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Mathematical modelling to investigate infectious disease dynamics and control strategies

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Abstract

Infectious diseases still represent one of the major threats for human health due to both their direct and indirect effects on public health and worldwide economies. Despite the current possibility to eradicate or control certain infections like smallpox, polio and measles, the increase in incidence of new infections (so called emerging diseases), or the increase in incidence or geographic range of ones that have existed previously (re-emerging diseases), poses a new threat to public health. To further complicate things, the role of animals in the insurgence and spread of new diseases is central. Of all emerging diseases indeed, the 60.3% originate by, or involve into their cycles, animals and represent the so called zoonoses. The increase in number of emerging and re-emerging infections and their potential to fast spread into animal and human populations make central the development of tools to reduce human infection risk. Epidemiological studies become then central to understand the relationship among events, investigate their causal effects and understand risk factors. Despite of that, classical epidemiology, centred on the study of the relationships between events, show limits in the investigation of mechanisms underlying infection spread, and in considering the interactions among populations, thus possibly leading to simplistic and spurious conclusions. Mathematical modelling instead, and the development of a "dynamical epidemiology", allows the investigation of dynamics of infections, thus providing us a mechanistic point of view to understand infection spread. The strengths of mathematical modelling applied to epidemiological studies are several. At first, they can investigate the extent to which an event can mechanistically influence another consequential event. This characteristic of

mathematical modelling has a great application in public health, as it allows to prioritize interventions or studies on those events that have a major impact on disease outbreak. Another strength of mathematical modelling is its ability to describe the dynamics of an infectious disease by accounting for interactions among populations and sub-groups of populations within the same population. At least, mathematical modelling permits for theoretical investigations of mechanisms of transmission and to answer to "what if?" questions, allowing to explore theoretical scenarios which have not yet occurred or which needs to be preventively tested, like the application of an intervention strategy.

With the present work we then provide four applications of mathematical modelling to infectious disease. We focused on two wildlife-originating infections: West Nile virus (WNV) and *baylisascariasis*. Both infections are emerging or re-emerging in Italy and can represent a threat for human beings due to their possible severe outcomes. Due to the potential harm they are for human beings, a thorough surveillance and a wide intervention and control plans are ongoing both to promptly identify the presence and circulation of their causative agents and to reduce human infection risk. A full understanding of WNV cycle is fundamental to reduce human infection risk, but several knowledge gaps still exist, especially on the role played by different bird species involved in its spread. For both infections moreover, despite several are the control strategies proposed a quantitative analysis of their performance has never been performed. Aimed at filling these gaps, we developed a mathematical model to simulate WNV spread, and used it to explore mechanisms driving infection spread. We found birds recovery rate and mosquito biting rate having the major influence on disease spread and thus being the most urgent mechanisms to be investigated via field and laboratory experiments. Birds'

susceptibility and their competence to infection have a negligible influence on disease spread, thus making investigations to understand them of secondary importance. These results might be of aid also in defining the characteristics of a bird species to be a good WNV spreader, by focusing the attention on species that have a small recovery rate or are frequently bitten by mosquitoes. Moreover, we found a negative effect of birds' abundance in affecting WNV prevalence in mosquitoes, further helping us in distinguishing among species that are suitable to have a role in WNV spread. We then exploited the built model to explore intervention strategies against WNV. We showed that a reduction of the vector population is more effective than a reduction of birds' abundance in an area. In particular, the best efficacy is shown by the reduction of mosquito breeding sites, followed by the active elimination of their eggs and larvae. On the contrary, reducing the abundance of competent birds or their reproductive sites can obtain an increase in human infection risk. Similarly, we also studied the effectiveness of different intervention strategies to reduce the number of *Baylisascaris procyonis* eggs in the environment. The ingestion of *B. procyonis* eggs indeed is the cause of *baylisascariasis*, an infection that can have severe health consequences in human beings. With our work we explore the effects both in terms of efficacy (i.e., potential to eliminate eggs from environment) and efficiency (i.e. the timing needed) of three different intervention strategies. The interventions tested are: the active culling of raccoons, raccoons' anthelmintic treatment and faeces removal. We found that raccoon culling might have the best and faster results, highlighting the importance of assessing the intervention on the base of an objective prove on its efficacy. With the proposed work then, we highlighted the role of mathematical modelling in epidemiological studies, by,

at first, exploiting their potential to investigate the extent to which an event can influence another consequential event. Secondly, we used them to describe the dynamics of an infectious disease, by accounting for interactions among populations, and focusing on mechanisms underlying infection spread. Moreover, we also exploited them for theoretical investigations, like the simulation of the application of an intervention strategy to reduce human infection risk is.

In conclusion, mathematical modelling can widely help our understanding and management of infectious diseases through a new and different point of view from that provided by classical epidemiology. Mathematical modelling indeed includes the investigation of spreading mechanisms and non-linearity of interactions among individuals and subgroups of populations, thus allowing a more complete comprehension of diseases spread. The cooperation of diverse health professionals is fundamental to fully exploit both classical epidemiological studies and dynamics ones, and the effects of their cooperation can lead to a better knowledge of infections and a consequent reduction of human infection risk.

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CHAPTER 1

General Introduction

Introduction

Infectious diseases as an ongoing thread

In the nineteenth century, medicine progresses lead by antibiotics, pesticides, and vaccines, produced a growing of optimism about the possibility that infectious disease would have finally been eradicated, especially in high income countries [1]. Quite the opposite, in the last 80 years we have seen a reversal of the trend, with the resurgence of infectious disease to an extent now meriting the highest concern level [2–6]. In particular, one of the majors threatens for global health and economies is now represented by the emergence of new infections, or of ones that have existed previously, but are rapidly increasing in incidence or geographic range, so called respectively *emerging*, or *re-emerging diseases* [5,7,8]. In about 60 years (from 1940 to 2004), 335 new emerging infectious diseases have been reported [9], among which AIDS, SARS and MERS, but also Malaria and Tuberculosis. AIDS alone is thought to have infected more than 60 million people worldwide up to 2004 [10], with the devoting of funds for HIV vaccine research estimated around $200-250 million/year [11]. Or also vector-borne diseases, that cause more than 700 000 deaths annually, and a massive earmarking of funds for their control and elimination (in 2019, funding for malaria alone reached US$ 3 billion) [12], evidencing both the impact on human health and economies that can be caused by the emergence or re-emergence of an infectious disease. With the more recent COVID-19 pandemic the importance of preventing, or at least managing, new diseases emergence shows its full importance. With more that 211 millions of confirmed cases and more than 4 millions deaths from the beginning of the pandemic to the end of August

[13], COVID-19 kneed global health and worldwide economy, making clear the need of strengthening both our surveillance capability of new infections, and of developing effective intervention strategies to limit infection spread.

The number of human emerging infectious diseases has found to be rising since the second half of the 1900s, and due to the increased frequency of human-animal contact, travelling and global environmental changes it is probably bound to grow further [9]. Consequently, the so-called spill-over events, namely the transmission of a pathogen from a vertebrate animal to a human, are now the cause of big concern, but are still poorly understood [14]. Among human emerging infectious diseases, the 60.3% are zoonoses (namely those infections having a non-human animal sources), of which the 71.8% have a wildlife origin [9], highlighting the important role of not-human animal sources as a threat to public health. Then, the importance to safeguard animal health takes on now a new relevance, not only considering productive and economic losses due to livestock infections, but also as a potential source of infection, of increasing importance, for human beings [15]. Consequently, the coordination of different expertise becomes central in preventing spill-over events and to develop efficient surveillance and control strategies. Moreover, to contain the spread of new infections the cooperation of politics and economies worldwide is required, implying a transition from a public health to a more comprehensive global health [16]. But also, is important to focus, not only on human health, but on a so called one health inclusive of both human and animal health [15,17–19]. Among zoonotic disease, spreading worldwide and representing a global threat to human health, are vector-borne diseases. Vector-borne diseases, includes malaria, dengue, zika fever and West Nile disease, and account for more than 17% of all

infectious diseases, causing more then 700 000 deaths annually [12]. Their spread is due to the complex interaction between hosts and vectors, usually involving a high number of species, making their monitor and control very complex and demanding in terms of time and resources. Many of vectors are bloodsucking arthropods, but them can also be any living organisms that can transmit infectious pathogens between humans, between animals, or from animals to humans. The ubiquity and variety of vectors make vector-borne diseases one of the main issues for public health worldwide [20]. Moreover, vector-borne diseases are usually more spread in low-income countries, where a combination of climatic conditions and low economic resources, make monitor and control of vector spread poorly effective [20]. Malaria for example, caused by parasites of the genus *Plasmodium* and spread to people through the bites of infected mosquitoes, in 2019 alone is estimated to have caused 229 million cases and 409'000 deaths worldwide, of which the 94% in African Region [12].

Infections as a dynamic process

The impact of diseases on global health and economies is then undeniable. Classical epidemiological studies are then central to extract the relationships between events to interpret disease spread and to individuate risk factors [21]. Their thorough comprehension becomes fundamental to safeguard human health and to improve diagnoses and tracing techniques. But infectious diseases add a level of complexity respect chronic conditions such as cancer or heart disease, that only depends on individual-level characteristics (e.g., genetics or individual health status). Infection risk also depends upon the state of other individuals in the population, making the spread of

infectious diseases strictly related on the interaction between susceptible and infectious individuals [21,22]. The complexity of mechanisms causing infection transmission and the nonlinearity of interactions among subgroups in a population, make the approach of classical epidemiology, although practical, simplistic, so as to lead to spurious conclusions [22–24]. To catch the effects of interactions within and between populations, another possible approach is provided by dynamical epidemiology. In dynamical epidemiology the focus is posed on understanding disease dynamics by accounting for interaction between individuals, and mathematical modelling (nonlinear transmission models) is the tool for excellence [21]. A dynamical approach, hence, allows the understanding of processes and mechanisms underlying infection spread, thus going beyond the comprehension of the mere statistical and geospatial relationship within events. With respect to a classical epidemiolocal investigation, this approach also gives us the possibility to describe the dynamics of an infection, exploring the and formulating hypotheses on the mechanisms involved. Mathematical models can thus provide a description of disease dynamics but can also be used to predict the future trends in disease spread or can be used to explore the possible intervention strategies. In particular, the use of mathematical modelling can be of aid in case diseases, that have complicated cycles, such most of zoonotic ones, and of which infection mechanisms heavily relies on interactions among populations and subgroups of populations. Moreover, their use is very beneficial when investigating emerging diseases, dealing with which we cannot rely on previous experience.

Mathematical modelling for infectious diseases

Due to their potential to account for interaction among individuals, in the second half of 18th century, mathematics bashfully began to be applied to the study of infectious diseases, gradually showing their ability to interpret epidemiological trends and guide data collection. At the end of 20th century, the number of studies concerning with mathematics applied to epidemiological literature increased and proved to be potentially crucial in our understanding of infectious diseases [25–29]. Despite of that, insights obtained showed to have a low effect on public health choices compared with their potential. The most accredited theories about the regular recurrence of measles and the relationship between number of mosquitoes and malaria incidence have been developed with the use of mathematical modelling [30,31]. But also, more recently, public health choices on SARS outbreak management have been influenced by mathematical modelling obtained results [32–34]. Their potential to describe the course of an epidemics from a dynamics point of view, and contemporary to explore mechanisms driving such dynamics, is the innovation and strength of mathematical modelling.

Their ability to catch the complexity of diseases and interactions among population subgroups indeed, provides a new point of view in epidemiological studies, incorporating mechanisms of infections and obtaining a global overview of processes and dynamics of spread. Different are the available techniques and diverse can be the uses of mathematical modelling, depending on the aim of the study and background information available. Their most intuitive use relies on producing simulations and predictions of investigated systems, but mathematical models can also have an important role in investigating and exploring epidemiological theory. They can be used indeed in answering

the so called “What if?” questions, thus simulating hypothetic or alternative scenarios and exploring the consequences of actions before they are implemented. Another way of exploiting mathematical modelling potential is to recognise those mechanisms that have the greatest impact on system dynamics. Considering that those biological mechanisms underlying the system are represented by model parameters and knowing that some of these parameters have a greater influence on dynamics then others (namely are more sensitive), we can theoretically explore the extent in model output of a change in parameter estimation thus recognising those mechanisms that have the major influence on the system. Moreover, knowing which parameters are more sensitive than others, can aid in understanding which one need further attentions and studies. Furthermore, new and quantifiable concepts have been introduced via modelling, as it happens with the introduction of the concept of the basic reproduction number (R_0), or the identification of critical threshold for epidemic development, leading to a deeper comprehension of phenomena and providing epidemiologists with a tool to objectively quantify some characteristics of the spread of a disease [35–38]. The potential of mathematical modelling is therefore particularly useful when dealing with new infections, for which it is hard to rely on precise background information, or with wildlife-borne infectious diseases, for whom the data collection can be hard and demanding.

Despite its potential, the formulation of a mathematical model is a complex process, that requires to operate suitable choices to obtain a model as simple as possible and yet adequate to address the answering of the question posed. One of the main challenges when dealing with modelling is to find the right balance between simplicity and complexity in model formulation. Keeping a model as simple as possible is fundamental

to rely on solving strategies, moreover, the less parameters we have, the less approximation we need to estimate them. On the other hand, to obtain a meaningful model it is important not to neglect or underestimate variables determining the course of infection, thus also an over-simplification of the model should be avoided. The deviation from reality due to oversimplification is rarely testable or measurable, thus making results interpretation depending on experience and expertise of modellers or epidemiologists. Moreover, mathematical modelling is often cryptic for non-experts, thus relegating this tool to a niche of modellers with the risk of establishing a vicious circle in which models are complicated to time to time, losing touch with reality and consequently being excluded by public health choices.

Seen the variety of mathematical techniques available and the extent of their potential uses, several diverse mathematical models have been built to be used in epidemiological studies, we here propose an overview of the most used.

Predator-prey- interaction and parasitism

Predator-prey theory is one of the most exploited theories in describing the interaction between two different species and assumes that the number of preys limits the number of predators and vice-versa. Broadening the concept of interaction beyond the active killing of preys by predators, the relation between parasites and their hosts can be seen as a particular manifestation of the general predator-prey interaction [39]. In parasitism indeed, parasites interact with their host by depending upon them for nutrients, and contemporary harming or damaging them with an extent that largely depend on

characteristics of both species. Consequently, the appropriate modification of the base predator-prey model can be used to describe any kind of host-parasite interaction.

To model infectious diseases then, Anderson and May with their work [39,40], proposed to look at them all as a host-parasite interaction, with characteristics depending on the mutual relationship existing between the infective agent and its host thus proposing a classification of infectious diseases in micro- and macro- parasitic infections.

- *Micro-parasitic infections:*

Micro-parasitic infections, generally caused by bacteria and viruses, are characterized by a rapid increase in parasites number when introduced into a susceptible host. In micro-parasitic infections, the number of parasites harbouring their host is hardly quantifiable, thus, to comprehend the mechanisms driving disease spread, it is crucial to explore the interaction between infectious and susceptible hosts, regardless to intensity infection. The host population is then divided in subgroups depending only on the infection status of individuals. The simplest subgroup division for a population is represented by the SI model, in which we only consider the interaction between susceptible individuals (S) and infectious ones (I), with rates describing the passage between subgroups depending on infection characteristics. The number and the characteristics of population subgroups depend on infection characteristics and model assumptions, and we can thus have diverse subdivisions. We can divide the population in susceptible (S), infectious (I) and recovered I individuals, resulting in an SIR model, but by considering an incubation period for the disease, we can also include exposed individuals (E) thus obtaining a SEIR model, and so on. In these models, the progression of individuals between subgroups of the

population depends on disease characteristics and is represented in the model by infection-specific rates.

- *Macro-parasitic infections:*

Macro-parasitic infections are generally caused by bigger organisms like helminths (worms) and arthropods, and their cycle generally includes one or more free-living stages that pass from one host to the next. Infection tends to be chronic, and mortality, morbidity, host fertility reduction, depend on the number of parasites harboured by the host. These characteristics make essential to measure infection in terms of parasite burden for each subject, and not just their infected/non-infected status. The resulting model then includes diverse developmental stages of both hosts and parasites as subgroups of the population. The effect of parasite burden on host's chances of surviving or reproducing varies greatly depending on both host and parasite characteristics and usually depends on parasite number into hosts. Moreover, the pattern of distribution of parasites among hosts is usually over-dispersed, with few members of the host population harbouring most of the total parasite population, thus heavily affecting disease dynamics.

Deterministic or stochastic models?

Rates in mathematical modelling represent the speed of the occurrence of an event that causes the passage of an individual from a subgroup of population to another.

- *Deterministic models*

Deterministic models are the most traditionally used in epidemiological studies. In these models the role of chance in event occurrence is not included and rates at which an

individual moves from one population subgroup to another are certain and continuous on time. One of the main characteristics of deterministic models is that, given the starting conditions, they lead to exactly and only one solution. This trait, to the detriment of a loss in realism, makes them easy handling and capable of providing precise and fixed thresholds determining whether an epidemic will occur. On the other hand, they do not account for the reliability or the confidence in the results. Due to their characteristics deterministic models are usually considered reliable when simulating an epidemic but loosing reliability when simulating the very beginning or the end of an epidemics, where the low number of cases increases the effect of chance in the probability of spread of an infection.

- *Stochastic models*

On the contrary, stochastic models assume that the passage from one population subgroup to another include a varying chance in transition probability and is not certain to occur. Although starting from the same set of initial conditions then, we can obtain a range of predictions whom width depends on the variance of the transition probability. Stochastic models then incorporate chances, provide a more realistic description of phenomena, but is harder to get analytical results for these models. Some of them uses are the investigation for example of probability that an epidemic will occur and the mean time to extinction of a disease.

Compartmental or individual based?

Another possible difference in mathematical model structure is among compartmental or individual based ones.

- *Compartmental models*

Compartmental models are the most common and widely used. In compartmental models the population is divided in subgroups of individuals that are homogeneous in some feature, like for example in SIR models their epidemiological status. Individuals move from one population status to another according with model assumptions and rates (e.g., infection or recovery rates). Compartmental models can be both deterministic and stochastic. Their main limitation is that them do not account for differences among single individual hosts, but their limitation is widely surpassed by their efficacy in predicting infection spread in populations and estimating the various meaningful epidemiological parameters.

- *Individual-based models*

In contrast, to account for differences among individual hosts we can rely on individual based models. Them investigate the infection spread in a population focusing on the individual organisms that compose it, with their set of state variables or attributes and behaviours. Several are the variables changing among individuals and can include spatial location, physiological or behavioural traits, but also age and growth of dispersal. In individual-based models, the dynamics at the population-level is investigated, but it is the result of the interactions among individual with their own characteristics, with each other and their abiotic environment. Despite their major complexity if compared with compartmental ones, individual-based models can be useful when specific difference at

individual-level is suspected to be essential for answering some population-level question.

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Outline of the Thesis

In the present work we propose four applications of mathematical modelling to solve epidemiological questions related to wildlife-borne infectious diseases.

Within the first two chapters, we used the potential of mathematical modelling of exploring infection mechanisms of action to ameliorate our knowledge of disease spread. The infection investigated is West Nile Virus (WNV) for both chapters, a vector-borne disease currently worldwide spread. WNV is naturally maintained in an enzootic cycle involving birds and ornithophilic mosquitoes. It can also affect human beings and horses, that however are only considered to act as dead-end hosts. In human beings the infection is usually asymptomatic, but, when they occur, symptoms can range from a mild influence-like syndrome to a severe neurological disease. The possible invalidating consequences of neurologic disease, and its worldwide spread, make WNV a concern for public health of several countries, but, due to the complexity of its cycle, several epidemiological aspects are still unknown. Moreover, the role played by different bird species in spread and maintenance of infection is still debated, and only some of the involved species, European bird species especially, have been deeply investigated to estimate their epidemiological parameters. Also, considering that not all epidemiological parameters are equally sensitive on infection spread, a deeper comprehension of mechanisms having the major impact on system dynamics becomes important in giving a hierarchy to future studies and investigations. To meet this need, with chapter 1, we used and adapted a validated mathematical model by Marini (2020), to perform a

sensitivity analysis aimed at understanding which epidemiological parameter has the major effect on the infection spread. This analysis allowed us to discriminate among epidemiological mechanisms which of them affects, and which one instead do not, the infection spread. We thus, at first, provided solid and objective results to understand which of the possible future investigations would lead to the best improving of knowledge about WNV. On the other hand, exploiting model results, we can highlight which species-specific characteristics affects more disease spread, and thus understanding which avian species better encounter the characteristics of a good spreader or of a reservoir for WNV. With chapter 2 we further investigated characteristics making an avian species a potentially involved in WNV spread by focusing on the specie-specific demographic characteristics instead of the epidemiological ones. Indeed, one of the main knowledge gaps about WNV is the role of both different avian species and the overall avian community. But field and laboratory data collection of species-specific characteristics can be hard and demanding. For this reason, we propose a theoretical investigation to understand the characteristics of a bird species that makes it suitable of being involved in WNV spread. In this chapter we exploited mathematical modelling to explore if differences in demographic species-specific characteristics could lead to differences in predicted WNV spread.

In the following two chapters instead, we exploited the potential of mathematical modelling to answer to “what if?” questions. The common thread of chapter 3 and 4 is that they both provide a theoretical analysis of the effectiveness of diverse intervention strategies in reducing human infection risk. The strength of these last two chapters is that they both provide us with objective proves on intervention strategies effects, that are

usually only gained from experience and for which most of the time there is no scientific proof.

Chapter 3 is again focused on WNV and investigate the efficacy of six different intervention strategy to reduce the risk for human beings to be infected. Despite its importance indeed, only few studies exploring the efficacy of the existing intervention strategies to reduce human infection risk are available, and we still cannot rely on a full awareness of the best strategy to apply. For this reason, we further adapted the mathematical model used in chapter 1, to simulate six different scenarios, in each of which we simulated a different intervention strategy. Then we compared the reduction of human infection risk obtained with each of the intervention tested to determine the most suitable one to be performed in the study area (Lombardy region, Italy), thus concluding that intervention strategies on the vector population are the most efficacy.

In chapter 4 instead we focused on *baylisascariasis*. *Baylisascariasys* is a zoonotic infection caused by *Baylisascaris procyonis*, a roundworm parasite having as definitive host raccoons (*Procyon lotor*). The accidental ingestion of *B. procyonis* eggs by human beings can lead them to develop a severe neurological disease, and the recent expansion of raccoons in new areas, makes baylisascariasis a threaten for public health in diverse countries. Italy is one of the countries where raccoons have been recently introduced, and with them the risk for human beings of being infected by B. procyonis. This fact leads to the need of developing an efficient intervention strategy to avoid, or at list reduce, human infection risk. Currently available strategies are diverse, but a global analysis of their efficacy was lacking. We then built a mathematical compartmental model to describe infection spread in the raccoon population and then tested three different

intervention strategies to investigate which of them could obtain the best results both in terms of reduction of parasite infective stages dispersed in the environment and speed to reach it.

