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Quantifying West Nile virus circulation in the avian host population in Northern Italy

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ABSTRACT

West Nile virus (WNV) is one of the most threatening mosquito-borne pathogens in Italy where hundreds of human cases were recorded during the last decade. Here, we estimated the WNV incidence in the avian population in the Emilia-Romagna region through a modelling framework which enabled us to eventually assess the fraction of birds that present anti-WNV antibodies at the end of each epidemiological season.

We fitted an SIR model to ornithological data, consisting of 18,989 specimens belonging to Corvidae species collected between 2013 and 2022: every year from May to November birds are captured or shot and tested for WNV genome presence. We found that the incidence peaks between mid-July and late August, infected corvids seem on average 17% more likely to be captured with respect to susceptible ones and seroprevalence was estimated to be larger than other years at the end of 2018, consistent with the anomalous number of recorded human infections.

Thanks to our modelling study we quantified WNV infection dynamics in the corvid community, which is still poorly investigated despite its importance for the virus circulation. To the best of our knowledge, this is among the first studies providing quantitative information on infection and immunity in the bird population, yielding new important insights on WNV transmission dynamics.

1. Introduction

West Nile virus (WNV) is a flavivirus that causes remarkable outbreaks in humans and represents one of the world's most widespread arboviruses (Chancey et al., 2015; Habarugira et al., 2020). WNV is maintained in nature through mosquito-bird-

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mosquito infection cycles, with *Culex* mosquitoes being the main vector for pathogen transmission (Habarugira et al., 2020; Petersen et al., 2013). Many passerine birds, especially of the Corvidae family, develop sufficient serum viremia to allow mosquito infection upon feeding. Mammals and humans can get infected through bites of infected mosquitoes but do not develop sufficient viremia to transmit the virus (dead-end hosts) (Petersen et al., 2013).

About 20% of humans infected with WNV develop flu-like symptoms, while less than 1% of the infections experience the West Nile neuroinvasive disease (WNND), which is accompanied by encephalitis and/or meningitis. This condition can lead to flaccid paralysis and respiratory failure, eventually leading to death in around 10% of WNND cases, especially among the elderly or individuals with pre-existing medical conditions (Petersen et al., 2013).

In Europe, reported infections are more frequent from early summer to early autumn (Young et al., 2021). Indeed, the risk of infection depends on the ecology of mosquitoes, which is influenced by climatic factors like temperature, rainfall and humidity (Giesen et al., 2023; Marini et al., 2022; Paz, 2015). The first major WNV outbreaks were reported in Romania in 1996, and in Italy in 1998 (Autorino et al., 2002; Tsai et al., 1998). In Italy, WNV has become an increasing public concern after the large outbreaks experienced in 2018 (230 neuro-invasive cases and 42 deaths) and in 2022 (295 neuro-invasive cases and 37 deaths) (EpiCentro, 2023).

Many modelling approaches have been proposed to describe and understand the dynamics of WNV epidemics (Barker, 2019; Bhowmick, Fritz, & Smith, 2024; Laperriere et al., 2011; Marini et al., 2020, 2022; Wonham et al., 2004). Recently, an entomological-epidemiological model (Marini et al., 2020) has been applied to mosquito data consisting of *Cx. pipiens* collections via $C0_2$ traps and of the prevalence of WNV-infected pools for the years 2013-18 in the Emilia-Romagna region (northern Italy). The study highlights the importance of spring temperature in determining the intensity of the WNV epidemic season, showing that the exceptionally high temperatures in April–May 2018 may explain the observed increase in both WNV prevalence in mosquitoes and the number of human cases.

Another factor in principle contributing to the very large epidemic observed in 2018 could have been a particularly large fraction of susceptible birds at the beginning of the year; this was ruled out by the aforementioned model, as it was predicted that immunity in birds at the beginning of 2018 was similar to the previous years; however, there was no empirical evidence for this, as no data on birds were used in the analysis. More generally, so far avian data have been used in a very limited number of studies in Europe (Giesen et al., 2023).

Given the complexity of WNV ecology, most modeling frameworks adopt a compartmental approach, simulating the population dynamics of mosquitoes and birds while accounting for environmental factors. Notable examples include (Bhowmick, Fritz, & Smith, 2024) where mosquito feeding preferences were incorporated. However, generating quantitative estimates of WNV circulation in the wild is often hampered by a lack of critical data, such as infection rates in avian populations, and challenges in parameterizing models that simulate infection dynamics between mosquitoes and birds. With limited available data, constructing a fully mechanistic model becomes a daunting task.

In response, we propose a simplified mathematical model that uses a phenomenological force of infection to approximate temporal variations in transmission within the host population. Focusing on a scenario where only ornithological surveillance data is available, we analyze detailed infection records in corvids from the Emilia-Romagna region between 2013 and 2022. This model allows us to assess the spatiotemporal heterogeneity of WNV circulation and estimate the proportion of corvids with anti-WNV antibodies.

2. Materials and methods

2.1. Study area and avian data

Data were gathered in the Italian region of Emilia-Romagna, which extends for 22,446 km^2 and has a population of 4.4 million inhabitants. Emilia-Romagna mostly lies in the Po Valley plain, which presents favorable ecological conditions for WNV circulation, such as *Cx. pipiens* breeding sites density and distribution, bird species and population, and climate (Bellini et al., 2014).

Data acquisition is regulated by the Italian Ministry of Health, in the context of the national arbovirosis prevention plan (Ministero della Salute, 2019). Ornithological data spanning the period 2013–2022 was provided by IZSLER (Lombardy and Emilia-Romagna Experimental Zooprophylactic Institute) and acquired according to the monitoring programs approved by the Emilia-Romagna regional government (Notes PG-2013-98988 and PG-2014-238156, regional approvals 1763 of November 13, 2017 and 810 of May 28, 2018).

