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Dear Editor,

Please find a revised copy of our manuscript that we wish to publish as an article in Peer Community In Infections: “*Relevance of the synergy of surveillance and populational networks in understanding the Usutu virus outbreak within common blackbirds (Turdus merula) in Metropolitan France, 2018*”; Bouchez-Zacria, M., Calenge C., Villers, A., Lecollinet, S., Gonzalez, G., Quintard, B., Leclerc, E., Baurier, F., Paty, M.-C., Faure, E., Eraud, C., Decors, A.).

We really would like to thank the two referees for the constructive and extensive feedbacks on the first submission. We have considered all the remarks of the referees, and we describe below how we accounted for these remarks in our revision.

We confirm that the work as submitted has not been published or accepted for publication, nor is being considered for publication elsewhere, either in whole or substantial part. Furthermore, all authors and relevant institutions have read the submitted version of the manuscript and approve its submission.

We hope our manuscript will meet your approval and we are looking to hear from you.

For the authors,

Sincerely,

Clément CALENGE

Revision round #1

Decision for round #1 : *Revision needed*

Dear colleagues,

Your manuscript has been reviewed by two referees. Your article has been positively evaluated, but it requires some revisions before it can be recommended.

I invite you to resubmit your manuscript, taking into account the reviewers' comments.

Yours sincerely

by [Florian Liégeois](#), 29 Oct 2024 10:12

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version: 1

Review by anonymous reviewer 1, 06 Sep 2024 19:28

Overall

The authors highlighted an important strategy on the potential use of data collected from different organization with different scope of work to describe an outbreak. I suggest that the utility of networks data synergies be added to the two objectives that are clearly indicated in lines (131-6). This clarifies that this aspect was part of the investigations as it is described throughout the manuscript, in the title and in the conclusion. The manuscript is sound, but it needs revision. My suggestions are provided below:

Abstract

- A clear statement of the knowledge gap and the main objective of the study are currently missing in the abstract. This information could be summarized from the one presented in Lines 128-136
- Thank you for this advice. We added the following sentence: *“Previous studies of USUV in France have focused on reconstructing pathways of introduction, but not on structural aspects of virus spread within the country.”* (lines 30-32)
- Write (SAGIR) in full at first mention (Line 31)
- Actually, SAGIR is not an acronym. It originally stood for ‘surveiller pour agir’ in French, which means ‘Monitor to Act’, but it is now just the name of the network, so that its full name is actually SAGIR. Nevertheless, we deleted the parentheses and completed the sentence: *“Data (RT-PCR of geolocated dead birds) on this 2018 outbreak were collected through both an event-based wildlife network named SAGIR...”* (lines 32-33)
- It is essential to specify the nature of the data collected regarding the 2018 USUV outbreak as part of the study details to provide context and support the research methodology (Lines 31-38).†
- We added parentheses *“(RT-PCR of geolocated dead birds)”* to the term data (line 32).
- Indicate the specific period of the USUV outbreak to give the reader an insight of what mid-July and August 2018 period meant in relation to the progression of the outbreak (Lines 31-38)
- We added *“during summer”* before “2018” (line 28).

- Reporting the statistical parameters/ data of the main significant findings (Lines 38-40) would help the readers understand the quantitative aspects of the key findings †
- We agree with the reviewer. We have modified the abstract accordingly, adding '*High human density (top 10.5% densest in France) and wetland concentration (top 19.3% most likely wetland) were significant factors in USUV case locations*'. (lines 40-41). We also completed the main text (lines 469-470 and 474-475).
- It would be beneficial to provide more specific details about the decline in findings by quoting relevant figures and statistical indicators (Lines 38-40).
- We provided information on quantitative population trends in medium and high USUV pressure areas (line 43-45).
- Mentioning the locations involved in the study and how they compare to one another would strengthen the abstract, which currently leans towards being primarily narrative.
- We completed with the following sentence: "*A large area (radius ~150 km) in the centre and centre-west of France, and smaller areas in the south-east, north and north-east of France (each with a radius ~ 50 km) were particularly affected.*" (lines 45-47)
- Conclusion currently absent
- We reworded the sentence to emphasise our conclusion (line 47) and added an opening about automation (lines 48-49).

Introduction

- No need to repeat the abbreviation again since it has already been defined (Line 46)
- Actually, this abbreviation is defined in the abstract, but not in the title. We think important to repeat the abbreviation in the introduction, as not all readers start to read the paper by reading the abstract.
- Almost 75 years now (Lines 68)
- We updated this countdown (line 74).
- There is a repetition of terms already defined eg 'event based' for SAGIR), *Turdus merula* for common blackbirds etc. This could be revised throughout the manuscript.
- We corrected these repetitions for SAGIR (line 144), *Turdus merula* (lines 102, 116, 436), *Strix nebulosa* (lines 103, 436).