CHAPTER 2

Understanding West Nile spread: mathematical modelling to disclose the most influential mechanisms on infection dynamics

Introduction

West Nile disease (WND) is an emergent vector borne disease caused by West Nile virus (WNV), a single-strand virus belonging to the Flavivirus genus [1]. Its cycle involves mosquitoes, mostly of the genus *Culex*, as vectors, and diverse bird species as maintenance vertebrate hosts [2–5]. Although acting as dead-end hosts, several mammal species, human beings included, can be infected via mosquito bite, and can develop symptoms ranging from mild fever to severe neurological disease [6,7]. Despite the low frequency of development of a severe illness (25% of infected persons develop symptoms, [6]), the recovery might take several weeks or months, and some effects to the central nervous system might be permanent [5,8]. In addition, WNV recent diffusion in several countries in Europe and North America make it one of the most spread flavivirus in the world [9]. Despite of that, the variety of bird species developing viraemia titres sufficient to infect feeding mosquitoes [10–12], and the differences in susceptibility and competence shown between and within families of birds [13–16], make WNV spread and diffusion still poorly understood, especially in the European continent [17]. Only few studies provide a complete analysis of different bird species viraemic responses, and the variety of the composition of local avian communities combined with the circulation of different WNV strains, makes inaccurate the extension of results to areas other than the ones tested [13,15,16,18,19].

To investigate spreading mechanisms of WNV, several mathematical modelling efforts have been attempted (e.g. [20–25]), but the above-mentioned uncertainties might hinder the precision of parameter estimates, thus impairing the reliability of simulations

obtained and the verisimilitude of simulated mechanisms. For this reason, the development of specific field research to investigate the epidemiological effects of different parameters is fundamental to improve the reliability of model simulations and our comprehension of WNV spread, especially in Europe where information about species involved in WNV spread and their epidemiological characteristics are currently lacking [14,17,26]. But, when a disease involves wildlife as WND does, field studies can be hard and demanding and not all parameters affect the dynamic of the disease in the same way. Small changes in some parameter can cause a huge variation in the dynamic itself, whereas big changes in some other can scarcely affect it. For this reason, the identification of those parameters having the biggest effect on disease dynamics can aid the prioritization of future research. Our work thus aims at investigating the effects on infection spread of different estimates of four parameters related to relatively unknown epidemiological characteristics of bird and mosquito species (mosquito biting rate on birds, avian competence, recovery, and susceptibility to infection).

Materials and Methods

Dataset and reference system

Entomological data was collected in Lombardy region, in the North of Italy, where WNV was first detected in 2008, and now is considered endemic (see **Fig.1**). Mosquito abundance records and their WNV status come from the Regional WNV mosquito surveillance, performed by Regione Lombardia and Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER). Mosquito abundance and their PCR positivity for WNV was determined, accordingly to national and regional guidelines for the entomological surveillance of WNV [27–29], by field collection of mosquitoes performed every two weeks by CO_2 traps covering an area at most of 400 Km^2 each (**Fig.1**). Temperature and precipitation data for each land unit, collected with ground stations, was obtained from ARPA Lombardia (Agenzia Regionale per la Protezione dell'Ambiente della Lombardia). We then divided Lombardy region into a northern sub-region, a western sub-region, and an eastern sub-region (respectively red, green and blue coloured regions in **Fig.1**), further details about clustering of trap locations are reported in **Supplementary Materials S2**. Because of the absence of WND in the northern cluster, we only investigated WNV transmission through the eastern and the western clusters. To estimate the number of birds composing the whole avian community and the number of magpies (i.e. the competent avian species) at the beginning of the summer we referred to records of the avifauna census provided by Regione Lombardia. The number of magpies was estimated as a proportion of the total number of birds estimated by a kriging

method calculating the number of individuals circulating in an area $A = \pi * r^2$,where r is the average *Cx. Pipiens* flight range and was considered equal to 500m [23].

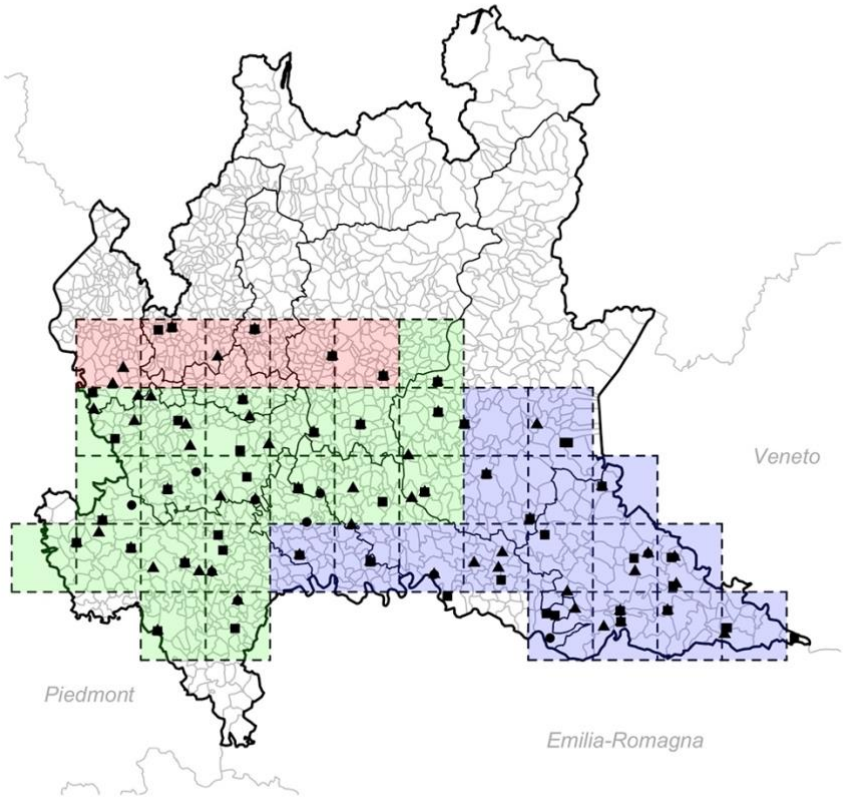

Figure 1: Capture site clustering proposed for Lombardy region. Red squares for the northern cluster, green squares for the western cluster, and blue squares for the eastern cluster. Black squares, circles and triangles represent mosquito capture site respectively for 2016, 2017 and 2018.

Model structure

The modelling framework follows the one proposed in Marini et al. [25] to investigate WNV dynamics in Emilia Romagna region, located south of Lombardy. First, the mosquito population dynamics is simulated through an "*entomological model"*, providing a daily estimate of the mosquito abundance for cluster and year. Then the estimated mosquito

abundance was included in an "*epidemiological model*", aimed at simulating the transmission dynamics of WNV in a competent bird population. Due to their abundance and competence for WNV, and in analogy with the model proposed by Marini [25], mosquitoes of the *Cx. Pipiens* species were considered as the only vector species. Analogously, as magpies are competent for WNV [18,30] and abundant in Lombardy region, the avian host population was considered to grow and die with rates estimated for magpies in literature [23]. The dynamics of the disease were simulated from May to October in 2016, 2017 and 2018 according with data provided by the entomological surveillance. For both the *entomological* and the *epidemiological model*, the posterior distributions of the unknown parameters were explored following a Markov Chain Monte Carlo (MCMC) approach as adopted in (Marini et al. 2020).

The entomological model

To estimate *Cx. Pipiens* abundance during summer season, we calibrated the temperature-dependent entomological model presented in [25] on the recorded captures in Lombardy region, averaged over each cluster. Mosquito abundance was estimated for three years (2016, 2017 and 2018), starting from April up to October. Such resulting mosquito abundance was then included as a known function ω(t) into the epidemiological model.

The epidemiological model

The WNV model is based on a system of eleven differential equations, representing *Cx. Pipiens* and a competent avian species (juveniles and adult ones) infectious stage. To account for the complexity of the avian population, the number of competent birds was

estimated as a fraction (a_i) of the total number of birds estimated to live in the area (B_0). Birds are considered to become infectious (B_{Ia} and B_{Ij}) after an incubation period and then, as a consequence of infection, recover and become immune to reinfections (B_{Ra} and B_{Rj}). At the start of the season (May) the avian population is considered to be fully composed by adult birds, reproducing and giving birth to juvenile ones until mid-July. Because the maturity age of birds can be considered as one year [31], the newly born are considered as juveniles throughout the entire season. Mortality due to infection in birds was neglected because of the limited mortality due to WNV infection observed in magpies in Europe [2]. Mosquitoes are considered to become infectious after a temperature-dependent incubation period (θ_M), and for the rest of their life. Birds can acquire infection according to their susceptibility (p_{MB}) and to a mosquito temperature-dependent biting rate over the bird population (b), depending on the proportion of mosquitoes' bites directed to the competent avian species (b_1 and b_2) and a function of temperature [32]. Two different biting rates were considered in the model, one for the initial part of the season (b_1) and one for the latest part of the season (b_2) because of the observed shift in mosquito biting rate between early and late season [33]. The infection rate of mosquitoes was estimated by a temperature dependent function representing the probability of WNV transmission from infectious birds to mosquitoes per bite (p_{BM}, [23]). Considering that the competence for WNV transmission of an avian species, which is influenced by the viraemic titres developed by birds, is often considered one of the key parameters to be urgently investigated, we included among varying parameters a scalar (p), that represents the competence of the avian species (i.e. the probability for the bird species to allow the transmission form birds to mosquitoes). This parameter is multiplied

to the mosquitoes' susceptibility (p_{BM}) to determine the overall transmission rate from bird to mosquitoes. The avian recovery rate (ν_B) is represented by the inverse of the duration of infectious period in birds. In analogy with the *entomological model*, the posterior distributions of the unknown parameters (see below) were explored through an MCMC approach, applied to the binomial likelihood of observing the weekly number of positive pools given the predicted mosquito prevalence.

The complete scheme of the model and the equations describing the system are reported in Supplementary materials S2.

Parameter estimates

All biological and epidemiological parameters for birds and mosquitoes refer to magpies and *Cx. Pipiens* are those reported in Marini et al 2020 for Emilia Romagna region. 10'000 iterations of the MCMC sampling were performed, obtaining 10'000 suitable sets of parameters. Simulations for WNV dynamics were then performed randomly choosing 100 different sets of parameters from the estimated posterior distributions, obtaining 100 different possible dynamics depending on the set of parameters chosen.

The unknown parameters estimated by the MCMC method for the entomological model are:

- K_1: density dependent scaling factor driving the carrying capacity for the larval population at the beginning of the season
- K_2: density dependent scaling factor driving the carrying capacity for the larval population at the end of the season
- M_0: the number of mosquitoes at the beginning of the season

We considered two different carrying capacities as during summer *Cx. Pipiens* breeding sites availability might change, causing a possible increase in larval mortality, for instance because of competition for resources with Ae. Albopictus at the larval stage [34].

Whereas for the epidemiological model are:

- a_i: the proportion of competent birds over the total avian population in cluster *i*
- b_1: the fraction of mosquitoes' bites on the competent avian population at the beginning of the season (from May up to mid-July).
- b_2: the fraction of mosquitoes' bites on the competent avian population at the end of the season (from mid-July up to October).
- i_b: the number of immune competent birds in each cluster at the beginning of the first year of simulations (2016)
- p : the avian competence, defined as the probability for a competent infectious bird to transmit the infection to a mosquito
- p_{MB}: the birds' susceptibility to infection, considered as the probability for a competent bird to become infected when bitten by an infectious mosquito
- ν_B: the bird recovery rate, considered as the reciprocal of the duration of viraemia

Being a characteristic of the infection itself, all epidemiological parameters were considered constant in time and space and were thus estimated across all years and clusters. Only the number of immune competent birds was considered different among years and between clusters and was estimated by the MCMC for 2016 and then considered as dependent from the estimated previous year final avian immunity. The prior distribution of all epidemiological parameters and of the number of immune competent birds in 2016 was considered to follow a uniform distribution. The total initial

number of birds was randomly chosen in 50-70 to account for the variability in the birds' number across sites and years. Mosquito WNV prevalence at the beginning of each season was randomly chosen into 0-0.001 [25].

To verify whether there is statistical relationship among unknown epidemiological parameters, we also check for their mutual correlation (*Pearson correlation coefficient*, with function *cor* in R 3.6.3 software).

R_0 estimate

We estimated WNV basic reproduction number (R_0) following the formula proposed in [35] for vector borne diseases. In this system R_0 represents the average number of secondary infected mosquitoes over the entire transmission cycle, following the introduction of an infected mosquito into fully susceptible mosquito and bird populations. The formula takes into consideration both the number of secondary infected birds following the introduction of an infectious mosquito into a fully susceptible bird population, and the number of secondary infected mosquitoes following the introduction of an infectious bird in a fully susceptible mosquito population.

The basic reproduction number is computed according to the following formula:

$$R_0 = R_0^{MB} * R_0^{BM}$$

with

$$R_0^{MB} = \frac{b * p_{MB}}{\mu_M}$$

$$R_0^{BM} = \frac{b * p * p_{BM}}{\delta_B} * \frac{\theta_M}{\mu_M + \theta_M} * \frac{N_M}{N_B}$$

R_0^{MB} (R_0^{BM}) is the number of hosts (mosquitoes) infected by an infectious mosquito (host). Following the formula proposed, the R_0 estimate depends on epidemiological parameters chosen to represent transmission rates and the vector-host ratio $\frac{N_M}{N_B}$, where N_M represents the mosquito number and N_B the number of competent birds. As mosquito death rate (μ_M), the probability of WNV transmission from infectious birds to mosquitoes (p_{BM}) and mosquito biting rate are a function of temperature, we estimate the R_0 of WNV at a temperature of 24°C. We chose a temperature of 24°C to exclude possible limitations in spread caused by temperature.

<u>R_t estimate</u>

To estimate the potential of WNV to spread during the summer season, we adapted the formula proposed for R_0 to estimate the effective reproduction number R_t, defined as the number of new infections caused by a single infected individual at time *t* in a partially susceptible population.

The effective reproduction number is thus computed according to the following formula:

$$R_t = R_t^{MB} * R_t^{BM}$$

with

$$R_t^{MB} = \frac{b * p_{MB}}{\mu_M} * \frac{B_S}{N_B}$$

$$R_t^{BM} = \frac{b * p * p_{BM}}{\delta_B} * \frac{\theta_M}{\mu_M + \theta_M} * \frac{M_s}{N_B}$$

Where, R_t^{MB} (R_t^{BM}) is the number of hosts (mosquitoes) infected by an infectious mosquito (host), M_S represents the number of susceptible mosquitoes, $B_S$the number of

susceptible birds and N_B the total number of competent birds. Consequently, the R_t estimate depends on the vector-host ratio ($\frac{M_S}{N_B}$) and on the proportion of susceptible hosts over the whole host population $\frac{B_S}{N_B}$.

Transmission spread and maintenance during summer season

To investigate the transmission of WNV throughout the summer season, for each set of parameters estimated by the MCMC, a daily R_t was estimated from May to October accounting for the daily mean temperature. Then, to investigate the probability of the infection to be maintained and spread during the season, we estimate the monthly frequency for R_t to lie above 1. Hereafter, we will refer to it as spread probability.

Sensitivity analysis of unknown epidemiological parameters

To investigate the effect of different parameter configurations, we performed a sensitivity analysis by varying each epidemiological parameter estimate and evaluating how that change affects R_t. A baseline effective reproduction number ($\overline{R_t}$) was estimated according to calibrated parameters, then by varying into 10%-200% each free parameter estimate (with a step of 10%) the new R_t was calculated. To understand the effect of the change in parameter estimate on the effective reproduction number, we studied the ratio between the baseline R_t and the varied one. We can notice that, by considering this ratio, we obtained an effect of the change of parameter of interest that is independent from the values of the others.

For a deeper comprehension of the effect of a parameter estimate change, we also compared the base monthly spread probability with the spread probability obtained with a parameter decrease of 90, 50 and 10%, and an increase of 10, 50 100%.

The investigated parameters are:

- Mosquito biting rate (b)
- Competence for WNV of the bird species (p)
- Competent birds' susceptibility (p_{MB})
- Duration of competent birds' infectious period (recovery rate,ν_B)

Temperature and host-vector ratio effect on R_0

To highlight the effect of temperature on WNV spread, we performed a sensitivity analysis by varying temperature from 10 to 30°C (with a step of 0.1°C) and estimating R_0 for each temperature for each set of parameters used for simulations. Then, the WNV spread probability at different temperatures was estimated as the frequency for R_0 to lie above 1. Despite temperature affects mosquito numbers during season, to obtain more generalizable results we investigate temperature effect only taking into consideration one at a time, four different vector-host ratios (i.e., 10, 100, 1000 and 10,000 mosquitoes/birds).

Results

Model calibration and fit:

Parameter estimates show a very high variability in the estimate of the percentage of immune birds at the beginning of the first simulated season ($i_{Bw}$and i_{Be}) and of competence (p), susceptibility (p_{MB}) and recovery rate (ν_B) of the bird population. The fractions of the mosquito biting rate on competent hosts (both b_1 and b_2) and the percentage of competent birds over the whole avian population (a_i) instead showed a lower variability. A full list of the parameter estimates (and their range) obtained by MCMC is reported in table 1. Model predictions confidence intervals include 97% of observed points, showing that, despite the wide confidence intervals, the model can well describe WNV dynamics in Lombardy region. For further details about model fit and obtained simulations see Supplementary materials S2.

Table 1: Estimated model parameters distributions (average and 95% credible intervals).

Parameter	Parameter biological meaning	Estimate range (2.5%-97.5% percentile)
B_0	Initial number of birds (whole avian community)	59.95 (50.53-69.57)
a_i	Proportion of competent birds over the whole avian community	0.0666 (0.0016-0.2228)
i_{Bw}	Proportion of immune birds at the beginning of the first simulated season, western sub-region	0.0445 (0.194-0.7929)[1]
i_{Be}	Proportion of immune birds at the beginning of the first simulated season, eastern sub-region	0.0445 (0.1972-0.7897)[1]
b_1	Proportion of mosquitoes' bites directed to the competent avian species during early season (day^{-1})	0.3641 (0.1552-0.4947)[1]
b_2	Proportion of mosquitoes' bites directed to the competent avian species during late season (day^{-1})	0.2203 (0.0131-0.4834)[1]
p	Competence of the competent avian population (day^{-1})	0.6191 (0.08-0.9841)[1]
p_{MB}	Susceptibility of the competent avian population (day^{-1})	0.7752 (0.3543-0.9948)[1]
v_B	Recovery rate (day^{-1})	0.3792 (0.0401-0.9253)[1]

[1] MCMC estimate

Among unknown epidemiological parameters a correlation lower than 0.5 was observed between all parameters, except between bird susceptibility (p_{MB}) and biting rate during late season (b_2) that show a correlation of 0.71.

Transmission maintenance during season

Model simulations showed that the spread probability of WNV in the mosquito population during summer (R_t>1) is about 0.7 in May, increases up to 1 in June and then decreases passing from 0.94 in July to less than 0.02 in October (**Fig.2**).

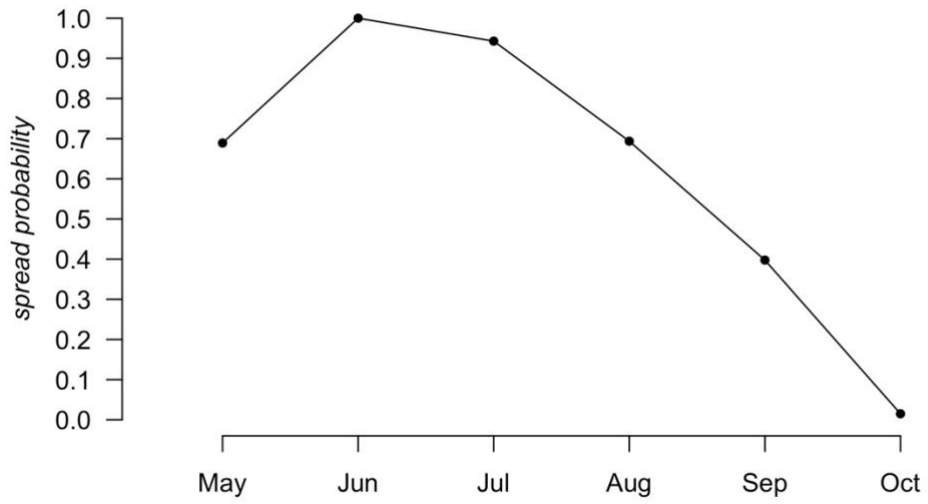

***Figure 2: R_t estimates**. Spread probability of WNV into the mosquito population during summer season.*

Unknown epidemiological parameters effect on R_t estimate

All the investigated epidemiological parameters affect the relative R_t estimate consistently with the formula applied for the R_t estimate. In detail, avian recovery rate (ν_B, **Fig.3** top-left box) is the most impacting parameter, dramatically decreasing R_t at higher values (i.e., shorter infectious period), with 90 and 50% decreased estimate causing respectively a 10-fold and 100% increase in ν_B estimate. The effect of parameter increase tends to lower if we increase parameter estimate, with 50 and 100% increased estimates causing respectively a 33 and 50% decrease in ν_B. Mosquito biting rate (b, **Fig.3**

bottom-right box) is very influential as well, decreasing $R_t$at decreased biting rates and increasing it at higher ones. Indeed a 50% decreased parameter estimate cause a 75% decrease of R_t and 50% increased parameter estimate a 125% increase of b Both recovery and biting rates have a non-linear effect on R_t estimate, with an enhanced effect for low recovery rates and high biting rates. Bird susceptibility to infection (p_{MB}, **Fig.3** bottom-left) and bird competence (p, **Fig.3** top-right) instead show the smallest effect on $R_t$range, moreover their effect is linear.

