grey line) and $L_r(t)$ (envelope of simulated point pattern under CSR hypothesis, grey area); **B**:
 569 Kernel smoothing of the point pattern (grey lines) and birds locations (black crosses) ; **C**: Mean
 570 number of cases (mobile average approach) reported per day and depending on date; **D**:
 571 Probabilities that spatio-temporal K function $K(u, v)$ simulated under the hypothesis of
 572 independence between spatial and temporal patterns, was higher than observed spatio-temporal K
 573 function $\hat{K}(u, v)$, depending on duration and distance (white line: contour limit of the set of

574 distance/duration pairs for which the proportion of simulation with $K(u, v) > \hat{K}(u, v)$ was lower
575 than 5%)

576

577 Impact on common blackbird population densities

578 The means and confidence intervals depicting cells of respectively low, medium and high USUV
579 cases density have been reported in Tab. [24](#), the distribution of those cases in Fig. [3A2A](#) and their
580 spatial distribution in Fig. [3B2B](#).

581 The estimation of common blackbird population trends estimated through variations of the mean
582 population indexes for 2015-2018 and 2019-2022 periods was not statistically different from 0 for
583 the low USUV area, ~~—~~but presented a significant decline for both medium and high USUV areas,
584 being more pronounced for the ~~latter second~~—than for the former (Tab. [24](#)). Two main results
585 were highlighted: i) a decreasing trend for common blackbird populations that have been *a priori*
586 exposed to USUV since the 2018 USUV outbreak and ii) the greater the pressure of infection was,
587 the greater the negative effect on common blackbird populations was (Fig. [3C2C](#)).