In Emilia-Romagna, each province has been divided into sectors of 1200–1600 km^2; every month, from May to November, no less than 20 birds are collected in each sector and tested for viral RNA in organs via polymerase chain reaction (PCR) techniques. Organ samples (heart, brain, kidney, and spleen) from each bird were pooled, mechanically homogenized, and tested by real-time PCRs. Sampling was carried out either by shooting or by capturing via walk-in cage traps the following resident Corvidae species: Eurasian jay (*Garrulus glandarius*), crows (*Corvus cornix* and *Corvus corone*) and Eurasian magpie (*Pica pica*). Additional details on the diagnostic and sampling procedures can be found in (Lauriano et al., 2021; Tamba et al., 2024).

Data include the date and municipality of sampling, species, and result of the PCR test. We considered data collected between the 20th and 47th week of each year. In order to address the spatial and temporal heterogeneity of data, while ensuring the availability of a sufficient number of data points for each unit of analysis, we divided each year into bi-weekly

periods, and the study area into 3 subregions (S1, S2 and S3), each including three administrative units (provinces). Specifically, S1 includes the provinces of Piacenza, Parma and Reggio Emilia (Fig. 1, green area), S2 includes the provinces of Modena, Bologna and Ferrara (Fig. 1, orange area) and S3 consists of the provinces of Rimini, Ravenna and Forlì-Cesena (Fig. 1, blue area). Provinces were grouped based on the qualitative pattern of WNV circulation; on this basis, a subdivision in 5 clusters was used in (Marini et al., 2020); here, for the sake of simplicity and in order to have a larger number of cases in each subregion, we chose to group clusters B and C proposed in (Marini et al., 2020) into subregion S2 (the central part of the region with the highest prevalence), and group clusters D and E (the Eastern part) into subregion S3. For each bi-weekly period and subregion, the fraction of infected specimens and its 95% Confidence Interval (CI) have been computed using the function `prop.test` in R (R Core Team, 2023).

A fraction of the samples collected from 2013 to 2015 and belonging to S2 had also been tested for the presence of WNV antibodies via enzyme-linked immunosorbent assay (ELISA). The procedure allowed us to discriminate between immune and recently infected birds (Tamba et al., 2017).

For further details on avian sample collection and specifics on the passive surveillance of human WNND cases, refer to (Tamba et al., 2024).

2.2. Epidemiological model

To estimate the fraction of immune birds, we fitted a deterministic SIR model to each subregion's ornithological data. The model is based on the following set of ordinary differential equations (for its mathematical derivation, see the Appendix), describing WNV circulation during a single epidemic season through the variables (x, y, z) indicating the fraction of susceptible, infected and recovered (immune) corvids, respectively:

$$\begin{cases} x'(t) = -\lambda(t)x(t) + \rho\gamma x(t)y(t) \\ y'(t) = \lambda(t)x(t) - \gamma y(t)(1 - \rho y(t)) \\ z'(t) = (1-\rho)\gamma y(t) + \rho\gamma y(t)z(t) \end{cases}$$

Thus, $z(t)$ provides a measure of the seroprevalence level in the avian population at time t.

As noted in (Marini et al., 2022), the distribution of symptom onset dates in recorded human cases is well-approximated by a Gaussian function, which was consequently used to model the force of infection in the human population. Similarly, here we assumed that the rate of new infections in birds during an epidemic season follows a Gaussian function of time f(t). The function is defined as $f(t) = \lambda(t)x(t) = C\varphi(\frac{t-m}{\sigma})$, where C represents the total fraction of infected corvids at the end of the season; φ is the standard Gaussian density function; m indicates when the peak of the infection incidence occurs in the season; σ defines the duration of the season, and t indicates the week of the year. The model includes two additional key parameters (see Table 1): ρ, defining the WNV-induced mortality in birds, and γ, representing the recovery rate which was assumed equal to the reciprocal of the average length of WNV detection via PCR (WNV PCR-positivity period). Furthermore, we assumed no overlap between the birds' breeding period and the epidemic season, therefore no birth occurs during the simulated season. Bird migration is assumed to be non-influential.

For each epidemic season (2013–2022), we simulated WNV transmission between the 20th and 47th week of the year (mid-May to mid-November). After each breeding period, seroprevalence declines due to the influx of immunologically naive newborns and the death of a fraction of adult birds. The initial condition of the system at each year Y depends on the final seroprevalence estimated at the end of the preceding season Y-1, according to the following relationship:

$$\left(x_{Y,S}(20), y_{Y,S}(20), z_{Y,S}(20)\right) = \left(1 - q \cdot z_{Y-1,S}(46), 0, q \cdot z_{Y-1,S}(46)\right)$$

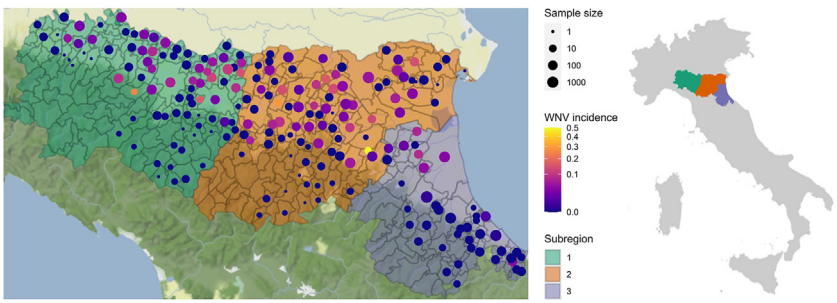

Fig. 1. Study area and collected data. Points represent the traps' locations, with size and color indicating the number of specimens and the overall fraction of positive birds collected between 2013 and 2022. Areas depicted in different colors represent the three subregions (S1: green, S2: orange, S3: blue).