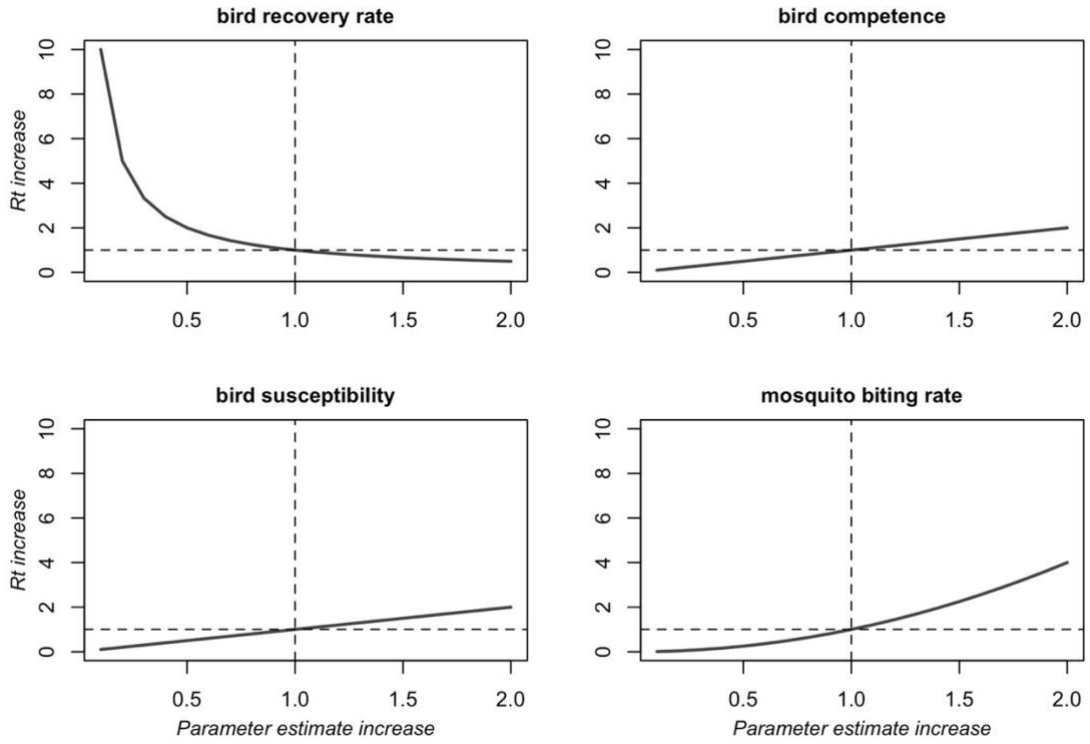

Figure 3: Sensitivity analysis on R_t. *Effect on the relative R_t of changes bird recovery rate (ν_B), mosquito biting rate (b), bird susceptibility to infection (p_{MB}), bird competence to infection (p), bird recovery rate (ν_B).*

The effect of a parameter estimate change on the probability of spread and maintenance of WNV during summer season (**Fig.4**), we can observe that regardless of the change carried out, the month with highest probability of WNV to spread is June, followed by July, while October remains a less suitable period for WNV circulation. The effect of a

change in parameter estimate on spread probability for WNV is very low in June, whereas is the highest in August/September regardless of the change in parameter estimate applied. A change in mosquito biting rate (b) shows to highly affect spread probability, with the highest effect in July and September. Recovery rate (ν_B), while highly affecting R_t estimate, less impacts spread probability, especially for enhanced estimates of the parameter. Birds' competence (p), and susceptibility (p_{MB}) show similar results when decreased, whereas a reduction in avian competence (p) shows a greater effect if compared with a reduction in avian susceptibility (p_{MB}).

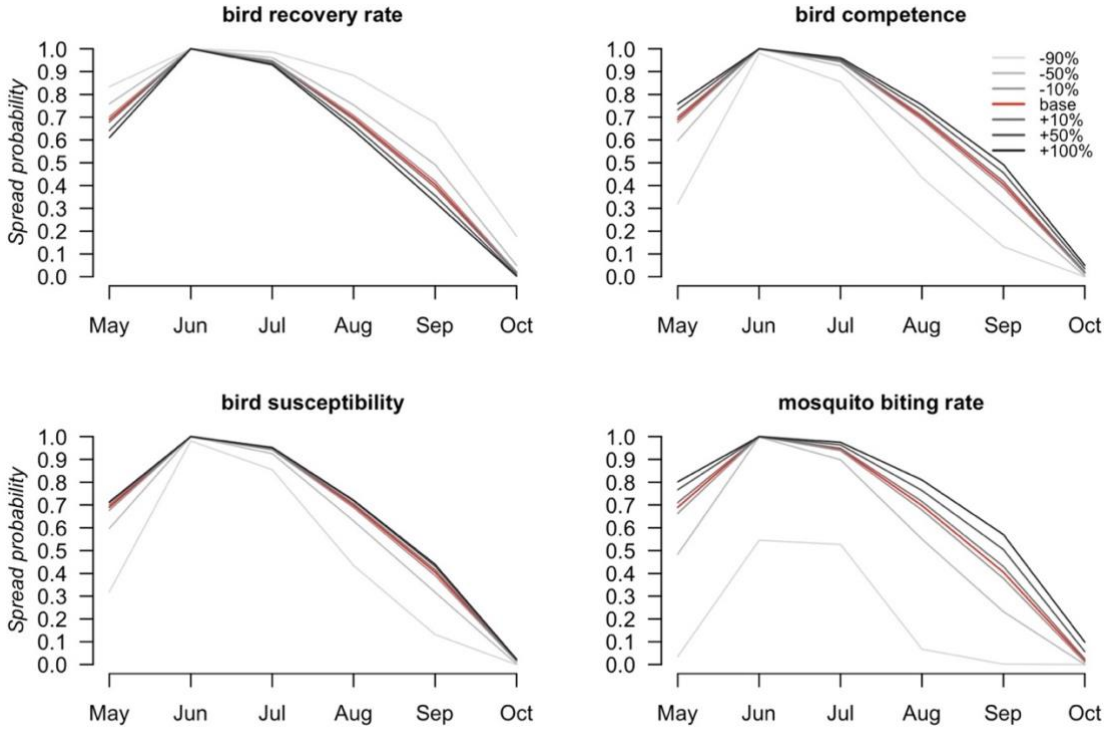

Figure 4: WNV spread. WNV spread probability into mosquito population during summer season in dependence of the increase in recovery rate ($\boldsymbol{\nu_B}$), bird competence ($\boldsymbol{p}$), bird susceptibility ($\boldsymbol{p_{MB}}$) and biting rate ($\boldsymbol{b}$) estimate. The greyscale shows from lighter to darker a change of parameter estimates of $-\mathbf{90}\%, -\mathbf{50}\%, -\mathbf{10}\%, +\mathbf{10}\%, +\mathbf{50}\%, +\mathbf{100}\%$. Red line represents the baseline.

Temperature and host-vector ratio effect on R_t (Fig.5)

As expected, R_t varies accordingly to temperature. Different vector-host ratios ($\frac{M_S}{N_B}$) show to strongly impact R_t, but do not change the trend of the effect of temperature on its estimate. For all tested vector-host ratios, R_t is always lower than one for temperatures below 14°C. For $\frac{M_S}{N_B} = 10'000$, and then it fast increases from 0 to 1 at 14.4 °C (long dashed line). Decreasing the vector-host ratio instead, the increase is less sharp and a spread probability of 1 is reached at 15.4°C and 19.8 respectively for $\frac{M_S}{N_B} = 1'000$ and

$\frac{M_S}{N_B} = 100$ (dashed and dotted line). With a vector host ratio of 10 instead (solid line), we can not reach a spread probability of 1, and the maximum probability is 0.43, reached for temperatures ranging in 27.1 and 28.4°C.

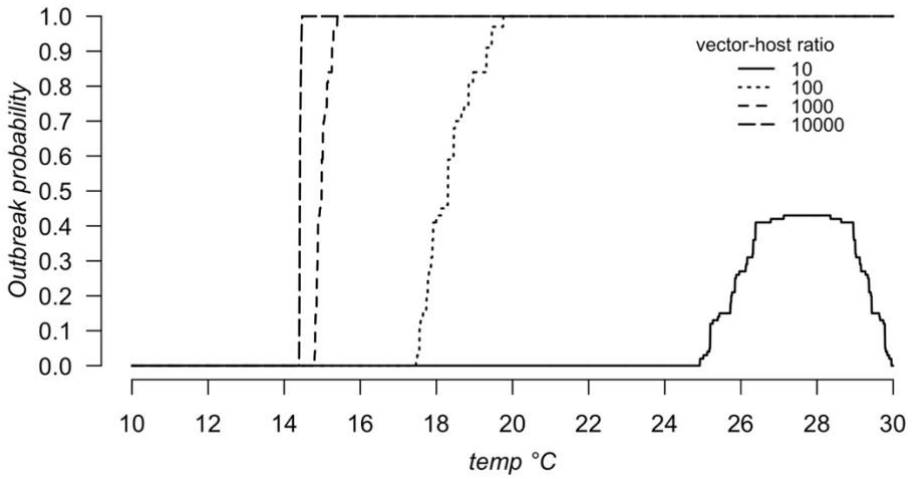

Figure 5: Effect of temperature on R_0. *R_0 as a function of temperature (10-30°C).* $\frac{M}{B} = 10 \frac{M}{B} = 100 \frac{M}{B} = 1000 \frac{M}{B} = 10000$

Discussion

With the present study we identified which of the WNV transmission mechanisms are those having the biggest effect on disease spread, thus highlighting our potential priorities in filling current knowledge gaps. We showed that the duration of infectious period in birds (ν_B) and the mosquito biting rate (b) have the biggest effect on disease dynamics, whereas birds' susceptibility to infection (p_{MB}) and birds' competence (p) showed a lower effect. Moreover, in agreement with [36], temperature is shown to highly affect spread probability, not allowing for any WNV spread if it is lower than 14°C. Lombardy region, our study area, shows all suitable characteristics to allow WNV spread during summer, especially in June where the environmental conditions seem to ensure the possibility of NWV to spread in mosquitoes.

Despite the progresses in health cares and preventive measures, the intervention to control the spread of infectious communicable diseases remains one of the main goals for public health [37,38], but this goal is often impaired by the lack of information and certainties about mechanisms driving infection transmission dynamics [17,39]. Mathematical modelling can be an efficient tool to aid in investigating infection dynamics and transmission mechanisms (e.g. [40] and [41]), but the adoption of this approach requires robust parameter estimates to be reliable and thus has often been hampered by existing limitations due to inadequate or partial data availability. If on the one hand the development of field investigations is fundamental to enhance our comprehension of spreading mechanisms, on the other hand it can be very long and demanding, making essential a careful choice of the most useful investigations to be performed. Moreover,

not all epidemiological mechanisms have the same impact on disease dynamics, and, consequently, also a small change in some model parameter estimate can cause a big variation in model predictions, and vice-versa [42]. Therefore, before developing any field study to focus on, it can be helpful to identify those parameters that have the biggest impact on the dynamic of the infection.

WNV is now considered one of the most widespread arboviruses in the world, with human cases identified worldwide [43]. Despite that, there are several mechanisms, that contribute to its spread and maintenance into wild populations, that are still unknown [17]. Many species are considered suitable as hosts or vectors, thus possibly giving different contributions to infection dynamics depending on individual and species-specific characteristics [16,18,22,44]. Moreover, the variety of involved species, the regional patterns shown by virus transmission cycles, and the existing differences in avian communities' composition among areas [12,19,45] further contribute to increase the sources of variability in infection dynamics, thus making hard and expensive the collection of field data required to fill knowledge gaps. Indeed, antibodies against WNV has been detected in a broad range of wild and domestic bird species all over the world, and virus isolation have been obtained from different avian species [12]. Not only, also viraemic titres developed by different birds have shown to strongly depend on both the host species and virus lineage [15,16,18,19]. Also, mosquito feeding behaviour can change among areas and mosquito species, depending on both host abundance and mosquito feeding preference [33,46,47]. All these characteristics are suitable to drive, or have an influence on, WNV spread.

In this context the present work aims at investigating how a change in WNV epidemiological parameter estimate can affect the spread of the disease. At first, we highlighted the importance of birds' recovery rate in driving WNV spread, showing that a change in this parameter estimate, especially if we consider low recovery rates (i.e., long infectious period duration), widely affects the effective reproduction number of the infection R_t (i.e. the number of secondary infected mosquitoes in a given day). A change in bird recovery rate estimate, despite highly affecting estimated number of infected mosquitoes, shows to affect the spread probability only when lowering it, evidencing the importance of having long durations of infectious period to allow for infection spread. These results on the one hand highlight the need of a careful estimate of species-specific recovery rates to obtain a reliable estimate of WNV probability and intensity of spreading, on the other hand point out the need of comparing species-specific rates to determine which of the investigated avian species plays the major role in spreading the infection. According to model simulations, also mosquito biting rate can widely affect WNV R_t, also influencing the probability of the infection to spread during summer. The interaction between birds and mosquitoes is known to play a central role in disease spread [48], moreover it is shown that mosquitoes can selectively choose where to feed on, preferring specific species to others [33,49]. According to our results, since the effect of biting rate estimate is quite substantial, and particularly high when increasing biting rate estimate, we can conclude that understanding which conditions and species-specific characteristics drive the probability of being bitten is critical to fully understand and predict the spread of WNV. In addition, this result again helps us to identify those avian species that are suitable to play an important role in WNV transmission, such as those highly bitten or

preferred by mosquitoes. Furthermore, by pointing out the role of mosquito biting rate in infection spread, this result highlights the importance of investigating the extent to which mosquitoes actively choose which species to feed on, and the extent to which the biting rate is driven by species abundance. For example, in northern Italy blackbirds are frequently bitten by mosquitoes, even if they are less abundant than other species [33]. This finding, coupled with the high number of blackbirds individuals living in the area, could suggest their role in the spread of WNV. Being aware of which avian species are primarily involved in the spread of infection would help us to fill some of the current knowledge gaps and improve our understanding of WND but would also allow us to efficiently estimate and predict the risk of infection for human beings, and consequently to develop appropriate intervention strategies to reduce it.

The avian competence, despite the high species-specific differences reported [16], seems to have a lower impact on the spread of the infection. It is assumed that to be capable of infecting mosquitoes (*Cx. Pipiens*), birds need to develop viraemic titres greater than 10^5 PFU/mL [5], but the collection of this information can be logistically demanding and hard to perform for wild birds. Moreover, experimental infections might not successfully mimic the natural infection occurring in wildlife, as mosquito inoculations can result in a higher viremia than needle injections [50]. Consequently, the efforts required for the estimate of bird competence may be greater than the benefit obtained.

As WNV antibodies have been detected in several birds belonging to different species, a number of bird species may be considered susceptible to WNV infection. The bird surveillance, ongoing in several countries, can therefore be widely useful to help us recognise between susceptible and potentially not susceptible avian species. Despite of

that, our analysis showed that the sole susceptibility of birds to WNV infection has a small effect on both the number of secondary infected mosquitoes and probability of spread of the disease. It implicates that, despite being informative on the possible circulation of WNV in the avian population, the investigation of the WNV-positivity of birds cannot be considered one of the most influential parameters.

Furthermore, this work supports the hypothesis that temperature and competent mosquito presence are limiting factors for WNV spread [36,51]. Indeed, according to model simulations, the R_t of the infection changes following a change in temperature and in the vector-host ratio. Despite that, the effect of all epidemiological parameter estimate does not change according to temperature and vector-host ratio, making our findings generalizable and extendible to areas other the one under testing. It is also important to notice that in our study area, temperatures and recorded mosquito densities always allow for WNV spread, highlighting the high human infection risk in this area. Moreover, simulating the dynamics of WNV, our model shows that very few infectious mosquitoes are enough to start and maintain WNV circulation during the season and a low number of birds is necessary to maintain the infection at the beginning of the season, implying that, to be able to early detect the infection, the testing of a large number of mosquitoes and birds is required. It follows that the entomological surveillance, allowing the fast and easier collection of numerous samples, is likely to be more informative to early detect WNV then the surveillance on birds. Moreover, due to the low birds and mosquitoes' numbers necessary to maintain the infection, and considering the long flying distance of birds, a surveillance plan could be beneficial also

in not endemic areas with suitable climatic conditions to early detect WNV introduction in new areas.

It is necessary to note that one limitation of the present model is the assumption of having only one competent avian species. Despite of this oversimplification, which could be overcome by future studies, this modelling approach have a proven ability to simulate and investigate WNV spread in nearby regions (i.e., Veneto and Emilia Romagna), suggesting its reliability despite its limitations [23,25].

In conclusion, WNV transmission and maintenance mechanisms still presents several knowledge gaps, thus impairing our capability of understanding and predicting its spread. Among them, duration of avian infectious period and mosquito biting rate are the most impacting on the number of secondary infected mosquitoes and on spread probability. These two mechanisms are thus the most urgent parameters to be fully studied, and their investigation could also be of aid in determining the avian species that plays the main role in WNV spread and maintenance. Furthermore, temperature and mosquitos' number can be limiting factors for WNV spread and areas with suitable conditions require the design of an efficient surveillance plan to keep disease spread under control. Finally, our results, obtained through mathematical model simulations, highlight how a synergic interaction among theoretical and field research, could be beneficial for a better comprehension of infectious disease spreading mechanisms by allowing the formulation of hypotheses to identify the most appropriate data required to cover knowledge gaps.

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Supplementary materials S2

Clustering of trap locations

The clustering of trap locations was performed using a K-means algorithm accounting for differences in temperature, precipitations, mosquito abundance and WNV circulation among trap locations, and it showed a wide variability among trap locations. For this reason, we based our clustering on a geographical division of the region by clustering together northern traps, eastern traps, and western traps.

Epidemiological-model Structure

According with the scheme reported in Fig.A we simulated WNV spread into Lombardy region through the following system of differential equations:

$$\begin{cases} M'_S(t) = \omega(t) - (b \cdot p \cdot p_{BM} \cdot \dfrac{B_{Ia}(t) + B_{Ij}(t)}{B_T(t)} + \mu_M) \cdot M_s(t) \\ M'_E(t) = b \cdot p \cdot p_{BM} \cdot \dfrac{B_{Ia}(t) + B_{Ij}(t)}{B_T(t)} \cdot M_s(t) - (\theta_M + \mu_M) \cdot M_E(t) \\ M'_I(t) = \theta_M \cdot M_E(t) - \mu_M \cdot M_I(t) \\ B'_{Sa}(t) = -(b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} + \mu_B) \cdot B_{Sa}(t) \\ B'_{Ea}(t) = b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} \cdot B_{Sa}(t) - (\mu_B + \theta_B) \cdot B_{Ea} \\ B'_{Ia}(t) = \theta_B \cdot B_{Ea} - (\mu_B + \sigma_B) \cdot B_{Ia} \\ B'_{Ra}(t) = \sigma_B \cdot B_{Ia} - \mu_B \cdot B_{Ra} \\ B'_{Sj}(t) = \gamma \cdot B_a(b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} + \mu_{Bj}) \cdot B_{Sj}(t) \\ B'_{Ej}(t) = b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} \cdot B_{Sj}(t) - (\mu_{Bj} + \theta_B) \cdot B_{Ej} \\ B'_{Ij}(t) = \theta_B \cdot B_{Ej} - (\mu_{Bj} + \sigma_B) \cdot B_{Ij} \\ B'_{Rj}(t) = \sigma_B \cdot B_{Ij} - \mu_{Bj} \cdot B_{Rj} \end{cases}$$

In the proposed system M_S, M_E and M_I respectively represent the susceptible, exposed and infectious mosquito population, whereas B_{Sa}, B_{Ea}, B_{Ia} and B_{Ra} susceptible,

exposed, infectious and recovered competent adult birds and B_{Sj}, B_{Ej}, B_{Ij} and B_{Rj} susceptible, exposed infectious and recovered competent juvenile birds. B_T and B_a represent the total avian population and the total adult avian population respectively.

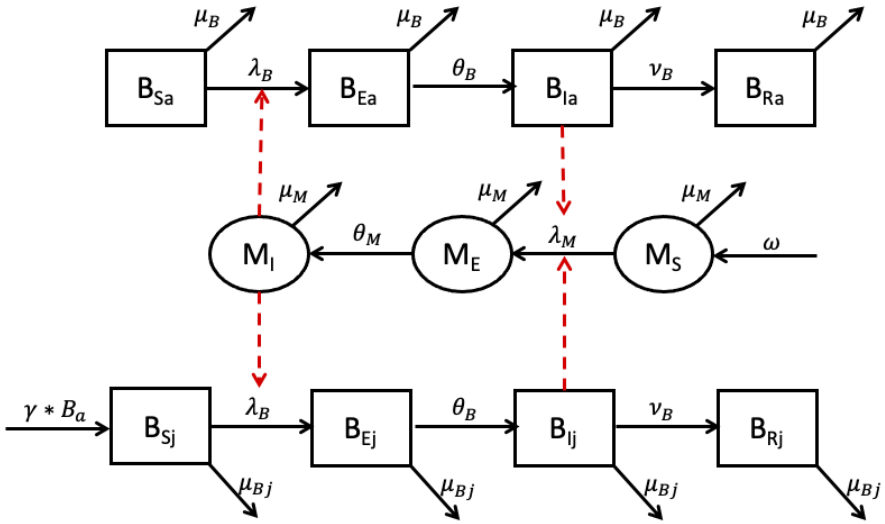

Figure A: Model scheme. Model flow chart for WNV transmission in birds (squares) and mosquitoes (circles) in an average trapped area. Compartments: $\boldsymbol{B_{Sa}}$, $\boldsymbol{B_{Ea}}$, $\boldsymbol{B_{Ia}}$ *and (*$\boldsymbol{B_{Sj}}$, $\boldsymbol{B_{Ej}}$, $\boldsymbol{B_{Ij}}$ *and* $\boldsymbol{B_{Rj}}$*): adult (juvenile) susceptible, exposed, infectious and immune birds; Ms, Me, Mi: susceptible, exposed and infectious mosquitoes. Parameters: λB and λM are the force of infection for birds and mosquitoes respectively and are computed as* $\boldsymbol{\lambda_B = b \cdot p \cdot p_{MB} \cdot \frac{M_I}{B_T}}$ *and* $\boldsymbol{\lambda_M = b \cdot p_{BM} \cdot \frac{(B_{Ia}+B_{Ij})}{B_T}}$*, with* B_T *being the total avian population and* B_a *the number of adult birds.*

Table A: **Model parameters.**

Parameter	Explanation	Value	Source
μ_M	Mosquito death rate (day^{-1})	$\frac{4.61}{151.6-4.75 \cdot T}$*	[1,2]
p_{BM}	Probability of WNV transmission from bird to mosquito per infectious bite	$\frac{e^{(-10.917+0.365 \cdot T)}}{1+e^{(-10.917+0.365 \cdot T)}}$*	[3]
θ_M	Extrinsic incubation period (day^{-1})	-0.132+0.0092	[4]
θ_B	Intrinsic incubation period (day^{-1})	0.5	[5]
$\gamma(t)$	Avian fertility rate ad day t (day^{-1})	0.5 (t $\leq$ July 20) 0 (t > July 20)	[6]
μ_B	Death rate of adult birds (day^{-1})	0.0015	[6]
μ_{Bj}	Death rate of juvenile birds (day^{-1})	0.0083	[6]

* T represents temperature expressed in Celsius degrees C°

Additional results

- *Model fit*

The model fit was quite satisfactory as 92% of the total (considering all years and clusters) number of weekly positive pools lies within the 95% CI predictions of the model (**Fig.B**).