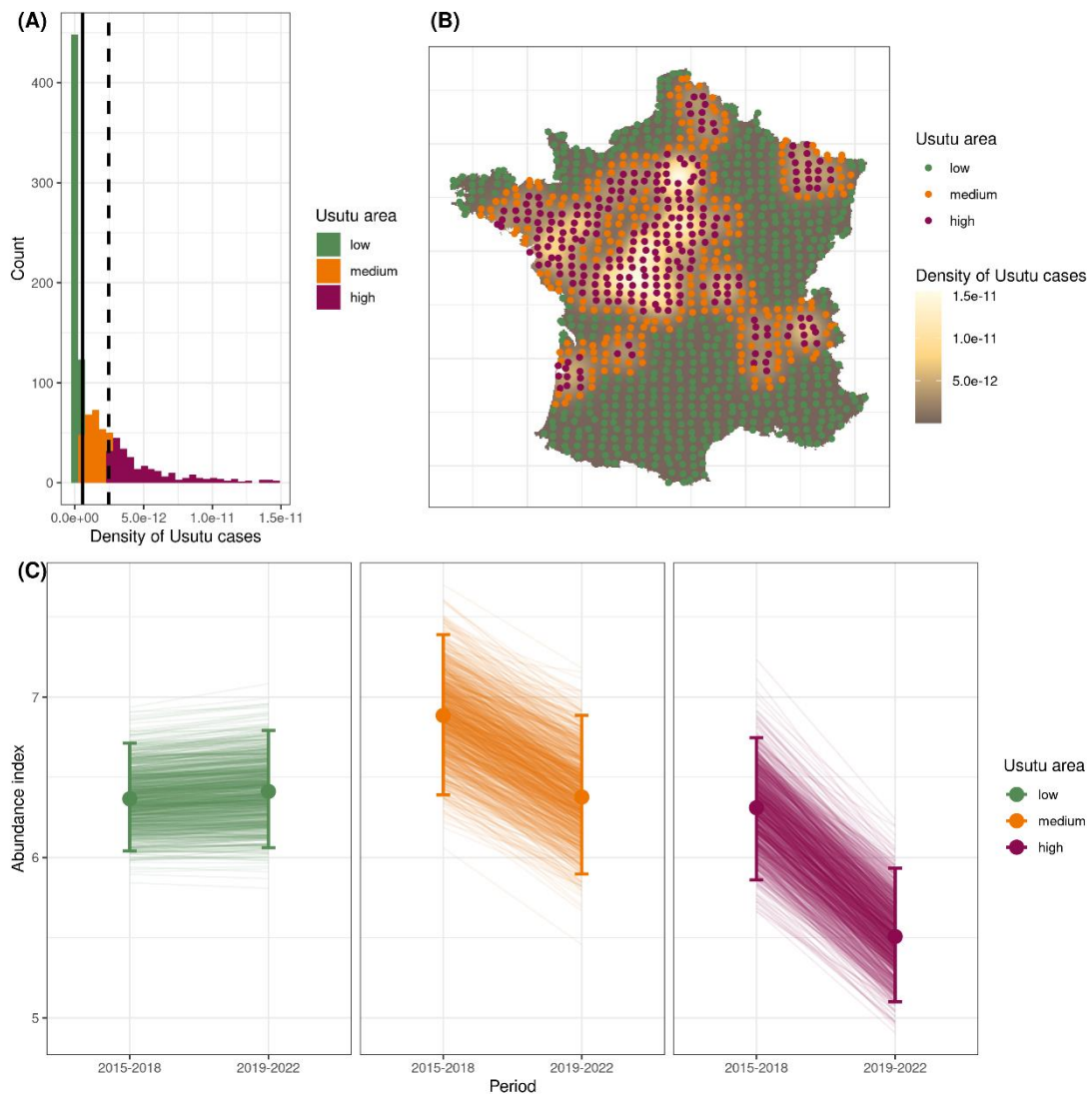
588

589 **Tab. 22.** Trends computed between 2015-2018 and 2019-2022 for the three different levels of
590 density of USUV common blackbird cases (CI: Confidence Interval).

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

591

592



593

594 **Fig. 3.** Common blackbird population trends in response to the USUV episode (A: Histogram of
 595 USUV cases density, from a kernel smoothing, for the routes where common blackbird were
 596 monitored (solid line: median of the distribution, dashed line: 75% percentile of the distribution);
 597 B: Maps of the kernel smoothing of USUV cases and locations of the centroids of REZOP road
 598 (pale green: low, orange: medium and dark purple: high densities of USUV cases as estimated by
 599 the kernel method); C: Predicted abundance index (mean values and their 95% confidence
 600 intervals) for the three classes of USUV cases density (pale green: low, orange: medium and dark
 601 purple: high densities) before (2015-2018) and after (2019-2022) the 2018 USUV outbreak. Each
 602 solid line represents changes in abundance value for a given simulation (1,000 simulated values
 603 were generated).

604

605

606 Discussion

607 Birds affected by USUV between July 15th and August 31st, 2018, in France were mainly common
608 blackbirds and to a lesser extent captive great grey owls. Cases were clustered at i) 5 km, ii) 125 km
609 (which was the average distance between two typical cases which we highlighted with a Thomas
610 process model) and iii) 200 km (which was the distance between main big clusters which appeared
611 simultaneously at the beginning of the outbreak and expanded thereafter). Three periods of USUV
612 diffusion have been featured: i) a first increase of USUV cases between July 15th and 20th (with
613 clustering at large distances of about 150 km between two and three days after a case and between
614 90-150 km a week after a case, corresponding to the emergence of main clusters – Brenne, Paris,
615 Orléans and Nantes), ii) a stagnation between July 20th and August 15th, and iii) an important
616 increase from August 15th (with strong clustering at small distances of about 10-20 km, between
617 two and three days after a case and expanding to 50-60 km five days after a case, corresponding to
618 the emergence of secondary clusters and the expansion of preexisting main clusters). Detected case
619 locations were statistically associated with wetlands and high human log-density population areas.
620 Common blackbird population trends estimated suggested an impact of the USUV infection on
621 them, with a decrease of the mean population index over 2015-2018 compared to 2019-2022. Areas
622 of *a priori* greater USUV pressure presented more pronounced negative trends than the two other
623 areas.

624 Species involved in the 2018 outbreak in the present study were those also found in other European
625 countries such as Germany (Becker et al. 2012; Lühken et al. 2017). Several hypotheses might be
626 put forward regarding the overrepresentation of common blackbirds: i) they are not trans-Saharan
627 migratory species and as a consequence probably deprived of an immunity they could have acquired
628 in Africa, ii) they might be more genetically susceptible than other birds species, iii) African strains
629 introduced in Europe might have adapted to common blackbirds and become more virulent for
630 this host (Bakonyi et al. 2004), iv) co-infections with *Plasmodium* might exist with a possible interplay
631 of the two agents, as highlighted in the Netherlands (Aglioni et al. 2023), v) a feeding preference
632 has been identified for them (when they were sufficiently abundant) by *Cx. pipiens* (Rizzoli et al.
633 2015) and vi) this species is abundant, ubiquitous, human commensal (Lühken et al. 2017) and thus
634 easy to detect by the SAGIR network.