Table 1
Model parameters.

Parameter	Interpretation	Assumed values or range	Reference
γ	Recovery rate (reciprocal of the PCR-positivity period)	0.44 weeks^{-1}	Komar et al. (2003)
ρ	WNV-induced mortality rate	0.2	Assumed. See text
q	Fraction of adult corvids after the breeding period	0.418	Marini et al. (2020)
C	Overall yearly infection strength	$(0, +\infty)$	Estimated
m	Seasonal peak of infection [weeks]	(20,46)	Estimated
σ	Temporal spread of infections [weeks]	$(0, +\infty)$	Estimated
b	Sampling bias parameter	$(0, +\infty)$	Estimated

where q indicates the fraction of adult corvids after the breeding period and depends on the birth and death rates. We assumed $(x_{2013,S}(20), y_{2013,S}(20), z_{2013,S}(20))=(1,0,0)$ for every subregion S, since no WNV infections had been reported in the area in the two years before 2013. Moreover, we allowed for a sampling bias in our data, as infected birds might be captured with a different probability with respect to susceptible and recovered ones. This bias has been modelled by the parameter b, defined as the relative risk of being sampled if infected, i.e. the ratio of the conditional probabilities of sampling a corvid given its infected or healthy status.

Parameters γ and q have been assumed from the literature (see Table 1), while the parameters C, m, σ and b have been estimated through a Markov chain Monte Carlo approach applied to the likelihood of observing the number of infected specimens reported in the data. As for ρ, we set it to 0.2, a value lower than laboratory estimates (Dridi et al., 2013; Komar et al., 2003; Lim et al., 2014, 2015) but seemingly more realistic, since in Europe WNV appears to exhibit limited pathogenicity in birds (Calistri et al., 2010). The sampling bias was assumed to be constant over the considered subregions and seasons.

All simulations were computed in R v4.0.2 (R Core Team, 2023) employing the package deSolve (Soetaert et al., 2010). Inference on the model's outputs and parameters has been carried out by means of a Monte Carlo Markov chain (MCMC) method; see the Appendix for details on the adopted procedure.

We then randomly extracted 200 parameters from the posterior distribution and used them to produce estimates of possible time series of the model variables; in particular, we computed $y(t)$, the model estimate of the weekly "true prevalence" curve (infected fraction in the total population), as well as of the "biased prevalence" curve (infected fraction in the sampled population) $y^*(t)$, that takes into account the estimated sampling bias.

We also explored correlations between observed yearly human cases (EpiCentro, 2023) and estimated avian incidence up to mid-summer (up to week 30), corresponding to the usual start of the epidemic season in the human population.

2.3. Validation & sensitivity analysis

Available antibody seroprevalence data was employed to validate our model's seroprevalence predictions. We also calibrated our model by assuming the initial 2013 conditions in subregion 2 as from the observed seroprevalence data, i.e. $(x_{2013,2}(20), y_{2013,2}(20), z_{2013,2}(20))=(0.55, 0, 0.45)$.

Sensitivity analysis was performed to test the relevance of the values we assumed for parameters γ, ρ and q in shaping our estimates. Specifically, we repeated the inference process, increasing or decreasing one parameter at a time by either 20% ($1/\gamma$) or by 50% (ρ and q). Thus, we employed the following alternative values: $\gamma_+ = 0.554$; $\gamma_- = 0.369$; $\rho_+ = 0.30$, $\rho_- = 0.10$; $q_+ = 0.627$, $q_- = 0.209$.

3. Results

3.1. Avian sampling

Available data consists of 18,989 captured specimens, of which 584 have been classified as positive for WNV genome presence in organs. Specimens have been classified as magpies (13,152 specimens; WNV+: 458), crows (3911 specimens; WNV+: 118) or Eurasian jays (1926 specimens; WNV+: 8). Table 2 summarizes the distribution of samples by year and subregion. Fig. 2 shows the observed WNV prevalence (points) and its 95% confidence interval (bars) for each biweekly period and subregion; clearly, in the bi-weeks and subregions in which the number of sampled birds was small, the confidence intervals are quite large.

Table 2
Number of tested and positive (within brackets) samples by year and subregion.

	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
S1	390 (26)	356 (15)	358 (13)	368 (21)	263 (14)	322 (16)	442 (11)	390 (14)	446 (13)	426 (16)
S2	1111 (115)	1047 (22)	797 (34)	458 (32)	475 (12)	804 (74)	1004 (13)	1232 (18)	861 (16)	788 (28)
S3	431 (3)	1042 (1)	338 (0)	461 (14)	557 (5)	485 (18)	341 (1)	708 (0)	1152 (0)	1136 (19)