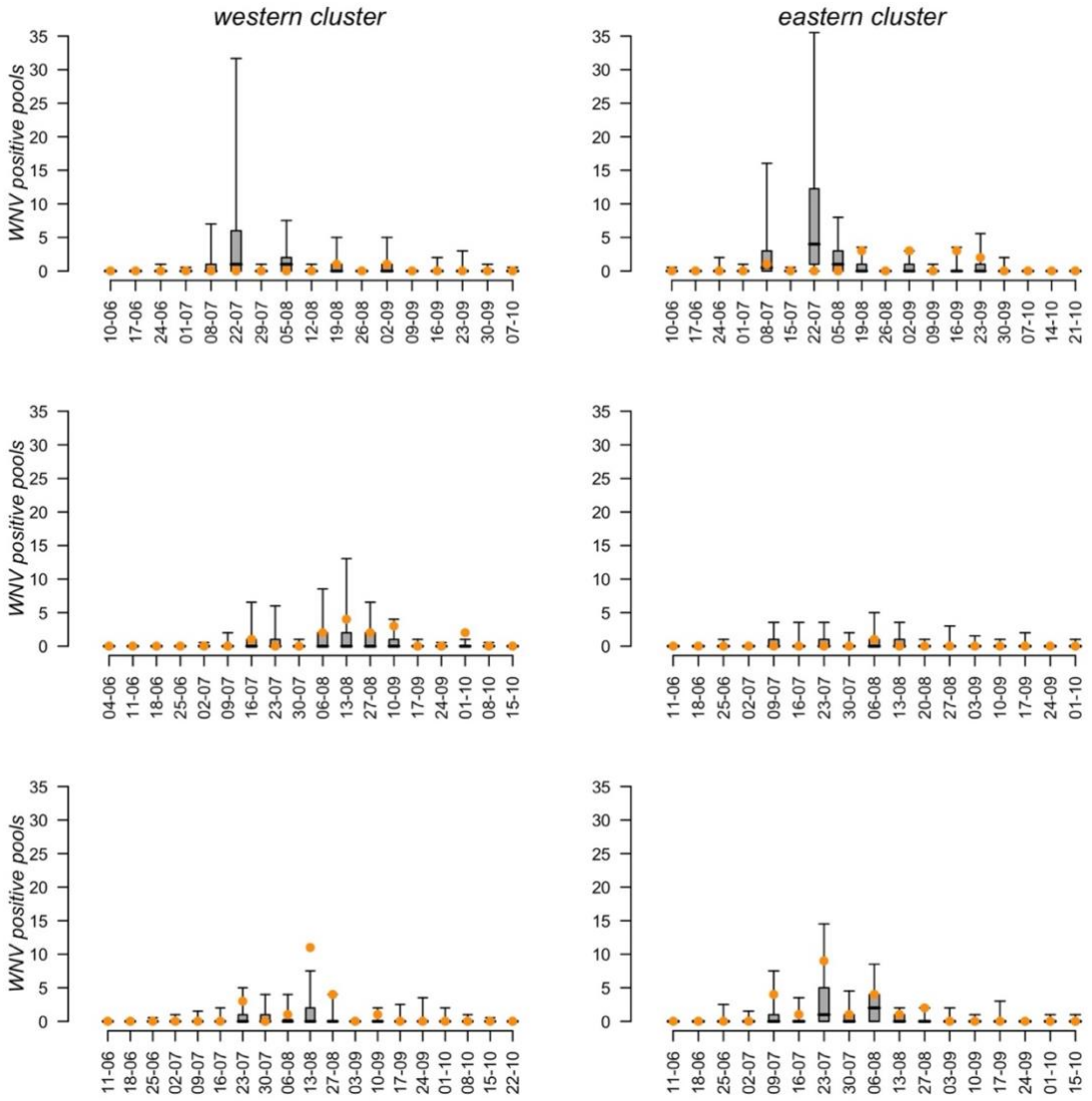

Figure B: Model predictions fit*. Predicted number of WNV positive pools for the three years (first line for 2016, second line for 2017 and third line for 2018) and the two areas. Orange points: observed weekly number of WNV positive pools; grey boxplots (median, 2.5 and 97.5% quantiles) show the predicted distributions of positive pools per week.*

- *WNV spread and prevalence*

We investigated WNV prevalence into mosquito (**Fig.C**) and birds (**Fig.D**) in Lombardy region, according with model assumptions. Model simulations predict a low prevalence of WNV for the mosquito population in all years and clusters, never exceeding a daily mean prevalence of 0.231%. In all years and clusters, the lowest prevalence is shown up to July, then we can see its increase, a peak in early/late August and a slight decrease and stabilization. The increase and decrease slope and the timing for prevalence peak both depends on the year and cluster considered. The lowest WNV circulation was predicted for the western cluster in 2018, with a mean prevalence $0.053\%, (0 - 0.301\%\ CI)$, whereas the highest was predicted in the eastern cluster in 2018, with a mean prevalence of 0.231% $(0.003 - 0.99\%\ CI)$.

On the other hand, avian prevalence is higher, reaching a daily mean 8.38% $(0.003 - 35.5\%\ CI)$. It increases between June and July, reaching the maximum between July and August and then slowly decreasing up to October. All confidence intervals are estimates as the $2.5 - 97.5$ quantiles.

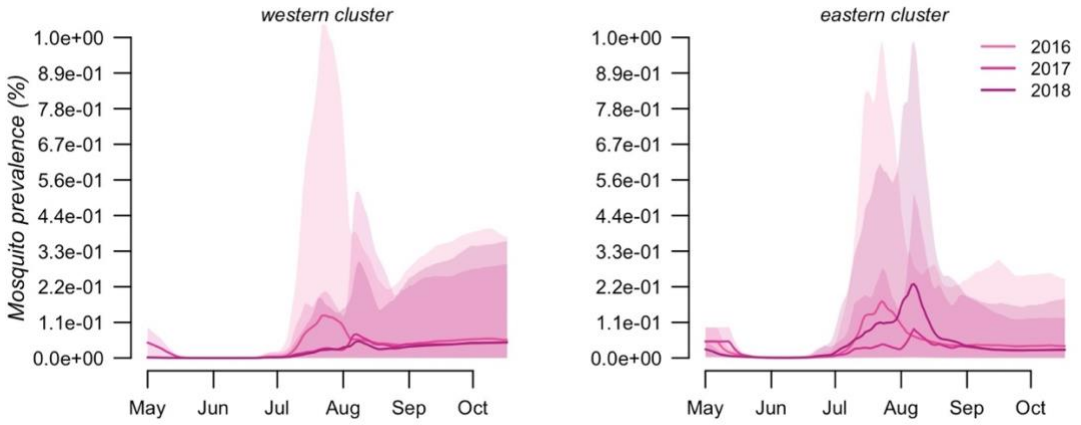

Figure C: Model predictions. *Predicted WNV prevalence in mosquitoes in clusters and years.*

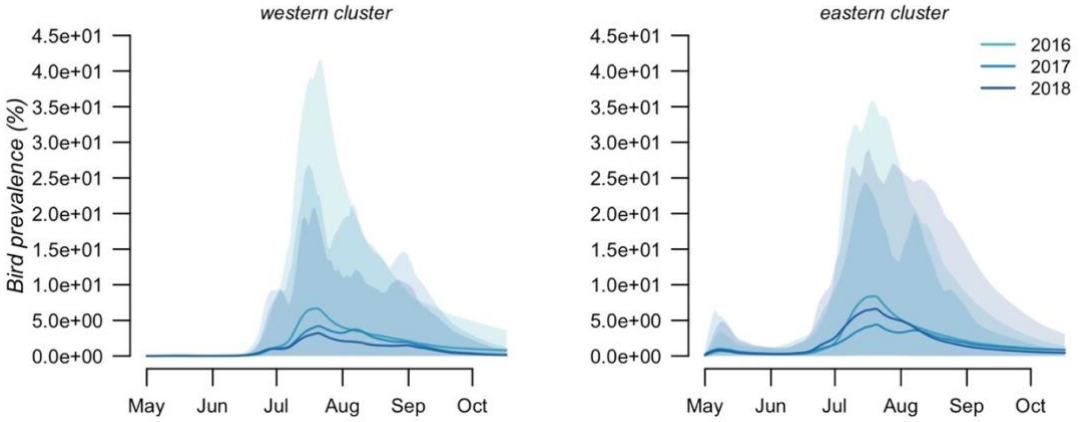

Figure D: Model predictions. *Predicted WNV prevalence in the competent avian population (magpies) in clusters and years.*

CHAPTER 3

Can different population dynamics of competent bird species characterize West Nile virus spread? A mathematical modelling investigation.

Introduction

West Nile Virus (WNV) is one of the most widespread arboviruses in the world and is the cause of West Nile disease (WND). It is maintained in an enzootic cycle involving mosquitoes (mainly of the genus *Culex*) and birds [1,2]. Human beings, as other mammals, can act as dead-end hosts and be infected via mosquito bite, developing symptoms that can range from influenza-like syndrome to severe neurological disease [3,4]. To control infection spread and avoid human infection risk, several studies have been carried out and a wide surveillance on mosquitoes and birds is currently ongoing in different countries, Italy included [5–7]. These efforts in monitoring the infection and in collecting information about spreading mechanisms and dynamics widely improved our awareness on the disease, but knowledge gaps persist [8]. As WNV involves several bird species, and the comprehension of multi-host systems is challenging, the role played by specific bird species potentially involved in the spread or maintenance of the infection still deserves a thorough attention [2,9–12]. In fact, WNV has been detected in more than 200 bird species in the Americas alone. Ecological and behavioural characteristics, like altitude and temperature of a specific species' ecological niche, and particular epidemiological characteristics, like competence and susceptibility to infection, make each species more or less likely to be responsible of infection spread [13,14]. Also, differences in birds' reaction to WNV infection, like disease-induced mortality, have been pointed among different species and countries [15]. Not only birds' reaction to infection changes among different species and countries, but also the composition of avian communities, in terms of species presence as well as their absolute and relative

abundance, can be very different among countries and areas [16]. All these features characterize the complexity of WNV dynamics, involving the overall avian community [12,17–19], making it hard to be investigated and comprehended. Given the complexity of the scenario, on the one hand it is fundamental to carry on field investigations and surveillance, acquiring new and more complete data to investigate the role of different bird species. On the other hand, a preventive identification of the main WNV reservoir hosts could help in circumscribing investigations. Although a different role of adult and juvenile birds in WNV infection dynamics has been hypothesized [20], bird population dynamics has not raised the principal attention as a discriminating factor in infection spread. Indeed, bird species present different biological features such as length of breeding season, survival, fecundity (i.e., number of offspring), thus having very different population dynamics during summer with a potential effect on WNV dynamics. For this reason, in the present work we investigate those demographic characteristics of the avian population, beyond the epidemiological ones, that might influence WNV infection. Based on our study area (Lombardy region in the north of Italy), we chose five different avian species, susceptible for WNV infection, that have different abundances and population dynamics during summer season (e.g., different length of breeding season, different number of new births per year or different life expectancies). Then, through a mathematical model, we investigated the influence of population dynamics traits on WNV mosquito prevalence, by considering each of them separately as the only WNV-competent avian species. We assumed different demographical features between species but, for the sake of simplicity, identical epidemiological parameters. Moreover, we also simulated the dynamics of WNV for three additional hypothetical scenarios. At

first, by considering the same avian species abundance at the beginning of the year, we investigated if and how differences in the only population dynamics can affect WNV spread. In the two successive scenarios, while considering fixed also birth and death rates, we varied respectively the initial month and the length of the breeding season.

Materials and Methods

The modelling framework follows the one proposed in [21], and then adapted to simulate WNV spread in Lombardy region (Chapter 2). In the proposed model we simulate WNV spread into a mosquito population, included as a Susceptible-Exposed-Infectious model, and a competent avian host population, included as a Susceptible-Exposed-Infectious-Recovered model, where birds are divided in two age-class (adults and juveniles). We considered as the competent host species five different bird species, and simulated WNV spread to investigate the possible effect of the specie-specific population dynamics on disease spread. Avian species included in model simulations are:

- Magpies (*Pica pica*)
- House sparrows (*Passer italiae*)
- Blackbird (*Turdus merula*)
- Hooded crow (*Corvus cornix*)
- Eurasian collared dove (*Streptopelia decaocto*)

Dataset and reference system

In analogy with ([21] and Chapter 2), to inform the proposed model we used data on mosquito abundance and their PCR-positivity to WNV gathered by Regione Lombardia (RL) and Istituto Zooprofilattico della Lombardia ed Emilia Romagna (IZSLER) under the entomological surveillance plan, carried out from April to October during the triennium 2016-2018.

The number of birds present in an averaged trapping area *A* was estimated for each species at the beginning of May by fitting the data obtained by the avifauna census

provided by Regione Lombardia with a kriging method (*autoKrige* function in package *automap* in R 3.6.3 software), with $A=\pi \cdot r^2$, where *r* is the average *Cx. pipiens* flight range (500m) [22,23]. The initial number of birds in each cluster was calculated as the average number estimated by the kriging method for the cluster and was considered the same for all the three years under study.

Following the clustering proposed in Chapter 2, the region was divided into three separated clusters homogeneous for temperature, precipitations, number of mosquitoes and WNV presence (Fig.1). Due to the absence of WNV in the northern cluster, we only investigated infection dynamics through the eastern and western ones (green and blue, Fig.1)

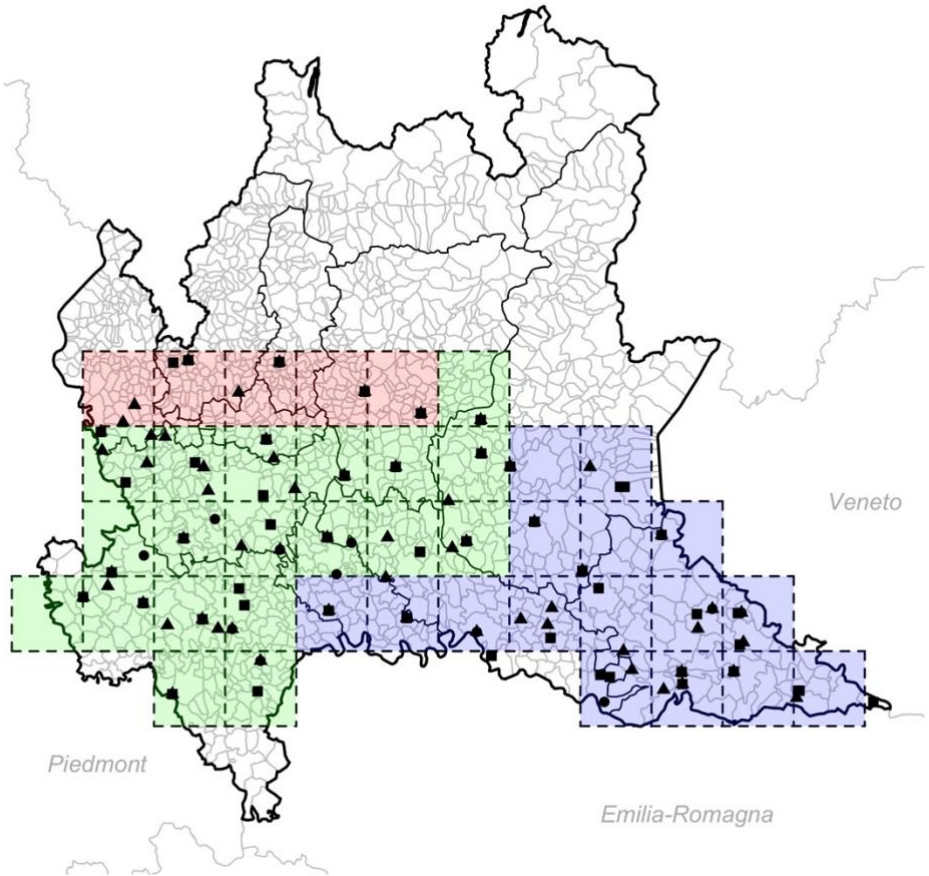

Figure 1: capture site clustering proposed for Lombardy region. Black squares, circles and triangles represent mosquito capture site respectively for 2016, 2017 and 2018.

The epidemiological model

An epidemiological model was built following the framework proposed in Chapter 2 to simulate the dynamics of WNV infection in a competent avian population in the study area (Chapter 2). The epidemiological model is based on a system of 11 equations following the scheme reported in **Fig.2.** Three of the compartments represent a mosquito population divided in susceptible (M_S), exposed (M_E) and infected mosquitoes (M_I). Eight of them represents a competent adult avian population divided in adult and juvenile

individuals divided in turn in susceptible (B_{Sa} and B_{Sj}), exposed (B_{Ea} and B_{Ej}), infectious (B_{Ia} and B_{Ij}) and recovered (B_{Ra} and B_{Rj}) birds. Both birds and mosquito dynamics are included as a known function derived respectively by two mathematical models: i) the avian population model ii) and the entomological model. The mosquito population abundance derived from the entomological model, as well as all unknown parameters values (for both entomological and epidemiological model), were taken from the work proposed in Chapter 2. Since mosquito feeding behaviour, and thus in the biting rate (b), might change from the early season (up to mid-July) to late season (from mid-July on) [24], two biting rates were considered, respectively b_1 for the early season and b_2 for the late season [25].

In analogy with Chapters 2 and 4 thus, and to consider the encountered differences in mosquito abundance among years and cluster, WNV mosquito prevalence was estimated for each year (2016-2018) and cluster (western and eastern).

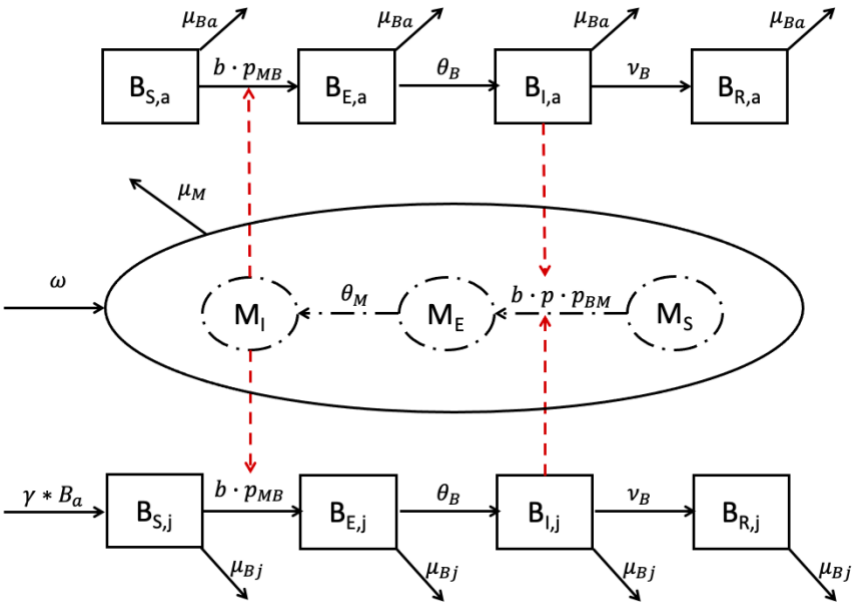

Figure 2. Flowchart for WNV transmission model.

The entomological model

The number of mosquitoes (*Cx. Pipiens*) to be included in model simulations was estimated through a four-compartment model representing mosquito developmental stages following the temperature-dependent entomological model presented in [21] and were calibrated on the recorded captures in Lombardy region in 2016, 2017 and 2018, averaged over each cluster, as presented in Chapter 2.

The avian population model

Due to the absence of field data and longitudinal observation on the number of adult and juvenile birds during summer season, we simulate a population dynamics of each species estimating the daily number of birds of three age classes through a three-compartmental model following the scheme reported in Fig.3. Such obtained dynamics were then used to estimate the daily adult and juvenile bird abundance to include in the epidemiological model as a known function.

The four compartments represent birds' developmental stages (pulli *P*, juveniles *J,* sub-adults *S,* adults *A*) and were chosen due to the marked existing differences of life expectancy of each developmental stage found in literature. Population dynamics of each species were estimated by using parameter estimates found in literature (a full table of parameters and references is reported in Tables A in Supplementary materials S3), adjusted in order to choose the most suitable set of parameters that keeps constant the avian population between two consequent years. In the proposed model, only adult birds are assumed to lay eggs with a specie-specific birth rate (*b*) depending on the mean number of eggs laid during each brooding and the mean number of broodings, and only during their breeding period. We considered one breeding season per year, with species-dependent length and timing. The number of pulli (*P* compartment) increases following the number of laid eggs, adjusted for the hatching probability. The number of juveniles and subadults was considered to vary following rates g_1 and g_2 respectively, which represent the inverse of the time spent in each age-class. All developmental stages die at a stage-specific death rate. We considered sub-adults as fully developed birds, but not sexually mature, thus they have the same death rate as adult birds, but do not participate in giving birth to new individuals. An environmental carrying capacity (K), depending on the mean number of birds reported in the area in literature, was considered to limit the number of juvenile birds. The complete system of equation is reported in Supplementary materials S3. All model parameters are considered species dependent.

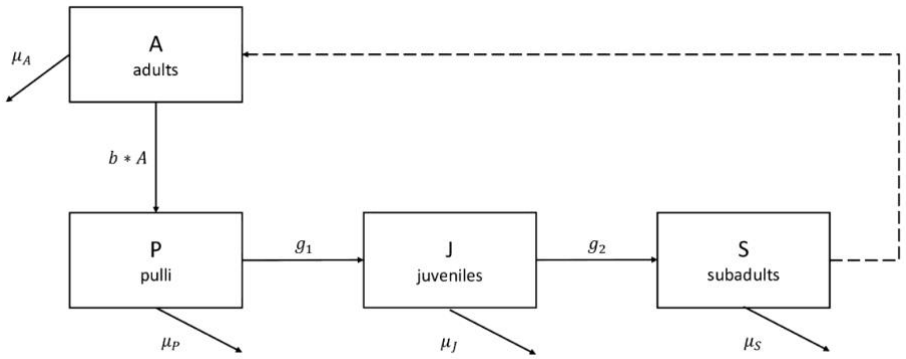

*Figure 3: Flowchart of population dynamics of birds. Compartments: adults (A), pulli (P), juveniles (J) and subadults (S). Parameters: μ (A, P, J, S) is the death rate; b*A is the birth rate multiplied by the number of adults; g (1, 2) are the growth rates for nestlings and juveniles respectively. For the sake of simplicity, to have no more than 11 differential equations, in the epidemiological model the simulated dynamics was included by considering as the daily number of juvenile birds the sum of the daily number of pulli, juveniles and sub-adult birds. The daily number of adults birds in both the epidemiological and the avian population coincide.*

Simulated scenarios

To investigate if species specific population dynamics affect WNV circulation, and which demographic characteristics have the highest influence, we proposed four different scenarios and then simulate WNV dynamics by using the five different population dynamics obtained with the 'avian population model'. Epidemiological parameter values were obtained from the work proposed in Chapter 2. The four proposed scenarios are:

1. "*base*" scenario: we considered the number of birds estimated from the bird census as the initial number of individuals for each species, and the species-specific parameters obtained by the avian population model to simulate birds, and consequently WNV, dynamics. Demographic parameters are reported in Table 1.