635 Any pattern identified by the analysis of data collected by a participatory program relying on non-
636 controlled sampling pressure can be caused by the opportunistic observation process (characterized
637 by a very heterogenous sampling pressure) as well as by the ecological or epidemiological process.
638 In particular, the data collection process differs across departments (differences in detectability, in

639 probability to report a carcass to the network, in probability to collect this carcass by the local
640 contact, mode of transport, mode of storage, etc.). Thus, when a pattern was identified in the data
641 (e.g. a cluster of cases), we could not decide between (i) the fact that this pattern was caused by the
642 observation process (e.g. higher local sampling pressure due to higher human density and thereby
643 detectability) and (ii) a biological cause (e.g. local factors favouring the diffusion of USUV in this
644 place). It would have been interesting to model the data collection process to draw firmer
645 conclusions on the biological process. However, the lack of information about the data collection
646 process within the SAGIR network has been a long-standing issue, hindering such modelling (as is
647 often the case with participatory networks). Our approach was therefore exploratory, and our
648 results could only help formulating hypotheses, and could not allow confirming them. Keeping in
649 mind this limit, we must stress that our results pointed out that such a network and statistical
650 analysis of admittedly imperfect data could however allow the understanding of diffusion pattern
651 and epidemiological process. Indeed, the network was alerted to the possible presence of USUV
652 cases in France and the level of attention given to surveillance was greater for this disease than for
653 other diseases. We considered the possibility that any pattern identified in our data could be caused
654 either by the observation process (e.g. the heterogenous sampling pressure) or by
655 ecological/epidemiological processes.

656 The short distance clustering (at 5 km) identified by our analysis could be explained by local
657 sampling effort. We could not exclude that observers would have been more proactive in detecting
658 dead birds around first detections. However, this short distance of 5 km is also close to flight
659 distances of *Cx. pipiens* estimated in a study carried out in Chicago, Illinois, on *Culex* female
660 mosquitoes. Indeed the mean dispersal distance was 1.15 km, and 90% of individuals stayed within
661 3 km from their larval habitat (Hamer et al. 2014). Another explanation for the 5 km clusters we
662 observed would be a USUV diffusion mainly due to mosquitoes. Sufficient amplification of the
663 virus in its various hosts would also have allowed a large number of mosquitoes to be infected.

664 The occurrence of different clusters simultaneously at the beginning of this 2018 USUV outbreak,
665 far apart from each other, is in favour of USUV endemicity throughout the country. The endemicity
666 has already been proven in Southern (Constant et al. 2022) and North-Eastern France (Johnson et
667 al. 2018), but the 2018 outbreak we studied and the diffusion pattern we showed raised the
668 hypothesis of a national endemicity. Previous outbreaks, even of lesser magnitude, might have
669 enabled certain lineages to settle. Indeed, in France, USUV Europe 3 strain has been identified in
670 common blackbirds in two north-eastern departments between 2015 and 2017. USUV Africa 2
671 strain has been isolated in common blackbirds, mosquitoes and humans in three south-eastern
672 departments and in Rhone between 2015 and 2016, while USUV Africa 3 strains have been

673 identified in mosquitoes and birds in two central and four south-eastern French departments
674 between 2015 and 2017 (Johnson et al. 2018, NRL data). Favourable conditions (e.g. sizes, naive
675 immune status and/or turnover of bird populations, temperature, rainfall, human population
676 movements) could then lead to new outbreaks thereafter. The early detection of this 2018 outbreak
677 was made possible by the well-informed SAGIR network, which vigilance has been reinforced
678 since the 2015 outbreak for USUV relevant species. Throughout Europe, endemic circulation and
679 spread of USUV infections have been reported, e.g. Northern Italy, Hungary or Austria (Constant
680 et al. 2022). The study of lineages involved within this 2018 outbreak could help validate
681 endemicity.

682 The different phases of the 2018 USUV diffusion we observed remained difficult to explain: a first
683 increase of cases, with clusters far from each other, a stagnation and then an important increase of
684 cases, with secondary clusters and the enlargement of the first ones. If endemicity might explain
685 the cases pattern of the first phase, the origins of the third phase remain unclear. Meteorological
686 variables, as mean temperature (Hamer et al. 2014), could explain this result. Indeed, the duration
687 of *Cx. pipiens* eggs development depends on temperature (hatching after only one day at 30°C, three
688 days at 20°C, ten days at 10°C and incomplete embryonic development below 7°C (Becker et al.
689 2010)). Temperatures were particularly high in July and August 2018 in France (respectively
690 +2.18°C and +1.1°C higher than the 1991-2020 baseline average
691 (<https://www.meteocontact.fr/climatologie/france/bilans-climatiques>)). *Cx. pipiens* density might
692 have increased in August due to these favourable conditions and promoted an increased possibility
693 of contact between common blackbirds and the arthropod. Moreover, laboratory experiments have
694 shown that higher temperatures resulted in higher infection rate of *Cx. pipiens* (Fros et al. 2015)).
695 These conditions could have allowed an important development of *Cx. pipiens* and the third phase
696 of USUV diffusion we could observe. Further studies collecting local relevant meteorological data
697 would be useful to assess this hypothesis. Nevertheless, we could not exclude that the diffusion of
698 information and knowledge of the presence of USUV in France would have also led to better
699 sampling effort from August 15th.