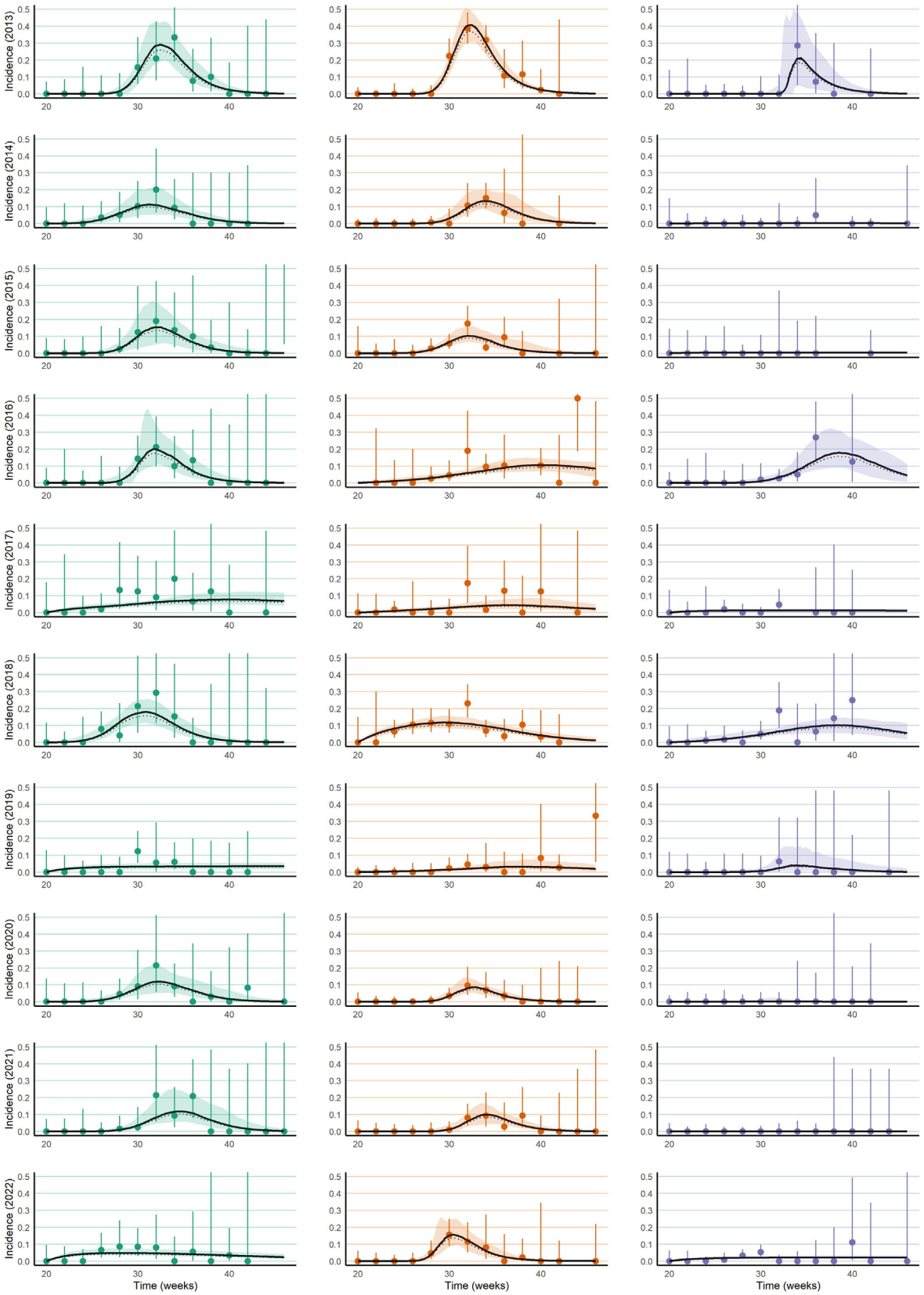

Fig. 2. Model estimates for the observed WNV incidence for each year (row) and subregion: S1 (green, first column), S2 (orange, second column) S3 (blue, third column). Shaded areas represent the 95% credible intervals of the "biased prevalence" $y^*(t)$, and the solid lines are their medians. The dashed lines are the median of the "true prevalence" $y(t)$. Points represent the mean observed incidence of each biweekly period with 95% CI (vertical bars).

A fraction (n = 558; 2.9%) of the observations collected from subregion 2 in 2013–2015 were also tested for WNV antibodies, resulting in 239 (42.8%) seropositive samples. Of those 558 samples, 71 (12.7%) were positive for the WNV genome, while no sample was positive for both antibodies and viral genome.

3.2. Parameter estimates and model accuracy

The adopted modelling approach effectively captured the temporal changes of WNV incidence in corvids across different subregions (Fig. 2), with 89% (327/366) of average estimates falling within the confidence intervals of data records. The posterior distributions of the free model's parameters are shown in Fig. 3 (values reported in Table S1 in the Appendix). Estimates are spatially and temporally heterogeneous. The estimated values for *m* (average: 31.3, 95% Credible Interval (CrI) 25.5–39.9) confirm that the peak in avian infection usually occurs between mid-July and late August.

The sampling bias *b* has been estimated on average equal to 1.17 (95%CrI: 1.02–1.43), indicating that infected birds were 17% more likely to be captured.