Table 1. Demographic daily rates used for epidemiological simulations in the *base scenario*.

Parameters	Blackbirds	Magpies	House sparrows	Collared doves	Hooded crows
Initial number of birds	7	5	16	9	15
Breeding season	75[a]-165[b]	133[a]-157[b]	75[a]-213[b]	61[a]-245[b]	110[a]-161[b]
Birth rate b	0.0344	0.0482	0.0522	0.0111	0.0576
Adults' death rate d_A	$3.1276 \cdot 10^{-6}$	$1.8764 \cdot 10^{-6}$	$1.2511 \cdot 10^{-6}$	$1.8766 \cdot 10^{-6}$	$1.8768 \cdot 10^{-6}$
Sub-adults' death rate d_{SA}	$3.1276 \cdot 10^{-6}$	$1.8764 \cdot 10^{-6}$	$1.2511 \cdot 10^{-6}$	$1.8766 \cdot 10^{-6}$	$1.8768 \cdot 10^{-6}$
Pulli death rate d_P	0.0194	0.0114	0.0146	0.0210	0.035
Juveniles' death rates d_J	0. 0.0128	0.0124	0.0181	0.018	0.0144
Growth rate pulli-juveniles g_1	0.0654	0.0521	0.0655	0.0575	0.0594
Growth rate juveniles-sub adults g_2	0.0244	0.0311	0.03444	0.063	0.0274

[a] start of the breeding season (Julian days)
[b] end of the breeding season (Julian days)

2. "fixed initial number of birds" scenario: to investigate the effect of demographic parameters on WNV prevalence in mosquitoes, we assumed all five bird species to start the season with the same number of individuals (i.e., 7 birds). Birth and death rates for each simulation were estimated from House sparrow rates. It is important to notice that, analogously as for the *base model*, for each bird species, all demographic rates were adjusted in order to obtain at the end of a one-year simulation a number of adult birds ranging into the 10% of the number of birds used for starting the simulation at the beginning of the year. For this reason, demographic parameters may differ among species, despite them all start from the same rates used for simulating the dynamics of house sparrow in the *base model*. Demographic parameters are reported in Table 2.

Table 2. Demographic daily rates used for epidemiological simulations in the *fixed initial number of birds scenario*.

Parameters	Blackbirds	Magpies	House sparrows	Collared doves	Hooded crows
Initial number of birds	7	7	7	7	7
Breeding season	75[a]-165[b]	133[a]-157[b]	75[a]-213[b]	61[a]-245[b]	110[a]-161[b]
Birth rate b	0.0379	0.0587	0.0348	0.0073	0.0425
Adults' death rate d_A	$3.1278 \cdot 10^{-6}$	$1.8761 \cdot 10^{-6}$	$1.2511 \cdot 10^{-6}$	$1.8766 \cdot 10^{-6}$	$1.8765 \cdot 10^{-6}$
Sub-adults' death rate d_{SA}	$3.1278 \cdot 10^{-6}$	$1.8761 \cdot 10^{-6}$	$1.2511 \cdot 10^{-6}$	$1.8766 \cdot 10^{-6}$	$1.8765 \cdot 10^{-6}$
Pulli death rate d_P	0.0164	0.0141	0.0113	0.02	0.0504
Juveniles' death rates d_J	0.0168	0.0141	0.0221	0.0148	0.0160
Growth rate pulli-juveniles g_1	0.0627	0.0522	0.0573	0.0615	0.0618
Growth rate juveniles- sub adults g_2	0.0321	0.0354	0.042	0.0519	0.0305

[a] start of the breeding season (Julian days)
[b] end of the breeding season (Julian days)

3. "*Shift of breeding season*" scenario: to investigate if different breeding periods can affect WNV transmission, we considered only one avian species with house sparrows' birth and death rates and varied only the initial month of the breeding season. Also, in this scenario the initial number of birds was fixed. Again, all birth and death rates were estimated starting from house sparrow's parameters, adjusted for each species to obtain at the end of one-year simulations a bird abundance of $\pm$10% of the number of birds used for starting the simulation. Demographic parameters are reported in Table 3.

Table 3. Demographic daily rates used for epidemiological simulations used in the *shift of breeding season scenario*.

Parameters	March	April	June	July
Initial number of birds	7	7	7	7
Breeding season	60[a]-90[b]	90[a]-120[b]	120[a]-150[b]	150[a]-180[b]
Birth rate b	0.1208	0.1244	0.1478	0.1634
Adults' death rate d_A	$3.1277 \cdot 10^{-6}$	$3.1279 \cdot 10^{-6}$	$3.1275 \cdot 10^{-6}$	$3.1270 \cdot 10^{-6}$
Sub-adults' death rate d_{SA}	$3.1277 \cdot 10^{-6}$	$3.1279 \cdot 10^{-6}$	$3.1275 \cdot 10^{-6}$	$3.127 \cdot 10^{-6}$
Pulli death rate d_P	0.0196	0.0216	0.019	0.0333
Juveniles' death rates d_J	0.0133	0.0187	0.0128	0.0129
Growth rate pulli-juveniles g_1	0.0575	0.0899	0.0753	0.09
Growth rate Juveniles- sub adults g_2	0.0254	0.0357	0.0243	0.0247

[a] start of the breeding season (Julian days)
[b] end of the breeding season (Julian days)

4. "*Different lengths of breeding season*" scenario: to investigate if different lengths of breeding periods can affect WNV spread, we considered only one avian species and varied only the length of the breeding season, assumed to start on March 1. Again, in this scenario the initial number of birds was fixed (7 individuals) and for each bird species, birth and death rates were based on house sparrow parameters and adjusted to obtain at the end of one-year simulations a number of adult birds ranging into $\pm$10% of the number of birds at the beginning of the year. Demographic parameters are reported in Table 4.

Table 4. Demographic daily rates used for epidemiological simulations used in the *Different lengths of breeding season scenario*.

Parameters	1 month	2 months	3 months	4 months
Initial number of birds	7	7	7	7
Breeding season	60[a]-90[b]	60[a]-120[b]	60[a]-150[b]	60[a]-180[b]
Birth rate b	0.0386	0.245	0.1062	0.081
Adults' death rate d_A	$3.1274 \cdot 10^{-6}$	$3.1276 \cdot 10^{-6}$	$3.1279 \cdot 10^{-6}$	$3.1278 \cdot 10^{-6}$
Sub-adults' death rate d_{SA}	$3.1274 \cdot 10^{-6}$	$3.1276 \cdot 10^{-6}$	$3.1279 \cdot 10^{-6}$	$3.1278 \cdot 10^{-6}$
Pulli death rate d_P	0.0233	0.02	0.021	0.0245
Juveniles' death rates d_J	0.0155	0.016	0.0136	0.0187
Growth rate pulli-juveniles g_1	0.0698	0.0779	0.0818	0.0673
Growth rate juveniles-sub adults g_2	0.0295	0.0305	0.0259	0.0356

[a] start of the breeding season (Julian days)
[b] end of the breeding season (Julian days)

Epidemiological parameter values were obtained from the work proposed in Chapter 2. Briefly, unknown epidemiological parameters of a competent avian species were estimated through a Bayesian approach considering the likelihood of observing the weekly number of positive pools given the predicted mosquito prevalence.

For each of the proposed scenarios, in analogy with Chapter 2, 100 different set of epidemiological parameters, drawn from the estimated posterior distributions, were used. Thus, for the sake of simplicity, we used the same epidemiological parameters for all considered scenarios. The full list of epidemiological parameters (average and 2.5%-97.5% percentile) used is reported in Table 5.

Table 5. Daily rates of epidemiological parameters used for simulations. T represents the mean daily temperature.

Parameter	Biological interpretation	Value
b_1	Mosquito biting rate during early season (91-200 Julian calendar day)	$0.373(0.159 - 0.496)$ [a]
b_2	Mosquito biting rate during late season (200-271 Julian calendar day)	$0.241(0.151 - 0.485)$ [a]
p	Avian competence	0.621 [a]
p_{MB}	Susceptibility of the competent avian population	$0.791(0.358 - 0.995)$ [a]
p_{BM}	Susceptibility of the mosquito population	$\frac{e^{(-10.197+0.365\cdot T)}}{1+e^{(-10.197+0.365\cdot T)}}$ [b]
θ_M	Extrinsic incubation period	$\frac{1}{0.0092\cdot T-0.132}$ [c]
θ_B	Intrinsic incubation period	2 [d]
ν_B	Recovery rate	$0.43(0.0425 - 0.982)$ [a]

[a] MCMC estimate range (average and 2.5%-97.5% percentile)
[b] [25]
[c] [26]
[d] [9]

In each scenario, we evaluated how different avian population dynamics affect WNV mosquito prevalence, as it can be considered as representative of human infection risk.

Results

The base-scenario

- *Birds' dynamics (two-age classes model)*

Investigating differences among birds dynamics, and according with the abundance derived on census data, we can observe that magpies (**Fig.4**, red line), blackbirds (**Fig.4**, blue line) and hooded crows (**Fig.4**, purple line) have an early breeding season resulting in a peak of the total number of individuals occurring between June and July, whereas doves (**Fig.4**, green line) and house sparrows (**Fig.4**, orange line) abundances peak later, respectively in August and September. Among the five species investigated, house sparrows are the most abundant during the whole season, followed by hooded crows and doves and then by blackbirds and magpies.

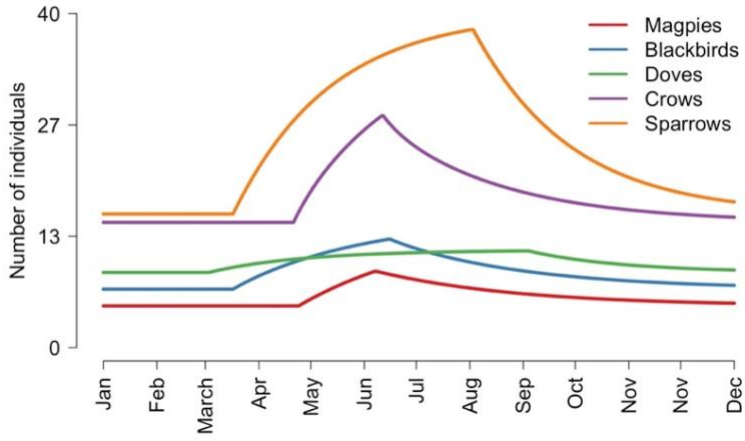

***Figure 4: Birds dynamics**. Comparison of birds' dynamics using the base scenario parameters (red line magpies, green line doves, purple line blackbirds, blue line hooded crows and orange line house sparrows).*

- *WNV prevalence in mosquitoes*

Comparing the obtained WNV mosquito prevalence, we can observe slight differences between clusters and years. Moreover, the overall trend results similar for all bird species investigated within clusters and years. Generally, we can observe a low WNV circulation up to July/mid-July, which subsequently increases up to August/mid-August, and then slight decreases and stabilizes. Only in 2016, in the western cluster, the highest prevalence was predicted after August, in October. Despite the similar trend shown by assuming different avian species as the only competent one, we can observe in all clusters and years a significantly higher prevalence for magpies (Fig.5 red lines), followed by blackbirds (Fig.5 blue line), and then doves (Fig.5 green line) and hooded crows (Fig.5 purple line). We can observe the lowest prevalence for house sparrows (Fig.5 orange line).

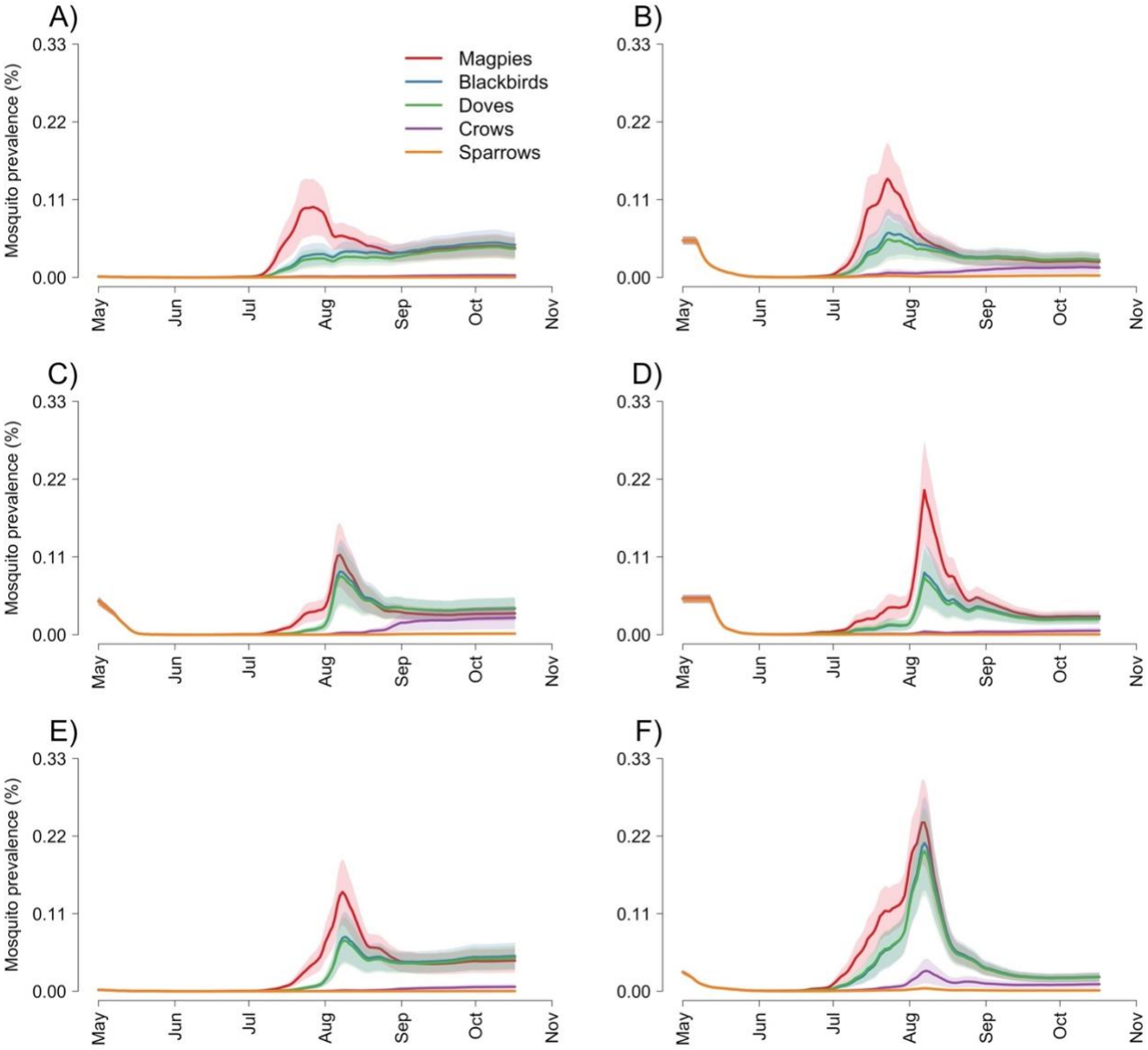

Figure 5: WNV predicted prevalence. WNV prevalence in mosquitoes considering five different competent bird species. Solid lines: average values; shaded regions: 95% confidence interval. Panels: A: mosquito WNV prevalence in western cluster in 2016, B: mosquito WNV prevalence in eastern cluster in 2016, C: mosquito WNV prevalence in western cluster in 2017, D: mosquito WNV prevalence in easter cluster in 2017, E: mosquito WNV prevalence in western cluster in 2018, F: mosquito WNV prevalence in eastern cluster in 2018.

The fixed initial number of birds -scenario

- *Birds dynamics*

In this scenario avian abundance is more homogeneous among different species. An earlier demographic peak is shown by magpies, hooded crows, and blackbirds (Fig.6, red, purple, and blue lines respectively) while house sparrows and doves (Fig.6, orange and green lines respectively) abundances peak later. Magpies, blackbirds, and house sparrow highest abundances are similar, but they are reached at different rates because of the different breeding season lengths.

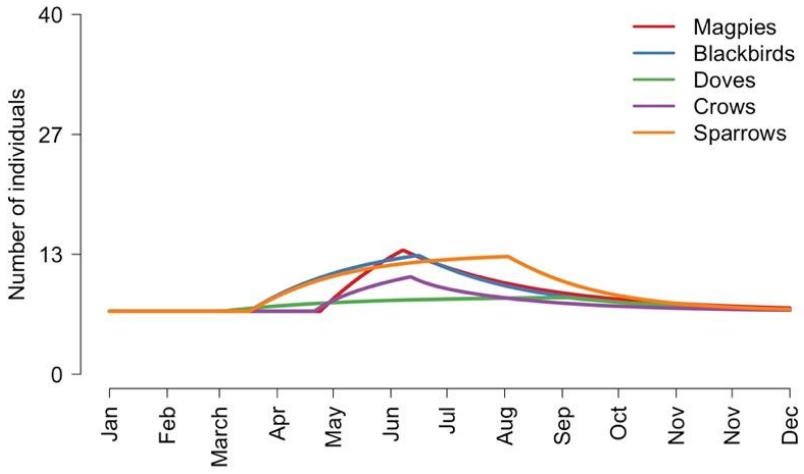

Figure 6: Birds dynamics*. Comparison of birds' dynamics using the fixed initial number of birds scenario parameters (red line magpies, green line doves, purple line blackbirds, blue line hooded crows and orange line house sparrows).*

- *WNV prevalence in mosquitoes*

The predicted trend for WNV prevalence in mosquitoes in clusters and years reflects the one predicted by the base scenario, with a low WNV circulation up to July/mid-July, a mild increase up to August/mid-August followed by a decrease and successive stabilization. Again in 2018 in the eastern cluster the peak is more marked. Avian prevalence is estimated to be quite similar between different species. Considering all clusters and years, the highest prevalence is obtained considering hooded crows and doves (Fig.7 purple and green lines respectively) as the competent species, while an avian population consisting of house sparrows only results in the lowest (Fig.7 orange line). Differences among species are higher between July and September and tend to decrease during the second part of the season.

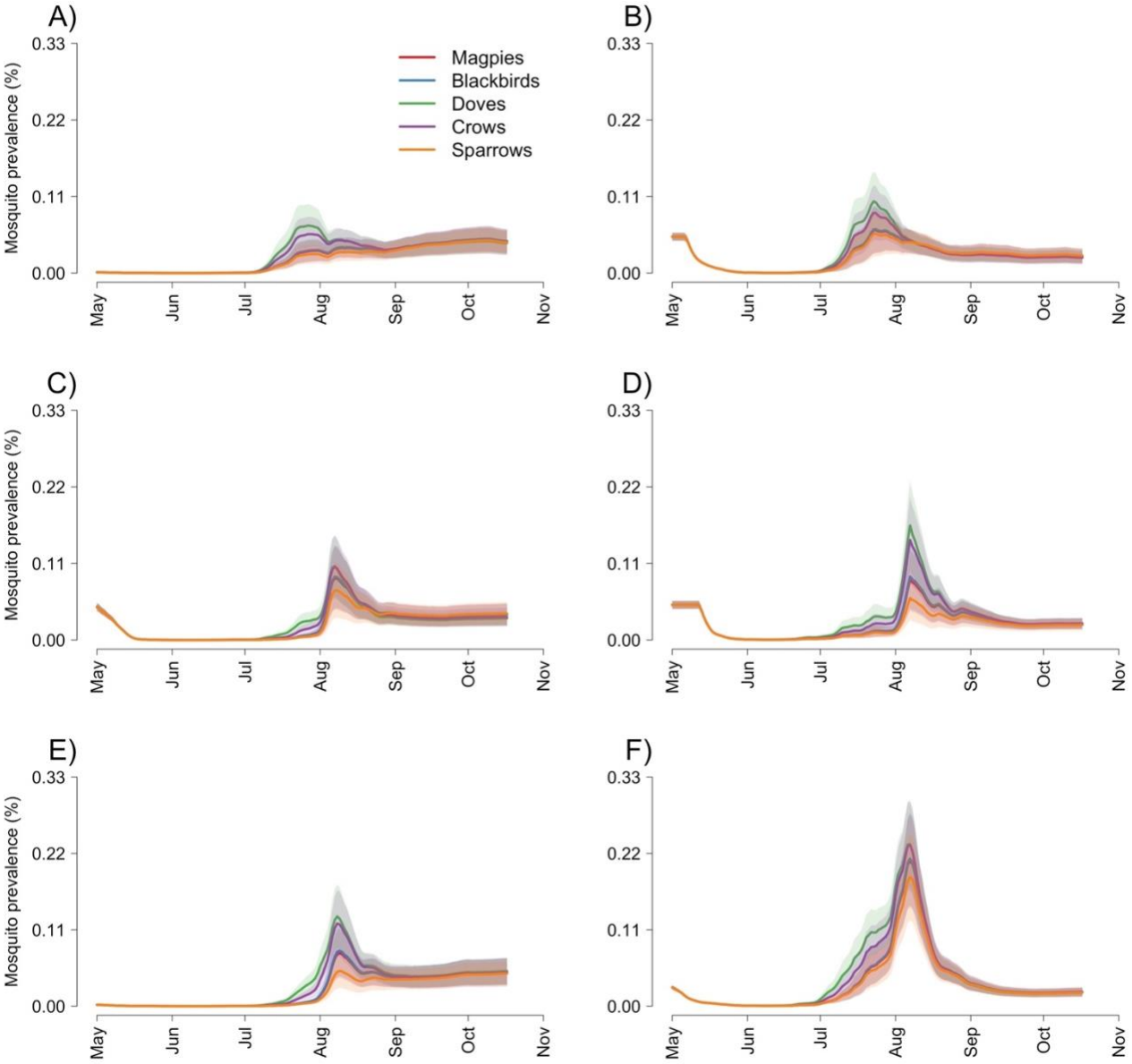

Figure 7: WNV predicted prevalence. WNV prevalence in mosquitoes considering five different competent bird species. Solid lines: average values; shaded regions: 95% confidence interval. Panels: A: mosquito WNV prevalence in western cluster in 2016, B: mosquito WNV prevalence in eastern cluster in 2016, C: mosquito WNV prevalence in western cluster in 2017, D: mosquito WNV prevalence in easter cluster in 2017, E: mosquito WNV prevalence in western cluster in 2018, F: mosquito WNV prevalence in eastern cluster in 2018.