700 In addition to the above-mentioned hypothesis related to the population dynamics of the main
701 vector of the disease, other non-mutually exclusive hypotheses could explain the appearance of
702 USUV clusters in late summer (after August 15th). This period coincides with the initiation of
703 movements in common blackbirds, particularly juveniles that disperse outside their parents'
704 territories (Snow 1958), and to a lesser extent, with the first mentions of post-breeding migrants
705 (Toulotte et al. 2022). Similar movements have been shown as the main drivers of seasonal
706 epizootics of the avian influenza in mallards (van Dijk et al. 2014), and the same processes might

707 be at play in the USUV episode reported here. USUV naïve birds such as juveniles, or individuals
708 whose original populations have not yet been exposed to USUV, might be more prone to develop
709 and spread the virus, easing the apparition of clusters.

710 In resident blackbirds, this period also overlaps with resource-demanding activities including
711 breeding for some late breeders (Sauvage 2016), post-breeding (complete) moult in adults (Snow
712 1969; Morrison et al. 2015) and partial moult in juveniles (Cramp 1988). Previous works have
713 shown that such activities may induce trade-offs with immunity in birds (e.g. Moreno et al. 1999;
714 Sanz et al. 2004; Martin II 2005; Moreno & rueda 2010). Accordingly, late summer may coincide
715 with a period of greater susceptibility to infections in blackbirds. Unfortunately, the lack of available
716 data on age, sex, or moulting status, both for the recorded USUV cases, but also for individuals in
717 the exposed populations, did not allow us to confirm these assumptions. This constitutes a relevant
718 area of investigation for future studies and highlights the need for a deeper description of
719 specimens found dead.

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

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

736 The association between wetlands and USUV was consistent with the life cycle of USUV, with
737 mosquitoes as vectors and wetlands known as suitable habitat for *Cx. pipiens* (Becker-Vogels et al.
738 [2010](#); [Haba and McBride 2022](#)~~2016~~). The association with high human population densities could
739 be more surprising at first sight. In an event-based network, the number of observers could partly

740 explain such a result. Indeed, the greater the number of people able to collect birds affected by the
741 disease would be, the greater the potential for the disease to be detected in densely populated areas
742 would be. In addition, common blackbirds are familiar to urbanised areas (Partecke and Gwinner
743 2007), and their size and colour (especially in males) are striking (Lühken et al. 2017): collecting
744 USUV dead common blackbirds would be easier in such areas. We therefore could not exclude
745 that the association between human high-density areas and USUV places could be explained by a
746 sampling bias, ~~although this hypothesis is difficult to confirm with our opportunistic data. Actually,~~
747 ~~as for wetlands, a selection for high human density areas by mosquitoes might be a part of the~~
748 ~~explanation for this.~~ ~~The contribution of AFVPZ data could be particularly interesting from this~~
749 ~~point of view, making it possible to multiply the sources and to reduce the bias. However, given~~
750 ~~the small number of birds from rescue centres (n=3), the only observation pressure hypothesis~~
751 ~~alone cannot explain the association with~~ the USUV cases. Indeed, areas of high human density.
752 Another part of the explanation could be *Cx. pipiens* can also find favourable breeding grounds in
753 , which have also been shown to be associated with urban (Haba and McBride 2022) or peri-urban
754 (Vogels et al. 2016) environments, ~~where breeding grounds favourable to mosquitoes could be~~
755 ~~found~~ (e.g. rainwater collection containers, ~~or~~ ponds in gardens, flooded cellars or construction
756 sites) (Becker et al. 2010). ~~This habitat preference might also explain the association we observed~~
757 ~~between cases of this vectorial disease and high human densities.~~