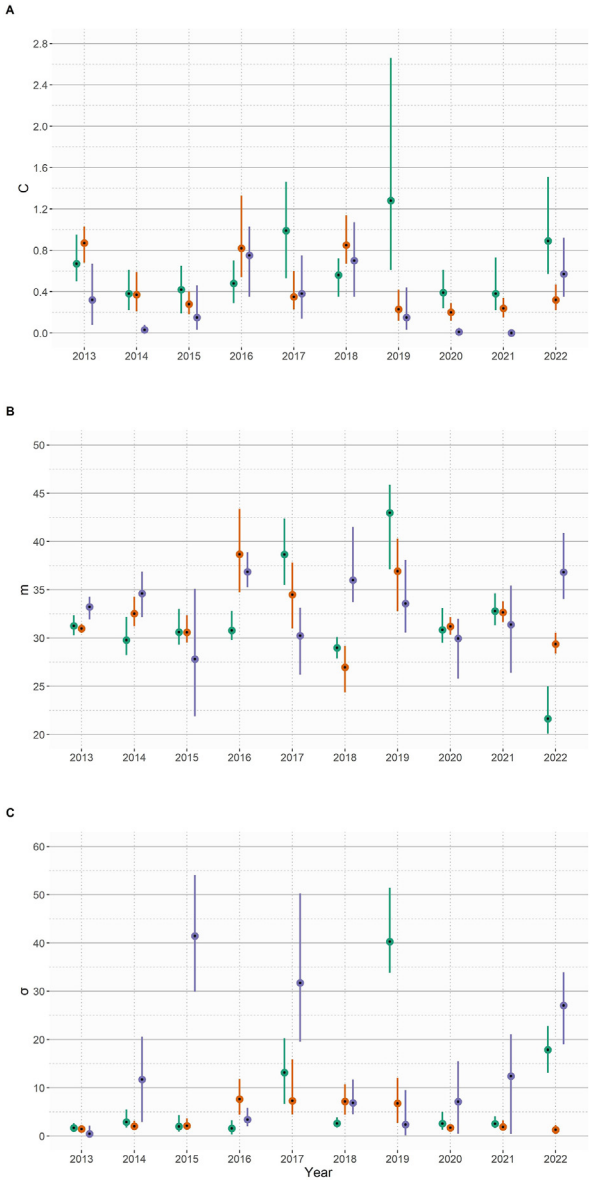

Fig. 3. Estimated distributions of free model's parameters *C* (panel A), *m* (panel B) and σ (panel C). Average (dots) and 95% CrIs for S1 (green), S2 (orange), S3 (blue).

Our estimates suggest a similar trend in the WNV incidence in corvid birds between subregions S1 and S2, with a significant level of WNV circulation in most years. In contrast, we found that subregion S3 was characterized by a generally lower WNV incidence, exhibiting significant WNV transmission in corvids only in 2013, 2016, and 2018.

The highest peaks in WNV incidence (up to 0.40 on average in subregion 2) occurred in 2013, 2018, and 2022, when larger epidemics in humans occurred (EpiCentro, 2023). For these years, significant levels of WNV circulation in birds were found, although comparable levels were identified for other years as well. None of the year-dependent parameters (C, m and σ) show a significant correlation with the observed WNV cases in humans. However, the estimated cumulative WNV incidence in birds up to the 30th week is significantly correlated with the number of human cases (see Fig. S1 in the Appendix); conversely, correlation is not significant between the number of human cases and the overall prevalence found in corvid samples (Fig. S2 in the Appendix).

3.3. WNV seroprevalence in birds

Fig. 4 shows the estimated seroprevalence in corvids ($z(t)$) for each subregion during the 10 years under study. The highest seroprevalence values were estimated for S2 in 2018.

High final seroprevalence values (38–89%) were consistently estimated for S1 and S2. Specifically, in the subregion S1, the estimated fraction of immune corvids at the end of the epidemic season (week 46) always falls between 58% and 89%. Seroprevalence levels associated with subregion 2 exhibit a more erratic trend, with peaks in 2013, 2016 and 2018. Peaks