Materials and Methods

- While this section is thoroughly described to enable replication, it is rather too detailed, hence too long. I suggest the following revisions:
- Add study design section -eg. This study employed an observational ecological design, using data from existing surveillance networks to assess the spatial and temporal distribution of Usutu virus (USUV) infections among bird populations in France. Data were collected opportunistically from mortality events reported through the SAGIR and AFVPZ surveillance networks, encompassing

wild and captive birds. The study design allowed for the examination of environmental and ecological factors influencing USUV distribution, such as proximity to wetlands and human population density, which serve as proxies for mosquito habitats and bird sampling pressures. We used spatial patterns and temporal trends at the population level, to understand the dynamics of USUV outbreaks and the identification of high-risk areas and informing future surveillance and management strategies.(comment 1)

- Thank you for this detailed suggestion. We added a study design section at the beginning of the Material and methods section (line 176). The paragraph you suggested was used with some modifications (lines 174-183) underlined in the following lines:

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

- Summarize the section titled "Epidemiological Surveillance Network and Provided Data" (L 170-249) focusing on the following key areas;(comment 1.1)
- Study Setting- Geographical area (Describe in 1 paragraph, the regions in France, covering the diverse habitats, urban and rural areas, wetland under study and where each of the network organizations operated). A map could help with the visualization of the study setting.(comment 1.2)
- Laboratory and surveillance infrastructure (A short summary of the scope of work of the accredited veterinary laboratories that conducted the various tests such as necropsy and virology etc.(comment 1.3)
- Data collection: Brief description of surveillance networks, SAGIR network, AFVPZ network and the type of data each collected (comment 1.4)
- Sampling and testing procedures (brief description of the necropsy and pathology testing - RT-PCR for USUV detection on tissue samples. This would not need to be detailed since this study used secondary data already gathered by the organizations. Provide details of the nature of data that was collected and used in this study and how it was reported and standardized (comment 1.5)
- Thank you for these tips and suggestions. This subsection was indeed a little long and too detailed. We have followed your recommendations and summarised what has been said (comment 1.1) and reorganised the different parts:1) study design (lines 173-183) (comment 1.2), 2) data collection and surveillance networks (comment 1.4) (lines 184-218), 3) laboratory procedures (comment 1.3 and 1.5) (lines 219-241), and 4) Epidemiological data analysis (line 242-353). We tried to be more concise in these sections. We have added a new (Fig. 1) (lines 193-198) to clarify the geographical description of collected data. We updated figure numbers.

Results

- Use Figure or Table references in place of (see below) (L278; L309)
- We removed the first “(see below)” because it was indeed superfluous (line 272). We replaced the second one by “(see Results section)” for clarification (line 303).
- Figure 1A needs to be interpreted further- It shows the comparison between the observed function and the simulated envelope under the complete spatial randomness (CSR) hypothesis (represented by the grey area) demonstrating significant spatial clustering of infected birds, indicating that cases are not randomly distributed but are concentrated in specific geographic areas.
- Thank you for this comment. We completed the sentence to clarify (lines 443-445).
- It is advisable to present the months in English since it is the language used in the manuscript. Alternatively provide key for English months (Figure 1C)
- Thank you for this comment. We corrected the Figure 2C (former Figure 1C) with English months (line 491).
- Provide key to Figure 1B to identify the places described in text, eg. Paris, OrlÈans, Nantes (west of France; Fig. 1B). (L454-58). Alternatively provide a map showing the study area in the materials and methods under study setting so that readers can follow the write-up.
- Thank you for this advice. We added the reference to Fig.1A for names of towns and regions (lines 448-449).
- Results could be tabulated for clearer view (L441-9). The table should also have a caption to describe the data presented in the table. This need to clear to show how these results are linked to the objectives of the study
- Thank you for this advice. We added the required table to improve the description of the data collected and replaced the list with a reference to the table. We hope that the lines we have chosen highlight the presence of both captive and wild birds and the complementarity of the networks (lines 434-441). We updated table numbers.

Discussion

Well discussed.

Review by David Roiz, 28 Oct 2024 14:09

The authors presented a very interesting analysis based on the SAGIR, AFVPZ and REZOP networks to explain the epidemiological impact of Usutu on common blackbirds during 2018 in France. A very positive point is that the authors developed a package in R and that throughout the vignette of the supplementary materials it greatly helped to understand the analysis.