The shift of breeding season scenario

- *Birds' dynamics*

In this scenario, we can observe a shift in the peak of the estimated population abundance depending on the starting month of the breeding season. If the breeding season starts in March (Fig.8, red line), then the peak occurs in April and the estimated number of birds between May and October is the lowest. A breeding season starting in April (Fig.8, blue line) delays the peak to May with a higher number of birds up to July. Analogously a breeding season starting in May causes a peak in June and a breeding season starting in June in July (Fig.8, green and purple line respectively), and their overall abundance is similar.

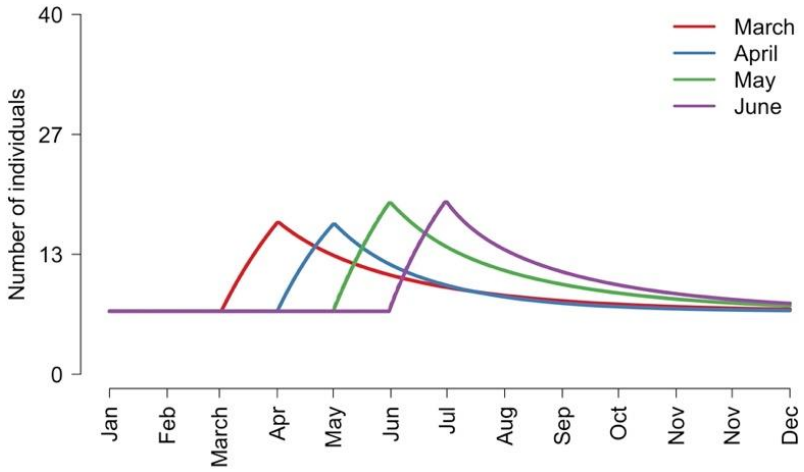

***Figure 8: Birds dynamics**. Comparison of birds' dynamics using in the shift of breeding season scenario, where the red line represents an avian species with breeding season starting in May, blue line in April, green line in May and purple in June.*

- *WNV prevalence in mosquitoes*

The predicted WNV prevalence has a similar trend between clusters and years, but also marked differences depending on the breeding season initial month. A season starting in

March and April (Fig.9 red and blue line respectively) results in the highest peak, and globally the highest prevalence. Conversely the prevalence is markedly lower if simulating a starting breeding season in May or June (Fig.9 green and purple line).

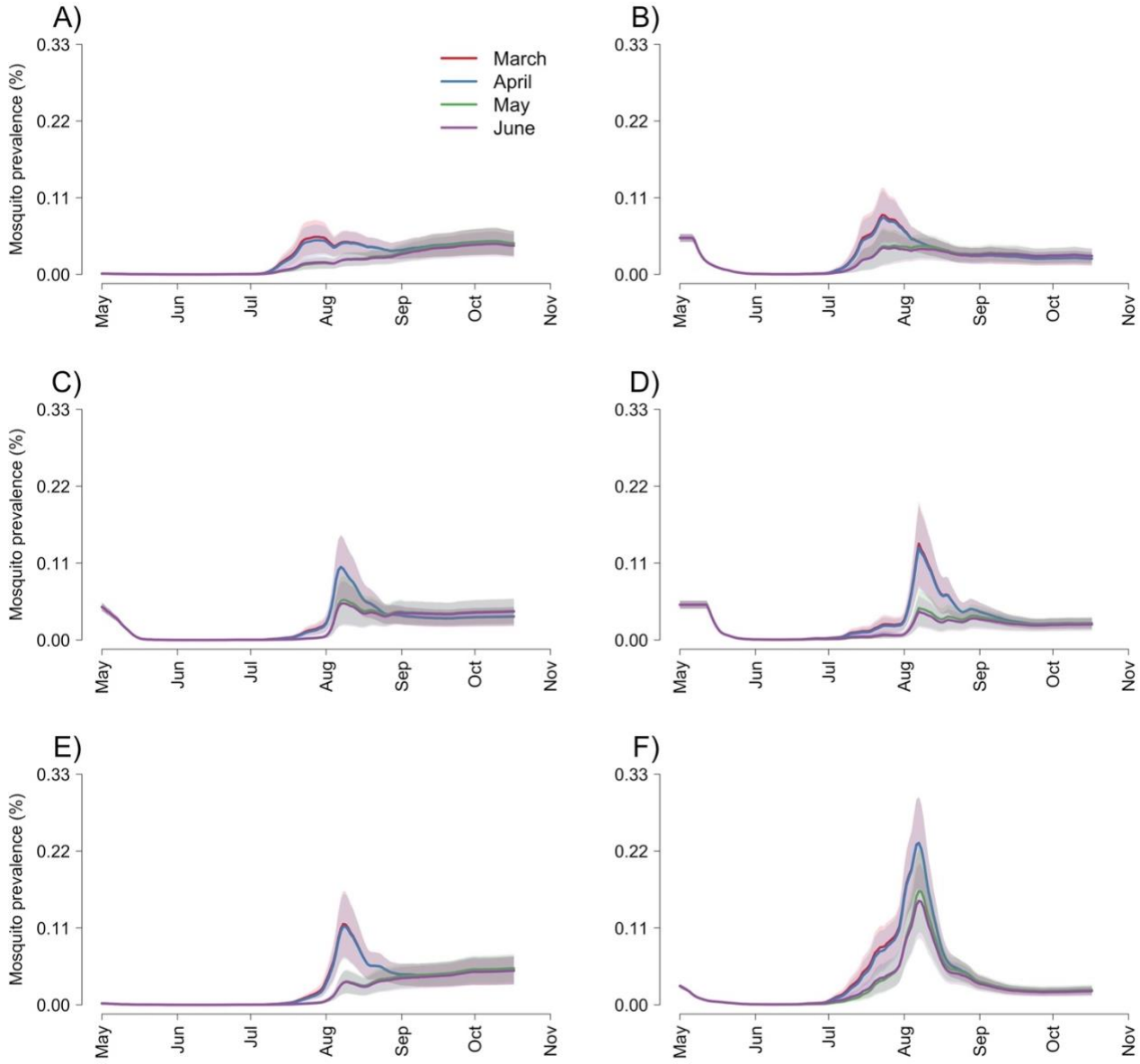

Figure 9: WNV predicted prevalence. WNV prevalence in mosquitoes considering four different breeding seasons for birds (avian breeding season beginning in March, or in April, or in May, or in June). Solid lines: average values; shaded regions: 95% confidence interval. Panels: A: mosquito WNV prevalence in western cluster in 2016, B: mosquito WNV prevalence in eastern cluster in 2016, C: mosquito WNV prevalence in western cluster in 2017, D: mosquito WNV prevalence in easter cluster in 2017, E: mosquito WNV prevalence in western cluster in 2018, F: mosquito WNV prevalence in eastern cluster in 2018.

The different lengths of breeding season scenario

- *Birds' dynamics*

By simulating different lengths for the avian breeding season, we can observe a general slighter variation in the number of birds at peak, but more marked difference in daily bird abundance. In the case of one- and two-months breeding seasons (Fig.10 red and blue lines respectively) the largest abundance is predicted to occur before May, whereas three- and four-months breeding seasons (Fig.10 green and purple lines respectively) make it happen later, in June and July respectively. The longer is the reproductive season the milder is the peak, with an exception for 1- and 2-months lengths, where the second one shows a higher peak. The highest number of birds at peak is estimated when considering a two-months reproductive season, followed by a one month-long one and then the three month-long one. The four-month long season showed the lowest predicted number of birds.

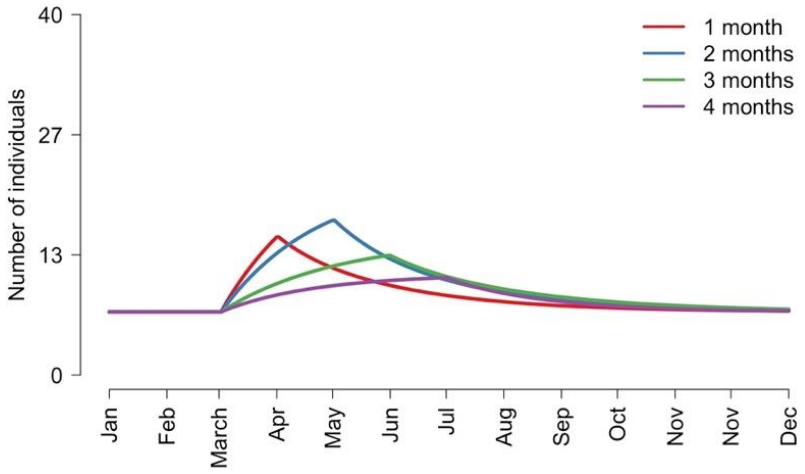

Figure 10: Birds dynamics. Comparison of birds' dynamics using in the different lengths of breeding season scenario, where the red line represents an avian species with one-month, blue line two months, green line three months and purple four months breeding season.

- *WNV prevalence in mosquitoes*

The predicted WNV mosquito prevalence seems to slightly depend on the length of the breeding season, although we can observe similar trends between clusters and years when considering the same length. One and four month-long breeding seasons (Fig.11 red and purple lines respectively) correspond to the highest prevalence in mosquitoes, with more marked peaks. Conversely, two and three month-long breeding seasons (Fig.11 blue and green lines respectively) result in a slightly lower mosquito prevalence.

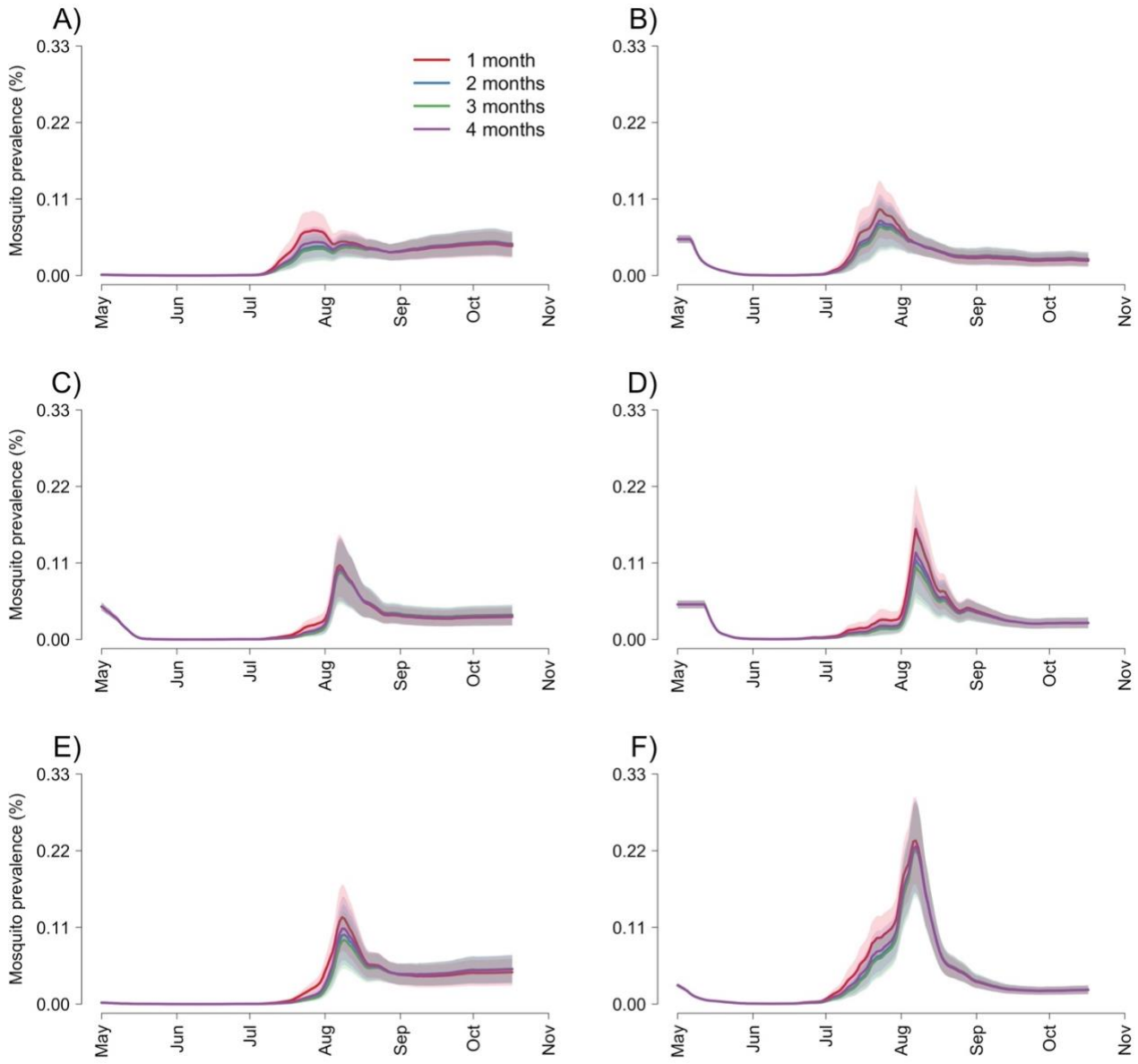

Figure 11: WNV predicted prevalence. WNV prevalence in mosquitoes considering four different lengths of birds' breeding season (one month, two months, three month and four-month breeding seasons). Solid lines: average values; shaded regions: 95% confidence interval. Panels: A: mosquito WNV prevalence in western cluster in 2016, B: mosquito WNV prevalence in eastern cluster in 2016, C: mosquito WNV prevalence in western cluster in 2017, D: mosquito WNV prevalence in easter cluster in 2017, E: mosquito WNV prevalence in western cluster in 2018, F: mosquito WNV prevalence in eastern cluster in 2018.

Discussion

The present analyses show that avian abundance during summer (June-August) widely affects WNV mosquito prevalence. We showed that species-specific bird demographic characteristics influence the magnitude of WNV infection in mosquito, whereas do not affect its dynamics.

The worldwide spread and emergence of WNV, highlights the increasing need of investigating its causes and mechanisms of spread. To protect human beings from infection by mosquitos' bites, understanding and predicting WNV presence and prevalence in the vector population is fundamental to define efficient intervention strategies and reduce human infection risk. But WNV cycle involves different vector and host species, and transmission outcomes might change substantially both temporally and spatially due to the composition of hosts communities [2,9,11,12]. So far, while the influence of vectors on WNV dynamics has been extensively analysed, the contribution of avian hosts presents greater knowledge gaps. Epidemiological characteristics of each avian species are clearly involved in infection spread and can drive the potential of an avian species to be involved in WNV cycle, but also the vector-host ratio plays a role in infection spread (Chapter 2), implying a possible involvement of avian demographic characteristics. For this reason, we here proposed an investigation to quantify how different demographic characteristics of different bird species can drive WNV transmission. This analysis can thus support the development of further studies to identify those avian species more involved in WNV maintenance.

We found that avian abundance during summer (June-August) crucially affects WNV mosquito prevalence. Lower abundance of the competent avian species is associated with a higher predicted WNV prevalence in mosquitoes. Several studies showed that blood meal richness (namely the number of species which mosquitoes have been found to feed on) is significantly different from species richness [18,27], thus implying that mosquito biting rate is driven primarily by an active choice of mosquitoes of where to feed on, and only subsequently by species abundance. Our study stresses out that large host abundances are not correlated with a higher WNV mosquito prevalence. On the contrary, it suggests that, if a species is competent for spreading WNV and is bitten by mosquitoes, the lower the number of individuals belonging to the species, the higher is infection in mosquitoes. This result is certainly driven by model assumptions stating that the rate at which a species is bitten is not dependant on the densities of the species relative to the density of other species. Mosquitoes indeed are shown to actively choose the species where to feed on, according to their feeding preference and not merely on species abundance [27]. On the other hand, is also likely that they revert to feed to other species, if the density of the preferred one is very low. Then, if we assume that mosquito biting rate is fully independent to bird species abundance, we are oversimplifying reality, and this must be considered when analysing results. Despite that, we showed that less individuals can cause a higher viral circulation under model assumptions, thus posing the attention on the actual existing knowledge gaps about the birds' role in WNV spread. Moreover, it highlights the relevance of mosquito feeding preference in the comprehension of the real role of bird species in the maintenance and spread of this infection. Indeed, independently of the initial number of birds, the highest WNV

prevalence in mosquitoes was registered in all scenarios when simulating with the less abundant avian species. In the first two scenarios (the *base-scenario* and the *fixed initial number of birds -scenario*) we can note that house sparrows, that are the most abundant species, produce a very low prevalence in mosquitoes, whereas magpies and doves, that are less abundant, are associated with a higher WNV circulation. As we considered epidemiological parameters fixed for all bird species, we suggest that this difference in infection spread is mainly attributable to the bird population size. Since the basic reproduction number (R_0) of a vector-borne disease is determined both by epidemiological parameters and by the ratio between hosts and vectors (Chapter 2), this result can be interpreted by considering that, a lower number of birds implies a larger vector-host ratio and consequently a higher R_0 value. Consequently, also the avian probability of being infected increases. On the contrary, if the number of birds is high, we can account for a dispersion of mosquito bites on not-infectious birds, with a consequent lower spread of the disease.

The role of abundance and composition of the avian community in WNV spread is still debated. For instance, in the United States, it was shown that host heterogeneity affects WNV transmission [12]. Several studies suggest mosquito biting rate to be driven only partially by avian species abundance, since mosquitoes actively pursue their favourite host species to feed on, regardless of its abundance [18,24,27]. In our model, only one avian species is included, thus not accounting for interaction between different species and neglecting any kind dilution effect on not competent species [19]. Despite that, the choice of including only one avian species can be supported by considering the selective biting of mosquitoes, that feed preferentially on certain bird species thus enhancing the

importance of selected species importance in WNV spread. Thus, our results can contribute to the interpretation of differences in WNV spread among areas and years, possibly linking them with fluctuations of the abundance of the competent and preferred avian species. Moreover, our analyses can help in determining which features characterize a bird species suitable to spread WNV by showing that species abundance could be predictive of its importance in WNV spread. On the other hand, this result further highlights the importance of a careful estimate of the real mosquito biting rate. Indeed, a quantification of the mutual relationship between feeding preferences and birds' abundance can be of aid in driving our understanding of the role of different avian species and in evaluating the reliability of the obtained results.

By further investigating the other two proposed scenarios (the *different lengths of breeding season scenario* and the *shift of breeding season scenario*), we can also observe that the timing with which the differences in abundance occur during season can influence WNV circulation. Indeed, observing the population with breeding season starting in April and the one with breeding season starting in May, the predicted WNV-prevalence in mosquito population is very similar, despite a very different number of birds during early season. We can note that, although the abundances of birds during early season are very different before June, from June on they are very close, thus suggesting a more relevant role of species abundance from June. All results obtained with the four scenarios seem to confirm this assumption, with increasing birds' abundance during summer negatively affecting WNV-prevalence in mosquitoes. This result gives us a further indication on the characteristics needed by an avian species to further amplify

viral circulation. Indeed, according with model results, the less abundant a competent bird species is in summer, the higher is its effect in driving infection spread.

The finding that only avian abundance, and no other breeding species-specific feature, affects WNV spread might suggest the possibility of considering the bird population included in model simulation as a uniform avian community. Despite that, this choice neglects the important role of species-specific epidemiological characteristics of birds, rising the need of deeper investigations on epidemiological parameters to strengthen our knowledge on WNV dynamics (Chapter 2, [8]).

Despite the existing limitations, further model-based investigations can be performed to better highlight the role of different avian population dynamics on WNV spread. As a different role of adult and juvenile birds is suggested in literature [20], a future development of the present work is to include different epidemiological parameters between adults and juveniles (especially of different biting/transmission rates), to investigate if age classes can influence WNV spread.

In conclusion, with the present work we quantified how different avian demographic features affect WNV circulation. We found bird density to crucially drive pathogen transmission while reproductive and breeding characteristics seem to play and indirect role. Also, we here highlighted the need of a deeper understanding of factors driving mosquito feeding preference. Finally, the present model can be updated with different epidemiological parameters, to investigate which species might have a major role in WNV spread.

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Supplementary materials S3

Model Structure

According with the scheme reported in Fig.2 and chapter 1, we simulated WNV spread into Lombardy region through the following system of differential equations:

$$\begin{cases} M'_S(t) = \omega(t) - (b \cdot p \cdot p_{BM} \cdot \dfrac{B_{Ia}(t) + B_{Ij}(t)}{B_T(t)} + \mu_M) \cdot M_s(t) \\ M'_E(t) = b \cdot p \cdot p_{BM} \cdot \dfrac{B_{Ia}(t) + B_{Ij}(t)}{B_T(t)} \cdot M_s(t) - (\theta_M + \mu_M) \cdot M_E(t) \\ M'_I(t) = \theta_M \cdot M_E(t) - \mu_M \cdot M_I(t) \\ B'_{Sa}(t) = -(b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} + \mu_B) \cdot B_{Sa}(t) \\ B'_{Ea}(t) = b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} \cdot B_{Sa}(t) - (\mu_B + \theta_B) \cdot B_{Ea} \\ B'_{Ia}(t) = \theta_B \cdot B_{Ea} - (\mu_B + \sigma_B) \cdot B_{Ia} \\ B'_{Ra}(t) = \sigma_B \cdot B_{Ia} - \mu_B \cdot B_{Ra} \\ B'_{Sj}(t) = \gamma \cdot B_a (b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} + \mu_{Bj}) \cdot B_{Sj}(t) \\ B'_{Ej}(t) = b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} \cdot B_{Sj}(t) - (\mu_{Bj} + \theta_B) \cdot B_{Ej} \\ B'_{Ij}(t) = \theta_B \cdot B_{Ej} - (\mu_{Bj} + \sigma_B) \cdot B_{Ij} \\ B'_{Rj}(t) = \sigma_B \cdot B_{Ij} - \mu_{Bj} \cdot B_{Rj} \end{cases}$$

In the proposed system M_S, M_E and M_I respectively represent the susceptible, exposed and infectious mosquito population, whereas B_{Sa}, B_{Ea}, B_{Ia} and B_{Ra} susceptible, exposed, infectious and recovered competent adult birds and B_{Sj}, B_{Ej}, B_{Ij} and B_{Rj} susceptible, exposed infectious and recovered competent juvenile birds. B_T and B_a instead represent the total and the adult bird populations, respectively. All model parameters are derived from chapter 1.