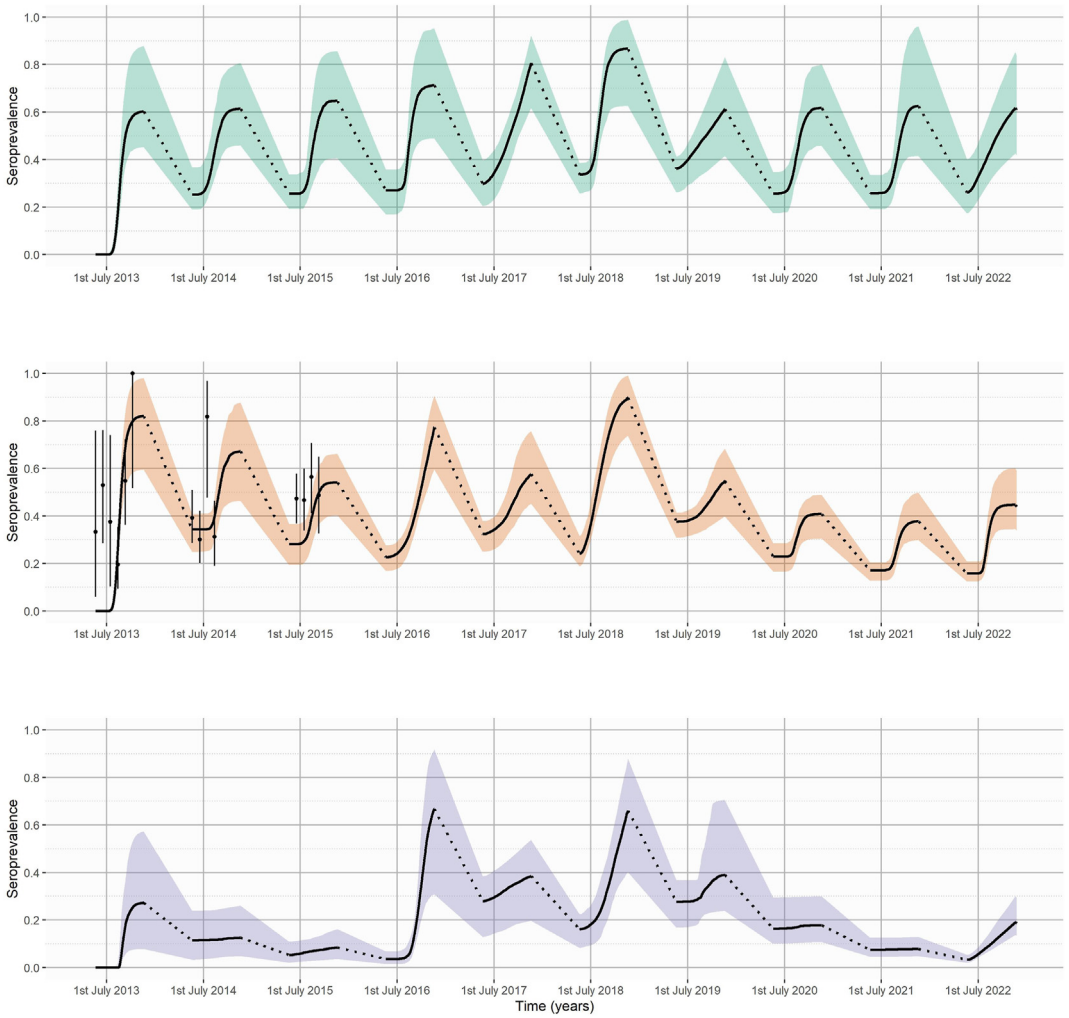

Fig. 4. Model estimates for the average (black line) seroprevalence dynamics (fraction of recovered corvids, $z(t)$) across each year and subregion (S1: green, first row; S2: orange, second row; S3: blue, third column), with their 95% CrIs (shaded coloured areas). The dashed lines represent the decline in seroprevalence resulting from bird breeding between consecutive epidemic seasons. For S2, the plot includes the observed monthly seroprevalence (2013–2015) along with its corresponding 95% CI.

following the years 2013, 2016 and 2018 were also found for S3. For the remaining years, the level of avian immunity is often negligible. Estimates for S2 are characterized by narrower CrIs due to the higher availability of samples for this area.

To validate our model results, we compared the seroprevalence records available for subregion S2 with the respective estimates. Although the fraction of data points whose credible intervals overlap our estimates is only 54% (7/13), the observed and estimated dynamics are qualitatively similar. The partial agreement between model estimates and observed seroprevalence records may be explained by the fact that, in our baseline analysis, we assumed a starting condition of $z(t) = 0$, leading to a mismatch between early 2013 predictions and available data (Fig. 4).

3.4. Sensitivity analysis

Fig. 5 shows the estimated seroprevalence for subregion S2 as obtained in the sensitivity analysis. Specifically, we found that perturbing the parameters γ (PCR-positivity period) and ρ (WNV-induced death rate) leads to estimated seroprevalence temporal patterns extremely similar to those obtained in our baseline analysis (i.e. alternative estimates always falling in the credible intervals of baseline estimates). On the other hand, perturbing q (fraction of adults at the beginning of the

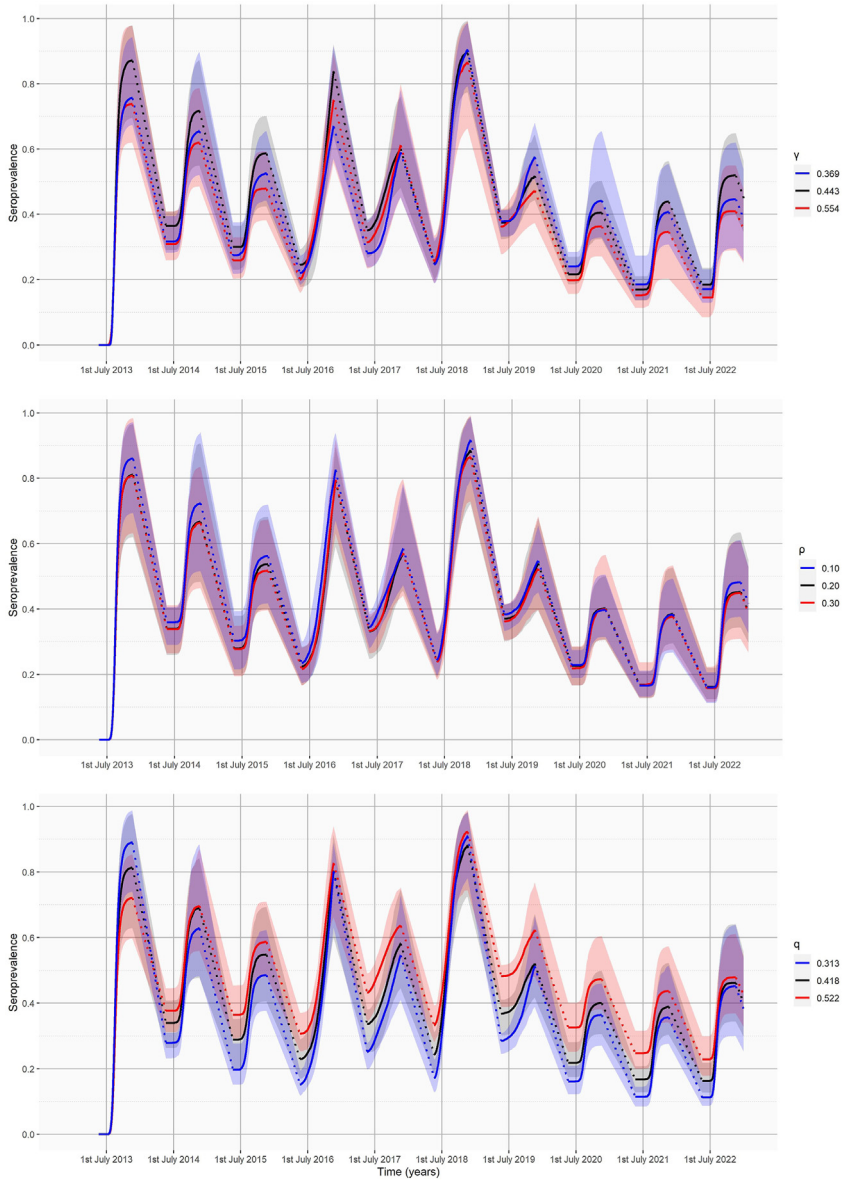

Fig. 5. Sensitivity analysis on the fixed parameters γ, ρ and q. Plots show the average (lines) effect of the perturbation of each parameter on the estimated seroprevalence in subregion S2. Shaded areas indicate each prediction's 95% CrI.