A negative point of the manuscript is the, not so clearly, explanations of the fact that the detected case locations were statistically associated with wetlands and high human log-density population areas. The

authors stated that “the density of USUV cases varied as a function of wetlands and human density, two variables that are supposed to reflect mainly mosquito abundance“. (lines 652-654)

However, the underlying mechanism that explains the relation of wetlands with USUV cases is not well justified. The authors said that “the association between wetlands and USUV are consistent with the life cycle of USUV, with mosquitoes as vectors and wetlands known as suitable habitat for *Cx. pipiens* (Vogels et al., 2016)”. However, when reading this publication, the content it is not supporting this hypothesis. In the paper is stated that the “biotype *Cx. pipiens pipiens* (that is the ornitophilic biotype) is more abundant in peri-urban areas than in wetlands”, and that “*Cx. pipiens* populations that are dominated by biotype *pipiens* play an important role in the natural transmission cycle of WNV (and USUV) in birds...”.

We chose to use Vogels et al. 2016 firstly because the study used favourable biotopes (farms, peri-urban and wetlands) and estimated the proportion of *Cx pipiens pipiens* versus *Cx pipiens molestus* and hybrids. They found that *Cx pipiens pipiens* was, in Sweden and the Netherlands, the overwhelming majority species in both peri-urban areas and wetlands. When these two environments were compared the proportion (although still in the majority) occupied by *Cx pipiens pipiens* was lower in peri-urban areas than in wetlands in the Netherlands, and was greater in peri-urban areas than in wetlands in Sweden. However, we changed this reference, which could indeed be confusing if used to justify wetlands and used Haba and McBride 2022 and Becker et al. 2010 instead (line 253 and lines 664-665).

Also, the underlying mechanism that explains the relation of high human population with USUV cases is not well justified. This might be mainly explained by the increasing reporting in these areas, and therefore, by a sampling bias. The solution is to weight by the possibility of reporting and make other analyses, if possible.

We agree with the fact that the high human population density might cause a higher reporting rate, and thereby explain the identified correlation. We also agree with the referee that firmer conclusions would be obtained from such data if we could model the data collection process to account for the possibility of a sampling bias. However, the lack of information on reporting rates has been a long-standing issue with the SAGIR network (as is often the case with participatory networks). We now indicate in the discussion (lines 571-574): “*It would have been interesting to model the data collection process to draw firmer conclusions on the biological process. However, the lack of information about the data collection process within the SAGIR network has been a long-standing issue, hindering such modelling (as is often the case with participatory networks)*”.

We also note (lines 671-673): “*We therefore could not exclude that the association between human high-density areas and USUV places could be explained by a sampling bias, although this hypothesis is difficult to confirm with our opportunistic data*”.

We however stress the importance of this bias in the paper (lines 666-669): “*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 fact, the authors said that “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 favorable 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 density”. (lines 676-681). This explanation based on the *Cx. pipiens* abundance is contradictory to the one that said before that the vector is common in wetlands, so the explanation is that this is a synanthropic species related to human habitats. The sentence in lines 259-260 “Wetlands and human density were supposed to be a proxy for the

density of *Culex pipiens* mosquitoes (Vogels et al., 2016)”

The discussion might be revised with more literature and particularly, adding more explanatory variables and covariables to explain accordingly the results of these interesting analyses.

We do not understand why the referee think that the two explanations are contradictory: *Cx Pipiens* is common both in wetlands and in human habitats (as reported by (Vogels et al. 2016)), where resources can be found. *Cx pipiens* “are able to inhabit nearly every kind of water sources” as we could read in Becker et al. 2010. However, we tried to formulate our idea more clearly to avoid confusion. We now note: “*Actually, as for wetlands, a selection for high human density areas by mosquitoes might be a part of the explanation for this association with the USUV cases. Indeed, Cx. pipiens can also find favourable breeding grounds in urban (Haba and McBride 2022) or peri-urban (Vogels et al. 2016) environments (e.g. rainwater collection containers or ponds in gardens (Becker et al. 2010)).*”(lines 673-678).

In addition, the wetlands in our database could include both anthropogenic wetlands and natural, wild wetlands. We had not distinguished between the two. We cannot rule out the possibility that the wetlands associated with the cases in our study are anthropogenic. Unfortunately, our dataset does not allow us to conclude. We have not altered the body of the text so as not to lose clarity. As we have not specified ‘wild’ or ‘natural’ wetlands, we believe that the reader will not be misled.

Moreover, while the inclusion of additional explanatory and covariate variables would undoubtedly enrich our analysis, the limited sample size and opportunistic nature of our data collection render such an endeavour challenging. Given these constraints, we believe that a more extensive analysis might introduce more noise than signal.