The system of equations to simulate the dynamics of the avian population instead was assessed according with Fig.3 as follows:

$$\begin{cases} \dfrac{dA}{dt} = -d_A * A \\ \dfrac{dP}{dt} = b * A - (d_P + g_1) * P \\ \dfrac{dJ}{dt} = g_1 * P - g_2 * J - \left(d_J + \dfrac{(g_1 - d_J) * J}{K_J}\right) * S \\ \dfrac{dS}{dt} = g_2 * J - \left(d_J + \dfrac{(g_1 - d_A) * J}{K_J}\right) * S \end{cases}$$

Where A, P J and S respectively represent adult birds, pulli, juvenile birds and subadult birds. The choice of using four different compartment was due to the differences in death rates among the age-classes, whereas the main distinction between adult and subadult birds was that latter were not considered sexually mature and thus did not participate to egg deposition. We considered an intraspecific competition, included in the model as the carrying capacity K_J, acting by limiting the number of juvenile and subadult birds only. According with the simulated scenarios, the full list of parameters used in simulations is reported in tables 1 to 4.

Table A. Demographic parameter estimates found in literature.

Parameters	Blackbirds	Magpies	Collared doves	House sparrows	Hooded crows
Breeding season [a]	75-165 [1,2]	113-157 [8]	61-245 [12,13]	75-213 [18]	110-161 [20]
Clutch size	2-4 [1]	6-7 [9]	1-2 12	2-8 [17,18]	2-9 [20,21,22]
Number of clutches (year^{-1})	2-3 [3,4]	1 *	3.8 12	2-3 [18]	1 *
Average life expectancy (years)	2-4 [5]	2-4 [10]	4 *	6 *	4 *
Hatching eggs (%)	92-95 [6]	57 [9]	59-60 [12,13]	75.5 [18]	69-81 [22,23]
Fledging period (days)	13-18 [1,3]	18 *	16-17 [14]	14-18 [19]	14 *
Stealing period (days)	14 [6]	26-30 [11]	17-22 [12,13,15]	27 [18]	18 [20]
Nestlings' mortality rate (%)	31-32 [1,3]	23 [9]	60 [12,13]	21 [18]	68.3 [12]
Juvenile's mortality rate (%)	70	53[9]	35[12,13]	70 *	70[24]

[a] beginning and end of the breeding season in Julian days
* no reference found in literature

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CHAPTER 4

Are we doing our best? Modeling control strategies against West Nile virus for quantitative assessment of efficacy

Introduction

Vector-borne diseases account for more than 17% of all infectious diseases and cause more than 700 000 deaths worldwide every year, thus being a current and actual burden for human being [1,2]. Mosquitoes are among the most known vectors, able to transmit different impacting infections like malaria, chikungunya, dengue, Zika and West Nile disease [3,4]. Due to climate and environmental conditions, some countries result more affected by mosquito-borne diseases, but the actual climatic and land use changes can enhance capability of several mosquito species to adapt and spread to new areas, making mosquito-borne disease a worldwide health issue [5–9]. Due to their impact on public health, to prevent vector-borne disease transmission to people, and to respond to current outbreaks, vector control has a central role in reducing human infection risk [2].

Among vector borne-diseases, West Nile disease (WND) is one of the most widespread, with outbreaks in Europe, Asia, Africa and also in the North of America [10–12]. It is maintained in an enzootic cycle involving mosquitoes of the genus *Culex* as vectors, and birds as vertebrate competent hosts species [13–15]. Also, humans and other mammals can be infected, mainly via mosquito bite [16]. Although severe symptoms are rare in human beings (only the 20% of infected people develop symptoms, of which only the 1% can be considered severe) [17,18], the increasing spread of the disease and the increasing number of human cases registered, makes the development of efficient surveillance plans and intervention strategies of primary importance [19,20]. Thus, the importance of understanding year-long fluctuations in WNV spread and the identification of the most efficient intervention plan to reduce human infection risk are now considered public

health central issues [19,21], especially at light of the unexpected increased number of human infections that have been observed in Italy in 2018 [22]. Despite that, the understanding of WNV cycle has proven to be very complicated to investigate, thus possibly hampering the assessment of intervention strategies that effectively reduce human infection risk.

Several mathematical models investigating infection dynamics have been developed to try to fill our knowledge gaps about WND [20,23–25]. In particular, in Veneto and Emilia Romagna regions (northern Italy), data coming from the entomological surveillance plan for arboviruses have been successfully used to investigate through mathematical modelling WNV spread and mechanisms of maintenance in both regions [22,26]. One of the advantages of investigating WND through mathematical models is that they can explore the dynamics of the system. Moreover, through their use we can simulate the potential outcome of different intervention strategies before applying them, with a small consumption of time and resources [20,27]. Despite their potential, their actual use in planning and developing future field studies and intervention strategies is still limited. For this reason, we here propose an analysis of the theoretical effect of different intervention strategies on human infection risk, to highlight their strengths and weaknesses at light of our interest to reduce the number of human infections. With the present work we aim to evidence the power of mathematical modelling in aiding public health management choices, but also to evaluate quantitatively the efficacy of the intervention strategies currently considered suitable. The present work is focused on the analysis of WNV spread in the Italian scenario, but the framework is easily applicable to other countries and vector-borne diseases.

Materials and Methods

The base model

A deterministic model based on a system of 13 equations was used to simulate the dynamics of WNV infection in a competent avian population and estimate mosquito prevalence throughout the summer season. The computational framework, summarized in Fig 1, follows the one proposed in (Marini 2020) for Emilia Romagna region, successively modified to simulate WNV dynamics in the study area (Chapter 2). The framework is composed by a first model ('entomological model') simulating mosquito abundance in the investigated area which is than included in a second model ('epidemiological model'), simulating WNV transmission between mosquitoes and birds. Further details on model scheme and equations are reported in the Supplementary materials S4. Following the clustering proposed in Chapter 2 Lombardy region was divided into three separate clusters (a northern, a western and an eastern one) including districts epidemiologically homogeneous for temperature, precipitations, mosquito abundance and their positivity for WNV. The northern cluster in following analyses is excluded because no circulation of WNV was found in it during the triennium 2016-2018. All parameters estimate follows the work proposed in Chapter 2, whereas the unknown ones for both the entomological and the epidemiological model are estimated by a Markov Chain Monte Carlo, respectively fitting the number of collected mosquitoes and WNV-positive mosquitoes. All data records about mosquito abundance and their positivity for WNV comes from the WNV entomological surveillance plan performed by Regione Lombardia and Istituto Zooprofilattico Sperimentale della Lombardia e

dell'Emilia Romagna, following the collection method and guidelines provided by Regione Lombardia and Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna [28]. In the entomological model the number of mosquitoes at the beginning of the mosquito activity period was considered to be the number of mosquitoes ending their diapause after overwintering and was estimated via Markov Chain Monte Carlo as a free parameter by the entomological model [22]. This choice was made because in temperate regions inseminated adult females of *Cx. pipiens* can survive the winter undergoing a quiescent phase termed diapause. WND spread was simulated from April to October, as it is the period of high activity for mosquitoes in the study area, thus we will refer to it as the *mosquito activity period*. Model scheme and equations are reported in Supplementary materials S4.

Inclusion of intervention strategies

The base model was then extended to include the following six different intervention strategies (Fig 1):

i. Use of adulticide targeting overwintering adult mosquitoes (*eliminating overwintering mosquitoes)*

ii. Use of larvicide during mosquito activity period to eliminate mosquito larvae and eggs (*larvicide treatment)*

iii. Use of adulticide applied to eliminate adult mosquitoes during *mosquito activity period: (adulticide treatment)*

iv. Reduction of breeding sites for the mosquito population (*mosquito breeding site reduction)*

v. Active removal of competent birds (*birds removal)*

vi. Reduction of the breeding sites for birds (*bird breeding site reduction)*

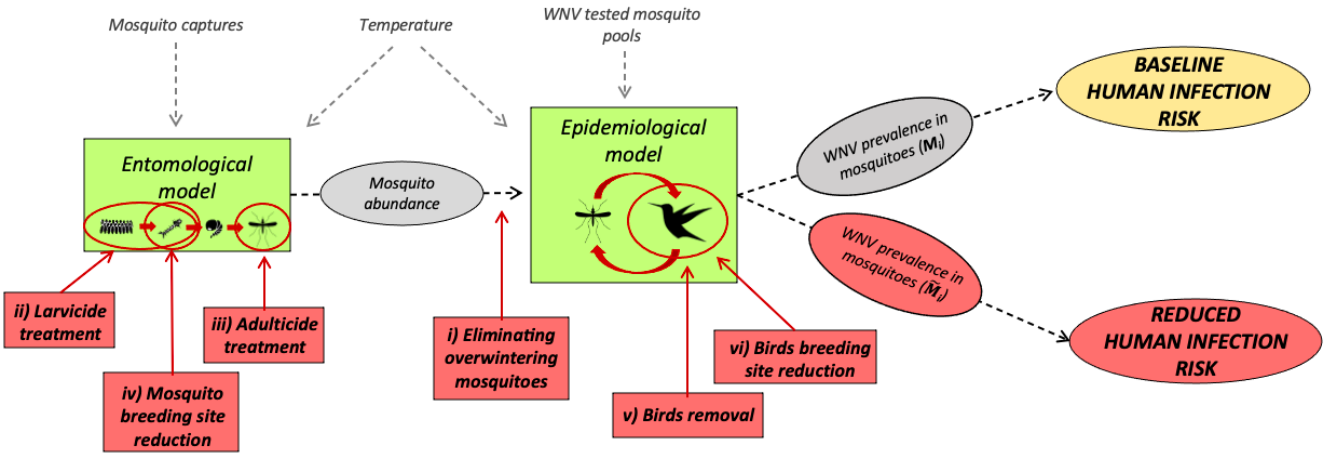

Figure 1: Schematic representation of computational framework and intervention strategies inclusion*. Mathematical models (green boxes) take as input temperature and entomological surveillance records (grey dashed arrows) to predict mosquito abundance or WNV prevalence in mosquitoes in the study area (grey ovals). WNV prevalence in mosquitoes was then used to estimate the human infection risk (orange oval). The scheme also includes the six different intervention strategies (red boxes). The target population of each of the six intervention strategies was highlighted with a red circle and the output of the modified model was shown as red ovals.*

A yearly simulation for each intervention strategy were performed over the three years (2016, 2017 and 2018) and over both region's clusters, then a mean relative daily risk of infection for human beings was estimated using the following formula:

$$RR = \frac{M_i}{\tilde{M}_i}$$

Where M_i represents the number of infected mosquitoes estimated by the "base model" and $\tilde{M}_i$ represents the number of infected mosquitoes estimated by the model including the intervention strategy. The efficiency of the intervention strategy was then considered as the effective reduction of the human infection relative risk. It was thus calculated as *Eff=1-RR* with *Eff* ranging between -100 and 100, implicating that an intervention strategy

that reduce the human infection risk of 100% has *Eff=100*, whereas an intervention strategy with no effect has *Eff=0*. Any intervention strategy that increases the human infection risk instead has *Eff* ranging between -100 and 0.

The system of differential equations used for intervention strategies is fully reported in Supplementary materials S4.

i. Eliminating overwintering mosquitoes

The reduction of the number of mosquitoes which survive the winter was simulated only by reducing the number of mosquitoes starting the yearly simulation, representing the number of mosquitoes just before the beginning of the mosquito activity period.

ii. Larvicide treatment

To simulate a larvicidal treatment, we included in the 'entomological model' an additional death rate for eggs and larvae. We considered this intervention strategy to have a daily constant effect during the whole mosquito activity period by assuming the effect of one treatment lasting up to the following treatment, without any efficacy loss.

iii. Adulticide treatment

To simulate an insecticide treatment, we included in the 'entomological model' an additional death rate for adult mosquitoes. We considered this intervention strategy to be performed once every two weeks from the beginning to the end of the mosquito activity period, immediately killing a given fraction of the adult population.

iv. Mosquito breeding sites reduction

To simulate the reduction of mosquito breeding sites, we reduced the density-dependent scaling factor driving the carrying capacity for the larval stages of the mosquito

population in the 'entomological model', thus obtaining new mosquito abundances to include in the epidemiological model to simulate WNV spread. This intervention strategy was considered to be implemented continuously during the mosquito activity period.

v. Birds removal

To simulate the active removal of competent birds, we included in the epidemiological model an additional death rate for both adult and young birds. We considered this intervention strategy to be performed once every month from the beginning to the end of mosquito activity period, immediately killing a given fraction of the adult population.

vi. Avian breeding sites reduction

To simulate the reduction of birds' breeding sites, we reduced the environmental carrying capacity of the bird population in the epidemiological model. This intervention strategy was considered to be performed constantly during the mosquito activity period.

We considered three different intensities for each intervention strategy (20, 50 and 80%). For instance, an intensity of 80% for strategy *iii* means that 80% of adult mosquitoes are immediately removed when the intervention is carried out. As mentioned above, the efficiency of the intervention strategy was then considered as the effective reduction of the human infection relative risk.

Results

i. Eliminating overwintering mosquitoes (Fig 2, top left box)

A reduction of the number of mosquitoes which survive winter affects the human infection risk mainly in the first part of the season (up to August), then the effect seems to wane, even potentially increasing human infection risk. To obtain a reduction of the risk of infection higher than 50%, it is necessary to remove more than 50% of overwintering mosquitoes. Despite that, regardless of intervention intensity, the effect lasts up to August and then decrease. Moreover, the higher is the benefit obtained before September, the higher and faster the effect worsens in the following part of the season.

ii. Larvicide treatment (Fig 2, top right box)

Using a larvicide to decrease the number of eggs and larvae efficiently reduces human infection risk, especially between mid-June and mid-August. The effect is almost negligible up to July, but then it quickly increases and maintains its efficacy up to October. A reduction of 20% of eggs and larvae is enough to obtain a substantial risk reduction (up to 52%), but by eliminating 50% of immature stages the efficacy increases up to 82%. The elimination of 80% of immature stages can reduce the risk up to 91%, pointing out that the increase in intensity of the intervention is not proportional to an increase in efficacy. From August on, for all tested treatments, we can observe a reduction of their efficacy. This reduction is particularly marked for the 50% treatment, showing the lowest efficacy from September on.

iii. Adulticide treatment (Fig 2, centre left box)

An adulticide treatment efficiently reduces human infection risk. The risk reduction starts in June and ends up in September. The highest effect is reached between mid-June and mid-August and a peak in late July. A reduction of 20% of adult mosquitoes is enough to obtain a substantial risk reduction (up to 36%), but the elimination of 50% of mosquitoes has even larger effect (up to 64%). The increase in intensity of the intervention is not proportional to an increase in efficacy.

iv. Mosquito breeding site reduction (Fig 2, centre right box)

According to model simulations, the reduction of mosquitoes breeding sites is the strategy with the highest efficacy all over the season. The reduction of 80% of breeding sites almost nullifies human infection risk (with a risk reduction up to 99%), but also a 20% intensity show marked results (with a risk reduction up to 55%). The elimination of 50% of breeding sites has an intermediate effect with respect to the other two teste intensities, with a risk decrease up to 91%.

v. Birds' removal (Fig 2, bottom left box)

The active removal of competent birds does not seem to have a noticeable effect on human infection risk up to June, whereas from August on such strategy increases human infection risk up to the end of October.

vi. bird breeding site reduction (Fig 2, bottom right box)

Similarly, to strategy *v*, active removal of competent birds breeding sites do not show any substantial effect in reducing human infection risk up to June. Afterwards it seems to increase human infection risk, with a peak in August.

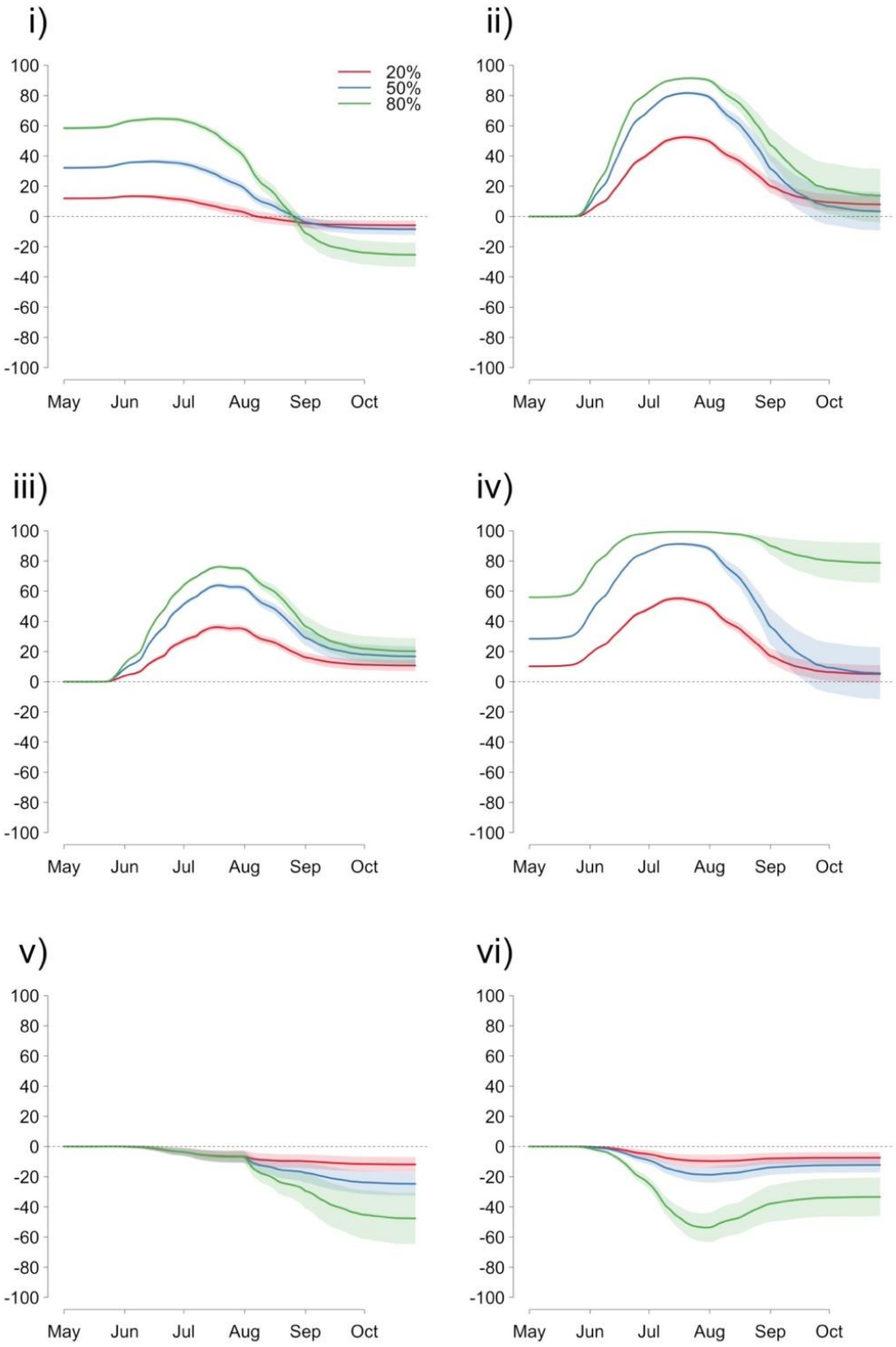

Figure 2: Intervention strategies effect on human infection risk. *Boxes from up-left to bottom-right shows the risk reduction following the application of intervention i,ii,iii,iv,v, and vi. Red, blue and green lines respectively represent an intervention intensity of 20,50 and 80%. Solid lines: average values; shaded regions: 95% confidence interval.*

Discussion

To provide a practical tool to investigate and quantitatively compare the efficacy of different intervention strategies, we adapted an existing and validated mathematical model [22], to simulate WNV spread in Lombardy region aimed at evaluating human infection risk. Six theoretical intervention strategies were then included in the model to estimate their effect on the predicted human infection risk. In accordance with previous findings evidencing vector control as a key method by which vector-borne diseases can be controlled [29–33], our findings show that interventions on the vector population have the best and more durable effects in lowering human infection risk. All interventions on the avian population instead show a moderate, if not opposite effect. In particular, the active removal of mosquito eggs and larvae and the reduction of breeding sites are the two most effective interventions, followed by the elimination of adult mosquitoes. None of the simulated intervention strategies shows a constant effect during all mosquito activity period, and all of them show a reduction in efficacy in late summer. This result highlights the importance of carrying treatments targeting the vector population at regular intervals during summer, or even to increase the effort from August on. Results obtained are in line with the current intervention choices performed in several areas, as well as in Lombardy region, primarily aimed to the elimination of larval populations and only occasionally and exceptionally directed against adult mosquitoes [34–36]. In addition, we showed that the reduction of human infection risk depends on the intensity of the intervention, but the obtained benefit (i.e., risk reduction) does not increase linearly with the effort applied. This highlights the importance of a quantitative evaluation

of the efficacy to set the most suitable intervention strategy even by a practical point of view, allowing for a careful cost-benefit evaluation [27]. The importance of a thorough evaluation of the effect of an intervention is double in developing a sustainable strategy. On the one hand, at light of pesticide resistance and environmental side effects of an over-abundant use of insecticides/pesticides [37–39], it is fundamental to be sure to minimize the effort maximising the result [34].

Considering the worldwide threat vector-borne diseases represent for human beings, they are now considered one of the main issues for worldwide public health [2]. But, due to unpredictability of disease spread and nonlinearity of interactions among population subgroups, the assessment of the most suitable intervention strategy to reduce human infection risk and the prediction of the whole range of interventions effects can be complex. WNV in particular (re-) emerged in several countries worldwide, Italy included. Its increasing spread and the complexity of its cycle raised up the need of carrying out an intensive surveillance on vectors and hosts populations. Moreover, in accordance with regional and national guidelines, several interventions are performed to reduce the vector population and decrease human infection risk [36]. Despite the ongoing huge effort on-course, and the recognized importance of developing an efficient intervention plan, the complexity of WNV impairs our assessment of efficient intervention plans.