Table 3
Estimated b (sample bias) values for the baseline scenario and the sensitivity analyses.

	Baseline	γ_-	γ_+	ρ_-	ρ_+	q_-	q_+	Observed seroprevalence
Average	1.17	0.95	1.56	1.19	1.07	0.97	1.31	1.57
95% CrI	1.09–1.55	0.84–1.09	1.34–1.95	1.01–1.50	0.92–1.29	0.83–1.21	1.14–1.54	1.34–1.91

epidemiological season) leads to a clear shift in the estimated seroprevalence, especially in the periods following the highest peaks in incidence; as expected, the mean shift is larger right after the breeding period (t = 20th week, average: 41%) than at the end of the epidemic season (t = 46th week, average: 35%).

Changes in γ and q strongly affect the estimated sampling bias b. As shown in Table 3, changing γ has the largest effect on the estimate of b, while different values of ρ (the WNV-induced mortality) result in similar outcomes. It can be observed that, if $\gamma = \gamma_-$, the 95% credible interval for b includes 1; in other words, if the length of PCR-positivity is around 19 days, data could be fitted without assuming any sampling bias. Similarly, by assuming a higher WNV-induced mortality ($\rho = \rho_+$) or a smaller fraction of adult corvids after the breeding period ($q = q_-$) we found a 95% CrI encompassing 1.

As a further sensitivity analysis, we re-calibrated the model, assuming that initial conditions in 2013 were given by the observed seroprevalence in the first part of that year in subregion S2 (no seroprevalence data is available for the other subregions). In Fig. S5 of the Appendix, we show the resulting estimated seroprevalence, which does not differ greatly from that obtained in the baseline, except for the year 2013 itself. Under this assumption, the estimate for the sampling bias b increases to 1.57 (see Table 3).

The complete results of the sensitivity analysis can be found in the Appendix.

4. Discussion

We leveraged novel and detailed WNV surveillance data in corvids to estimate the WNV incidence in the avian population using a simple yet reliable model, which was able to reproduce the observed patterns of infection. The model is used to provide quantitative information on WNV transmission dynamics in the corvid population in northern Italy and on the spatiotemporal evolution of WNV immunity in birds, representing a key hidden variable to understand the epidemiology of WNV and consequent infection risk for human populations. Analyzed data represent a unique opportunity to investigate WNV circulation in the host population, due to strong intrinsic difficulties in gathering reliable epidemiological records from wild birds.

It is important to acknowledge that wild bird collection was strongly regulated by the national arbovirosis plan (Ministero della Salute, 2019) and hunting legislation, which defined both the sampling period and the target species. This presents two key limitations: data concern only the epidemiological season, and the three species of corvids listed before, although having more extensive (in terms of time and bird species) samples would be beneficial for testing the model's predictions. Similar issues are also common in other studies, as surveillance often relies heavily on dead or easily captured birds, leading to an overrepresentation of species with high mortality rates (Simonin, 2024). The advantage of the samples used in this study is that they are obtained through active captures in the populations, thus limiting the sources of bias.

We found that the seroprevalence at the end of the infection season ranged between 7.8% and 89.1% (average: 52.5%, SD: 23.3%), depending on the year and the subregion. The high WNV seroprevalence we found in corvids is consistent with the limited evidence currently existing in the literature. For instance, seroprevalence in the house sparrow (*Passer domesticus*) and house finch (*Haemorhous mexicanus*) populations was observed to be as high as 30–40% in Los Angeles in October 2004 and 2009 (Kwan et al., 2012), while avian seroprevalence higher than 60% was recorded at the end of September 2005 in Chicago (Hamer et al., 2008). In Romania, 33.96% of wild birds tested positive between 2011 and 2012 (Paştiu et al., 2016), soon after a large (50 cases) WNV outbreak was observed in humans in 2010. Although the literature shows seroprevalence ranges similar to our estimates, it is important to note that our study focused solely on corvids, and comparisons with other Passeriformes or bird species may be misleading. For example, a study on the seroprevalence of anti-WNV antibodies in Germany during 2021–2022 revealed significant variation across bird species, with rates as high as 80% observed exclusively in Northern Goshawks from Eastern Germany (Schopf et al., 2024). Additionally, Usutu virus (USUV) infection has been shown to confer partial immunity to WNV in magpies (Escribano-Romero et al., 2021); since USUV co-circulates with WNV in Emilia-Romagna (Calzolari et al., 2010), our seroprevalence estimations may underestimate the level of anti-WNV immunity in the corvid population.

In a previous study (Marini et al., 2020) carried out in the same area between 2013 and 2018, we investigated WNV prevalence in the vector population through a modelling framework explicitly considering the transmission in birds as well. However, in that case, the model was informed only by entomological data. Anomalous high spring temperatures were found as the most likely driver of the large 2018 outbreak, ruling out a low immunity level in the avian population. In this study, we corroborate this hypothesis using ornithological data. In fact, we estimated that seroprevalence at the beginning of the 2018 epidemiological season was similar to that estimated for other years in all clusters.