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

773 The presence of USUV infection in zoological gardens also raised the question of conservation of
774 foreign species. As previously said, SAGIR network is a precious tool for early detection. But in
775 case of endemicity, control measures must also be considered (e.g. elimination of artificial container
776 habitats, reduction of shade sources over aquatic habitats (Tuten 2011), use of biological larvicides
777 such as *Bacillus thuringiensis* subsp. *israelensis* (Virgillito et al. 2022), indoor caging during periods of
778 high density of mosquitoes, mosquito nets). For the first time in 2018, the AFVPZ and SAGIR
779 networks were really coordinated to face the USUV outbreak. Data collected and related to both
780 captive and non-captive wild birds allowed the description and the characterization of the outbreak
781 within mainland France (time of onset and duration). Non-captive wild common blackbirds could
782 be seen in this context as USUV sentinel for captive wild birds and would be of great importance
783 for species with conservation issues. This role could also be extended to non-captive other sensitive
784 species (e.g. capercaillie or eagle-owl), and more specifically Passeriformes, as the birds collected in
785 rescue centres have highlighted.

786 Another issue of the diffusion of USUV in France in wild birds as common blackbirds, living close
787 to human populations, and in zoological species is contact with humans, who are known as USUV
788 incident hosts (the asymptomatic form being predominant and fever illness, or neurological
789 symptoms as encephalitis and meningitis (Vilibic-Cavlek et al. 2014) being rare (Angeloni et al.
790 2023)). Zoological parks are particularly favourable to the circulation of mosquitoes which can feed
791 on both humans and animals (Martínez-de la Puente et al. 2020). In addition, screening of
792 asymptomatic blood donors and seroprevalence studies have also revealed incidental findings of
793 the virus in asymptomatic human populations (Cadar et al. 2017; Bakonyi et al. 2017; Zaaijer et al.
794 2019; Angeloni et al. 2023). Their link to epizootic outbreaks has raised the question of the need
795 to strengthen USUV detection in blood donors, particularly during such periods (Cadar et al. 2017).
796 Long-term epidemiological studies would be necessary to provide a better understanding of viral
797 dynamics in potential wild reservoirs (Vittecoq et al. 2013). The USUV antigen detection in feather
798 (follicle shafts and bulbs) of infected birds raised the possibility for live bird testing (Giglia et al.
799 2021). Risk mapping of competent vectors for USUV (*Cx. pipiens* and *Ae. albopictus*) could also help
800 developing appropriate prevention and control measures (Martinet et al. 2023).

801 In a context of climate change (Walther et al. 2002; Parmesan and Yohe 2003), the impact on
802 arboviruses is complex to define (Franklinos et al. 2019), but the perspective of a shorter wintering
803 period and a longer period of activity cannot be ruled out. Such changes would not be without
804 effect on the distribution patterns of arboviruses such as USUV and recent research has shown the
805 role of climate change in the increase of the risk associated with West Nile Virus circulation through
806 Europe (Erazo et al. 2024). Moreover, the analysis of the origins and the within-France spatial

807 distribution of common blackbirds have highlighted that i) they mainly came from Western and
808 Central Europe and ii) were strongly segregated in autumn and winter in France (Lahournat et al.
809 2021). The coming seasonal disturbances in a context of climate change therefore raise the question
810 of the impact of USUV in France on its epidemiology on a European scale. The contributions of
811 networks such as SAGIR and REZOP are therefore more necessary than ever.

812 Following this analysis of surveillance data, one could question the potential impact of the reactivity
813 of a network such as SAGIR, coordinated with the other networks mentioned above, and what
814 strategy should be adopted in the event of a future outbreak. Indeed, we highlighted here the
815 relevance of combining data collected by different networks primarily dedicated to different
816 purposes (e.g., SAGIR and REZOP). For example, an automatization of alerts could enhance the
817 reactivity of the entire system of networks. The results of spatial analysis could be quickly accessible
818 and help the definition of areas of risk, in near real time, and where surveillance (and if possible,
819 control) efforts could be concentrated. Such an automatization could also be interesting in a
820 financial perspective: as reported above, the number of cases had to be limited to one per
821 department after August 31st, because of too high analysis costs associated with the growing
822 outbreak. This drawback could be circumvented with better reactivity and if necessary, control of
823 the virus diffusion. However, additional data (i.e. additional funding to analyse more samples even
824 during the peak of the outbreak) would make it possible to reconstruct the spread of the virus over
825 time by following different lineages more closely.

826

827

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844 **Compliance with ethical standards**

845 In their publications, the beneficiary and associated partners specify that the work was carried out
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847 **Conflict of interest**

848 The authors declare that they comply with the PCI rule of having no financial conflict of interest
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850 **Author contributions**

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

858 **Data, Scripts, code, and supplementary information availability**

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

864

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