Indeed, the estimates of seroprevalence we obtained must be considered just a first step, as they are model-based and empirical verification is extremely limited. Overall, however, our seroprevalence estimates proved to be quite robust to

different assumptions on parameter values surrounded by a large uncertainty. Nonetheless, we found that the fraction of adults surviving to the following year strongly affects this quantity, especially in years following a particularly high WNV circulation (e.g. 2018). This suggests that solid estimates of this quantity would be crucial for quantifying seroprevalence levels in birds. Furthermore, values assumed in the proposed work (Marini et al., 2020) are based on demographic studies (Birkhead, 1991) conducted in different regions of the globe. Consequently, the availability of demographic data from the considered study region would likely improve the accuracy of model estimates.

We indirectly estimated that infected birds are more likely to be captured. This appears consistent with the infection's clinical manifestation, which might cause lethargy and ataxia in birds (Del Amo et al., 2014; Komar et al., 2003; Oya et al., 2018); possibly this could make infected birds both an easier target for hunters and more prone to look for food in traps. Furthermore, juvenile birds are mostly susceptible to infection as they lose acquired maternal immunity, if any, at a few weeks of age (Bowen et al., 2008), and also more likely to be captured as they are less cautious. We remark, however, that by assuming a longer positivity period (lower recovery rate, see sensitivity analysis) the bias parameter could be neglected. Indeed, if birds are assumed to remain infectious for a longer period, the model can fit the data without increasing the sampling rate for positive birds. Note that what matters in this analysis is not the infectiousness period (that has been estimated to be at most around 7 days) but how long the WNV genome can be detected in birds; it would then be useful to obtain more accurate data on this quantity. The estimate of the sampling bias is affected, to a lower extent, also by the values of ρ, the fatality ratio, and q, the proportion of adults surviving to the following year.

Interestingly, the parameter shaping the magnitude of the force of infection (C) does not correlate with human incidence. In fact, a high number of WNV human cases was recorded in 2018 and 2022 (EpiCentro, 2023), but higher C values were estimated for instance in 2013 and 2016. On the other hand, our analysis suggests that human spillover is enhanced when the avian epidemiological season begins early; we identified a substantial correlation between the number of recorded human cases and the total estimated avian incidence up to the 30th week of the year. This is consistent with previous studies, suggesting that early WNV circulation is associated with higher epidemic risk in humans (Farooq et al., 2023; Marini et al., 2020, 2021, 2022; Riccardo et al., 2022). Note that in this study we only include human cases in which neurological symptoms are reported, since these should be less affected by reporting bias. Thus, surveillance of the avian population could be crucial to early detect an increased risk of transmission later in the season.

It must be remarked that the proposed model does not consider the whole infection cycle, but instead uses a phenomenological force of infection in corvids. However, this model allows us to estimate both the trend of seroprevalence and the WNV incidence curve in birds employing exclusively ornithological surveillance data. Despite the large drop in seroprevalence after the breeding season, the residual level of bird immunity entering the following season is significant and could possibly influence WNV dynamics. Globally, these results show that our model was able to extract informative knowledge that wasn't easily attainable from the available data. This knowledge could be used in a comprehensive mechanistic model of the whole infection cycle, informed by both entomological and avian data on WNV infection.

Note that in the phenomenological model, it is assumed that the rate of new infections, not the force of infection, is described by a Gaussian density function. From the mathematical point of view, this choice could lead to the density of susceptibles, $x(t)$, to become negative. The estimation method rejects parameters that would yield negative values of $x(t)$, thus leading to effective parameter estimates. In principle, one could then infer the time-varying values of the force of infection $\lambda(t)$ and compare them to estimates of the density of infectious mosquitoes.

Clearly, the model has several limitations, beyond the choice of a phenomenological law for the force of infection. For instance, absolute densities of corvids are assumed not to affect the infection or death rates, and we did not consider bird movement or migration, which could influence the dynamics of WNV spread. However, since we grouped data into large subregions, we focused on non-migratory bird species and WNV has been present over the whole region in the period under analysis, we believe that the impact of migration on this model should be negligible.

Finally, the estimated WNV infection patterns could be highly valuable, since data on bird prevalence is often highly uncertain and variable from one week to the next. This is likely due to convenience sampling which is often erratic both in space and time. While it is known from laboratory experiments that many bird species are competent for WNV infections, it is still unclear which species are the main driver of the WNV infection cycle in Europe (Nikolay, 2015). Estimates provided here could be used to inform models mimicking the whole transmission cycle between mosquitoes and birds, eventually allowing a better assessment of human spillover risk. Moreover, such models might provide a deeper understanding of whether corvids are among the most important hosts or whether other bird species are relevant for the WNV transmission dynamics.

CRediT authorship contribution statement

Alex De Nardi: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Conceptualization. **Giovanni Marini:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Ilaria Dorigatti:** Writing – review & editing, Conceptualization. **Roberto Rosà:** Writing – review & editing, Conceptualization. **Marco Tamba:** Writing – review & editing, Resources, Investigation. **Luca Gelmini:** Writing – review & editing, Resources, Investigation. **Alice Prosperi:** Writing – review & editing, Resources, Investigation. **Francesco Menegale:** Writing – review & editing, Methodology. **Piero Poletti:** Writing – review & editing, Methodology. **Mattia Calzolari:** Writing – review & editing, Resources, Investigation. **Andrea Pugliese:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

Data availability

All data and R codes used to obtain the presented results are available at https://github.com/dnaxel/WNV-seroprevalence.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.idm.2024.12.009.

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