By confirming the importance of vector control in reducing human infection risk for vector-borne diseases, we found the most efficient intervention strategies to be the reduction of mosquito breeding sites and a constant elimination of eggs and larvae during the mosquito activity period (April-October), followed by the elimination of adult mosquitoes. Conversely, simulated interventions against the avian population show to

have the poorest results, actually the potential to increase human risk. It is necessary to note that, in the present model, we only explicitly accounted for one competent avian species for the sake of simplicity and the mosquito biting rate is considered as a fixed fraction of bites directed to the competent avian species, thus possibly underestimating the effect in mosquito biting frequency following a change in the ratio among competent and not-competent hosts. On the other hand, it highlights the need of having a better estimate of the real mosquito biting rates to more realistically simulate WNV spread. If a better understanding of the relationship between mosquito bites frequency and bird abundance is known to play a role in the understanding of species involved in WNV maintenance (Chapter 1, Chapter 3, [40,41]), we here suggest that deeper studies are also necessary to evaluate the efficacy of interventions on the avian population. Indeed, model results show a negative effect of an active removal of birds of specific species on human infection risk, but such findings can be considered reliable only if avian abundance do not affect the frequency of mosquitoes' bites on the competent species [41].

Elimination of overwintering mosquitoes can decrease human infection risk only in the first part of the season. Interestingly, the higher is the benefit obtained in the first part of the season the lower is the risk reduction in the late season. Since human spill-over occurs usually during August/September [42], elimination of overwintering mosquitoes, at least if not supported by any other intervention during the *mosquito activity period*, might not be effective to reduce WNV spread.

We remark that in the present work we compare interventions having a different frequency and duration. This choice was made to try to include plausible interventions but can affect model results. Analyses considering different interventions to have the

same period of efficacy could then be useful for a deeper comparison between intervention efficacies. Deeper investigations on the real efficiency of currently applied intervention strategies are required to improve the precision in predicting the effect of the simulated intervention strategy, and, despite being beyond the scope of this work, the investigation of the effect of combinations of intervention strategies is fundamental for the development of an adequate intervention plan.

Finally, our results highlight that none of the considered strategies can eradicate WNV from Lombardy region. Eradicating an infection can be very complex and demanding. In particular WNV, involving in its cycle both vectors and wild animals, which might be difficult to survey and treat, is a big challenge for public institutions. Despite previous studies hypothesizing the feasibility of WNV eradication [20], our study suggests that to approach such goal, an intervention intensity lower than 80% could not be enough, thus implying a very high expense of resources. Moreover, since WNV might easily spread in new areas, after the eradication, the treatments should be carried out constantly in order to keep the area unsuited for WN re-emergence. Since we did not consider higher intensities, nor combinations of different interventions, and as the eradication of WNV is a very ambitious and coveted target, further investigations are certainly needed to evaluate the actual necessary effort.

In conclusion, our results suggest that among available intervention strategies the reduction of mosquito breeding sites and larvicide treatments are those showing the best efficacy. Despite that, a careful assessment of intervention strategy to be performed is fundamental to maximise its efficacy in reducing human infection risk. Indeed, the effect of interventions depends on their intensity, but the obtained reduction of human

infection risk does not increase linearly with the effort applied. Moreover, efficacies of interventions are not constant in time, with a best effect in July-August and a decrease in efficacy in September-October. For this reason, the outlining of an efficient intervention plan to reduce WNV human infection risk must take into careful consideration both the effort needed and the objective desired.

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Supplementary materials S4

Entomological-model Structure

We estimated the daily mosquito abundance following the differential reported in System 1, represented by the chart shown in Fig. A:

System 1:

$$\begin{cases} E'(t) = n_E \cdot d_A \cdot A(t) - (\mu_E + \tau_E) \cdot E(t) \\ L'(t) = \tau_E \cdot E(t) - (\tau_L + \mu_L \cdot (1 + \frac{L(t)}{K})) \cdot L(t) \\ P'(t) = \tau_L \cdot L(t) - (\tau_P + \mu_P) \cdot P(t) \\ A'(t) = (1 - \delta) \cdot 0.5 \cdot P(t) - \mu_A \cdot A(t) \end{cases}$$

In the proposed system E, L, P and A respectively represent eggs, larvae, pupae and adult non-diapausing female mosquitoes. Death rates (μ_E, μ_L, μ_P and μ_A) and developmental rates (τ_E, τ_L, and τ_P) specific of each age-class are all considered temperature dependent, in accordance with the work proposed by Marini (ref).

Here n_E represents the number of eggs laid in one oviposition, whereas δ represents the fraction of mosquitoes that undergo the diapause. Two different density dependant scaling factor driving the carrying capacity for the larval stage (K) were included, one for the early part of the season (up to June 30) and a different one for the late part of the season (from June 30) due to a possible change in *Cx. pipiens* breeding sites availability due to competition for resources with *Ae. Albopictus* (ref 25 Giovanni 2020). Considering only adult female mosquitoes, the term 0.5 in the equation for adults accounts for the

sex ratio. Since traps capture host seeking mosquitoes, only a fraction d_A of adult mosquitoes is considered to lay eggs.

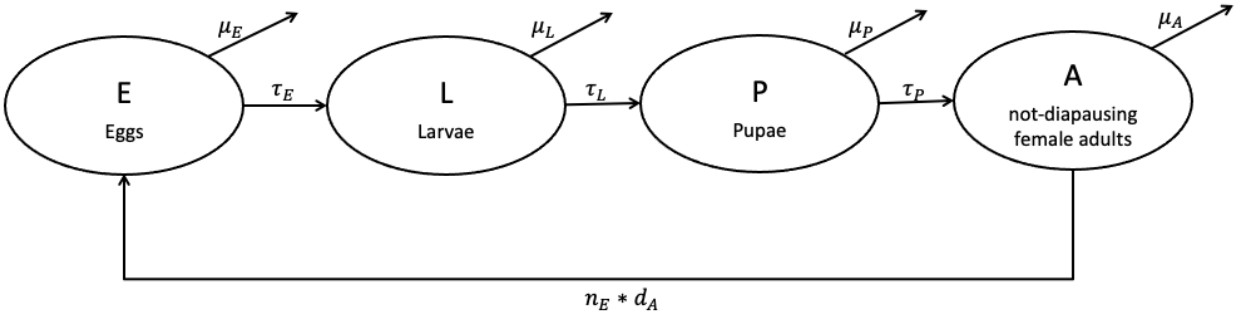

Figure A: Entomological-model scheme. Model flow chart for mosquito dynamics. Compartments (circles) represent four developmental stages of mosquitoes: eggs (E), larvae (L), pupae (P) and not-diapausing female adults (A).

Epidemiological-model Structure

According with the scheme reported in Fig.B we simulated WNV spread into Lombardy region through the following system of differential equations:

System 2:

$$\begin{cases} M'_S(t) = \omega(t) - (b \cdot p \cdot p_{BM} \cdot \dfrac{B_{Ia}(t) + B_{Ij}(t)}{B_T(t)} + \mu_M) \cdot M_s(t) \\ M'_E(t) = b \cdot p \cdot p_{BM} \cdot \dfrac{B_{Ia}(t) + B_{Ij}(t)}{B_T(t)} \cdot M_s(t) - (\theta_M + \mu_M) \cdot M_E(t) \\ M'_I(t) = \theta_M \cdot M_E(t) - \mu_M \cdot M_I(t) \\ B'_{Sa}(t) = -(b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} + \mu_B) \cdot B_{Sa}(t) \\ B'_{Ea}(t) = b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} \cdot B_{Sa}(t) - (\mu_B + \theta_B) \cdot B_{Ea}(t) \\ B'_{Ia}(t) = \theta_B \cdot B_{Ea}(t) - (\mu_B + \sigma_B) \cdot B_{Ia}(t) \\ B'_{Ra}(t) = \sigma_B \cdot B_{Ia}(t) - \mu_B \cdot B_{Ra}(t) \\ B'_{Sj}(t) = (\gamma - r \cdot \dfrac{B_j(t)}{K}) \cdot B_a(t) - (b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} + (\mu_{Bj} + r \cdot \dfrac{B_j(t)}{K})) \cdot B_{Sj}(t) \\ B'_{Ej}(t) = b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} \cdot B_{Sj}(t) - ((\mu_{Bj} + r \cdot \dfrac{B_j(t)}{K}) + \theta_B) \cdot B_{Ej}(t) \\ B'_{Ij}(t) = \theta_B \cdot B_{Ej}(t) - ((\mu_{Bj} + r \cdot \dfrac{B_j(t)}{K}) + \sigma_B) \cdot B_{Ij}(t) \\ B'_{Rj}(t) = \sigma_B \cdot B_{Ij} - (\mu_{Bj} + r \cdot \dfrac{B_j}{K}) \cdot B_{Rj} \end{cases}$$

In the proposed system M_S, M_E and M_I respectively represent the susceptible, exposed and infectious mosquito population, whereas B_{Sa}, B_{Ea}, B_{Ia} and B_{Ra} susceptible, exposed, infectious and recovered competent adult birds and B_{Sj}, B_{Ej}, B_{Ij} and B_{Rj} susceptible, exposed infectious and recovered competent juvenile birds. B_T represents the total competent avian community, and B_a the adult, and thus sexually mature, avian community.

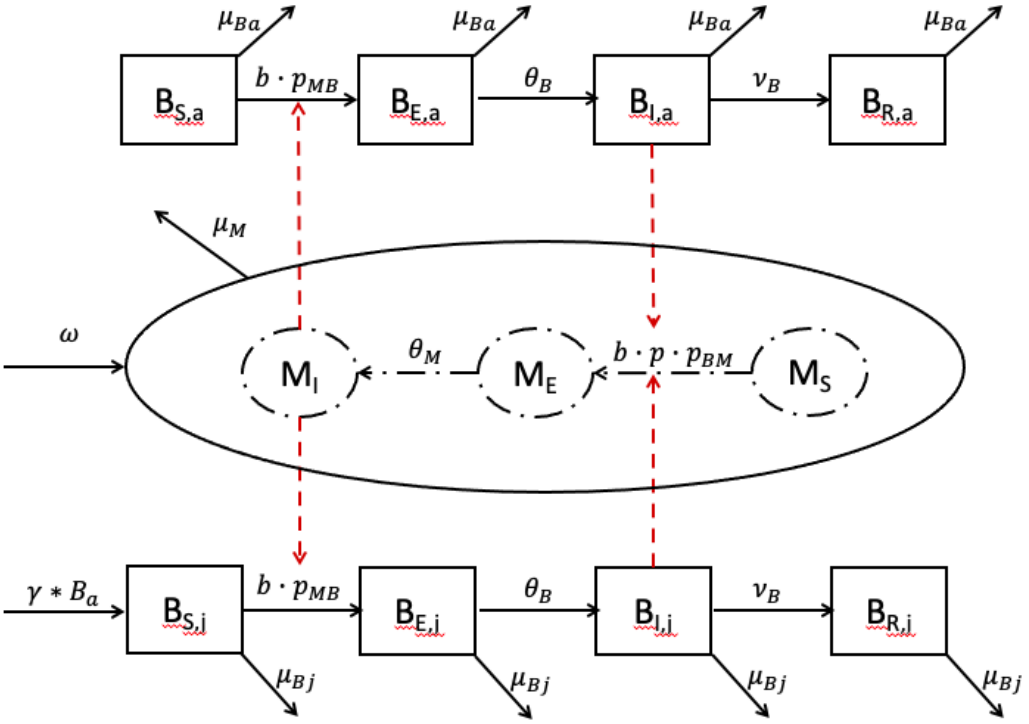

Figure B: Model scheme. Model flow chart for WNV transmission in birds (squares) and mosquitoes (circles) in an average trapped area. Compartments: $\boldsymbol{B_{S,a}}$, $\boldsymbol{B_{E,a}}$, $\boldsymbol{B_{I,a}}$ *and* ($\boldsymbol{B_{S,j}}$, $\boldsymbol{B_{E,j}}$, $\boldsymbol{B_{I,j}}$ *and* $\boldsymbol{B_{R,j}}$)*: adult (juvenile) susceptible, exposed, infectious and immune birds; Ms, Me, Mi: susceptible, exposed and infectious mosquitoes.*

Intervention strategies inclusion:

Intervention strategies on the vector population were included in model equations by adjusting *System 1* as follows:

System 3:

$$\begin{cases} E'(t) = n_E \cdot d_A \cdot A(t) - (\mu_E + \tau_E) \cdot E(t) - t_2 \cdot E\ (t) \\ L'(t) = \tau_E \cdot E(t) - \left(\tau_L + \mu_L \cdot \left(1 + \frac{L}{K \cdot t_4}\right)\right) \cdot L(t) - t_2 \cdot L(t) \\ P' = \tau_L \cdot L(t) - (\tau_P + \mu_P) \cdot P(t) \\ A'(t) = (1 - \delta) \cdot 0.5 \cdot L(t) - \mu_A \cdot A(t) - t_3 \cdot A(t) \end{cases}$$

Where t_2, t_3 and t_4, respectively represent the intensity of the larvicide treatment, the adulticide treatment and of the reduction of the mosquito breeding sites (strategies *ii-iv*).

Intervention strategies on the host population were included in model equations by adjusting *System 2* as follows:

System 4:

$$
\begin{cases}
M'_S(t) = \omega(t) - (b \cdot p \cdot p_{BM} \cdot \dfrac{B_{Ia}(t) + B_{Ij}(t)}{B_T(t)} + \mu_M) \cdot M_s(t) \\
M'_E(t) = b \cdot p \cdot p_{BM} \cdot \dfrac{B_{Ia}(t) + B_{Ij}(t)}{B_T(t)} \cdot M_s(t) - (\theta_M + \mu_M) \cdot M_E(t) \\
M'_I(t) = \theta_M \cdot M_E(t) - \mu_M \cdot M_I(t) \\
B'_{Sa}(t) = -\left(b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} + \mu_B\right) \cdot B_{Sa}(t) - i_5 \cdot B_{Sa}(t) \\
B'_{Ea}(t) = b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} \cdot B_{Sa}(t) - (\mu_B + \theta_B) \cdot B_{Ea}(t) - i_5 B_{Ea}(t) \\
B'_{Ia}(t) = \theta_B \cdot B_{Ea}(t) - (\mu_B + \sigma_B) \cdot B_{Ia}(t) - i_5 \cdot B_{Ia}(t) \\
B'_{Ra}(t) = \sigma_B \cdot B_{Ia}(t) - \mu_B \cdot B_{Ra}(t) - i_5 \cdot B_{Ra}(t) \\
B'_{Sj}(t) = \left(\gamma - r \cdot \dfrac{B_j}{K \cdot i_6}\right) \cdot B_a(t) - (b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} + (\mu_{Bj} + r \cdot \dfrac{B_j(t)}{K \cdot i_6})) \cdot B_{Sj}(t) - i_5 \cdot B_{Sj}(t) \\
B'_{Ej}(t) = b \cdot p_{MB} \cdot \dfrac{M_I(t)}{B_T(t)} \cdot B_{Sj}(t) - ((\mu_{Bj} + r \cdot \dfrac{B_j(t)}{K \cdot i_6}) + \theta_B) \cdot B_{Ej}(t) - i_5 \cdot B_{Ej}(t) \\
B'_{Ij}(t) = \theta_B \cdot B_{Ej}(t) - ((\mu_{Bj} + r \cdot \dfrac{B_j(t)}{K \cdot i_6}) + \sigma_B) \cdot B_{Ij}(t) - i_5 \cdot B_{Ij}(t) \\
B'_{Rj}(t) = \sigma_B \cdot B_{Ij}(t) - (\mu_{Bj} + r \cdot \dfrac{B_j(t)}{K \cdot i_6}) \cdot B_{Rj}(t) - i_5 \cdot B_{Rj}(t)
\end{cases}
$$

CHAPTER 5

How to choose the best control strategy? Mathematical models as a tool for pre-intervention evaluation on a macroparasitic disease

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Introduction

During the last century, the total number of infectious diseases has decreased globally, but an opposite trend has been observed for emerging and re-emerging diseases, which have increased in number and currently threaten human health [1–3]. Several diseases, like MERS, SARS, chikungunya and Ebola, emerged in recent years, causing severe epidemics and requiring a coordinated global response in terms of continued surveillance and research [1,4–6]. More recently, the COVID-19 pandemic dramatically and urgently highlighted the threat posed by emerging diseases to human health [7].

The development of efficient and tailored intervention strategies to control such diseases is thus essential, but the lack of previous field data, of background information on neglected or unknown pathogens, and the complexity of some systems, make the choice of the most efficient intervention strategy challenging. Additionally, most emerging infectious diseases (EIDs) are zoonoses (the 60.3% of EIDs), and most of them (71.8%) originate in wildlife [2]. Monitoring infected wild animals and identifying their role in the spread and maintenance of diseases complicates things further [8,9]. The range of approaches to cope with wildlife-originated zoonoses is wide, and most of applied strategies and techniques still give controversial results, often leading to a waste of time and resources [8–11]. As a consequence, on the one hand it is necessary to improve the current knowledge on emerging infectious diseases and their dynamics, on the other hand it is fundamental to develop tools for a better evaluation of the efficacy of potential intervention strategies.

Mathematical models can represent an efficient tool for an *a priori* simulation of host-pathogen interactions, thus improving our understanding of infectious diseases dynamics and helping to assess and evaluate different approaches to control diseases [12–15]. Mathematical models have been widely used in the investigation of disease dynamics and basic reproductive ratio (R_0) role in disease spread and maintenance. They have been used also to understand the role of different sources of heterogeneities in host populations in affecting the transmission and maintenance of diseases [12–14,16–19]. However, despite their potential, the use of mathematical models in empirical epidemiological studies and in the planning of public health policies still has limited practical application [20].

For this reason, we used a macroparasitic zoonotic disease as a model to apply and adapt a consolidated mathematical model [12] in order to provide a framework for the analysis of efficacy and efficiency of different intervention strategies.

B. procyonis is an ascarid nematode that infects North American raccoons as natural definitive hosts. The infective stage of the parasite is represented by eggs shed in the environment with raccoons' faeces, where they become infectious and can remain viable for years [21,22]. Birds and other mammals, humans included, may accidentally become infected and act as paratenic hosts [20,23–26]. Like with most of ascarids, ingestion of eggs by paratenic hosts may result in *larva migrans* syndrome, with larvae migrating from the gut and encysting into various host tissues. Compared to other ascarids, *larva migrans* by *B. procyonis* is particularly aggressive, often causing extensive neural damage. Several cases of severe or fatal neural *larva migrans* syndrome have been reported in humans in the last decades, most of them in children [21,25,27], and *baylisascariasis* is now

considered an emerging zoonosis [23]. The opportunistic behaviour of raccoons, that often coexist with humans in urban, suburban, and rural environments, combined with the high number of eggs shed and with their resistance, leads to extensive opportunities for contact and infection of human beings [21,22,28].

The sanitary relevance of *B. procyonis* and the high exposure risk make the development of prevention strategies for *B. procyonis* infection in humans necessary. Three main approaches are currently taken into consideration: the active removal of raccoons [21,29], the treatment of raccoons with anthelmintic baits [21,30,31], and the reduction of environmental contamination through faeces removal [21,29]. Each one of these strategies presents some strengths and weaknesses [21,26,29,30], but a systematic comparison of the efficacy (i.e. the capacity of the treatment to reach the egg elimination from the environment), and efficiency (i.e. the time needed to reach the egg elimination) of the strategies is still lacking. For this reason, we propose the use of a pre-existent and already validated mathematical model to (i) investigate the dynamics of the raccoon-B.*procyonis* system, and, in particular, (ii) compare both the efficacy and the efficiency of the three above-mentioned intervention strategies.

Methods

Study System

The investigated system consists of three interacting populations: the host population (raccoons, H), the parasite population (adult *B. procyonis*, P) and the free-living infective stage of the parasite (*B. procyonis* eggs, E). The simulated intervention strategies are: (i) raccoon depopulation, (ii) anthelmintic treatment and (iii) faeces removal.

Firstly, we performed a preliminary sensitivity analysis of the parameters representing the different intervention strategies, to compare their influence on the number of environmental eggs [32]. Then, we analysed the system by combining the analysis of equilibria (S1 Text) and simulations to evaluate both the efficacy and the efficiency of each intervention strategy. Multiple simulations were performed using the simple Euler forward integration method (function *euler* of "deSolve" package in R 3.6.3 software). In order to assess efficacy, we evaluated whether each specific intervention strategy was able to eliminate the egg population within 50 years. Efficacy was computed by using the equations for the analysis of the system equilibria reported in Supplementary materials S5. To assess the efficiency of each intervention strategy we evaluated the effective time needed to reach the new steady state. Since we focused our attention on intervention strategies eliminating the egg population and both adult parasite and egg populations consist of a discrete number of individuals, we considered that an intervention strategy reaches the steady state when the computed number of eggs and parasites is lower than one.

We chose to consider a 50-years time frame to clearly show and compare the effects of the intervention strategies on both the number of eggs and the time needed to reach the equilibrium. For this reason, interventions requiring more than 50 years to reach the equilibrium were not considered in the analysis and we only reported the number of eggs reached at the 50^{th} year.

To simplify the comparison between intervention strategies, we will focus hereafter on the proportion of subjects (raccoons/parasites/eggs, depending on the intervention strategy) treated per day, expressed as a percentage of the whole population size on that day and named hereafter "treatment coverage". It must be noted that the proportion of subjects treated does not strictly represent a constant number of raccoons/parasites/eggs treated per day, because it will depend on the population size of that day.

Due to the recent introduction of raccoons and *B. procyonis* in areas outside their natural North and Central American distribution range [21], two different scenarios have been explored:

- The 'native population' scenario: represented by a raccoon population in its native range, where the host population is close to its environmental carrying capacity (K) and the system is close to its steady state.
- The 'introduced population' scenario: represented by a raccoon population recently introduced in a new area, where neither the environmental carrying capacity nor the system's steady state have been reached yet. In this scenario, we considered, as initial sizes of H, P and L, the values reached from the system when the host population reaches 50% of its environmental carrying capacity.

We considered these two scenarios to take into account their epidemiological and demographic differences, as they differently affect the feasibility and efficacy of management and intervention strategies.

Dynamics of the system: mathematical model

Following Anderson and May [12], two deterministic models based on a system of three coupled differential equations have been implemented. Firstly, with the base model, we explored the dynamics of the system without any intervention, and secondly we introduced into the system the intervention strategies aimed at eliminating environmental *B. procyonis* eggs.

The dynamics of the base model, without any human intervention, can be described according to the following system of equations and are represented by the flow chart in Fig 1A:

$$\begin{cases} \dfrac{dH}{dt} = (b-d)H\left(\dfrac{K-H}{K}\right) \\ \dfrac{dP}{dt} = \beta EH - (d+\mu_1)P - \mu_2 H\left(\dfrac{P^2}{H^2}\dfrac{k+1}{k} + \dfrac{P}{H}\right) \\ \dfrac{dE}{dt} = hP - \beta EH - \delta E \end{cases} \quad (1)$$

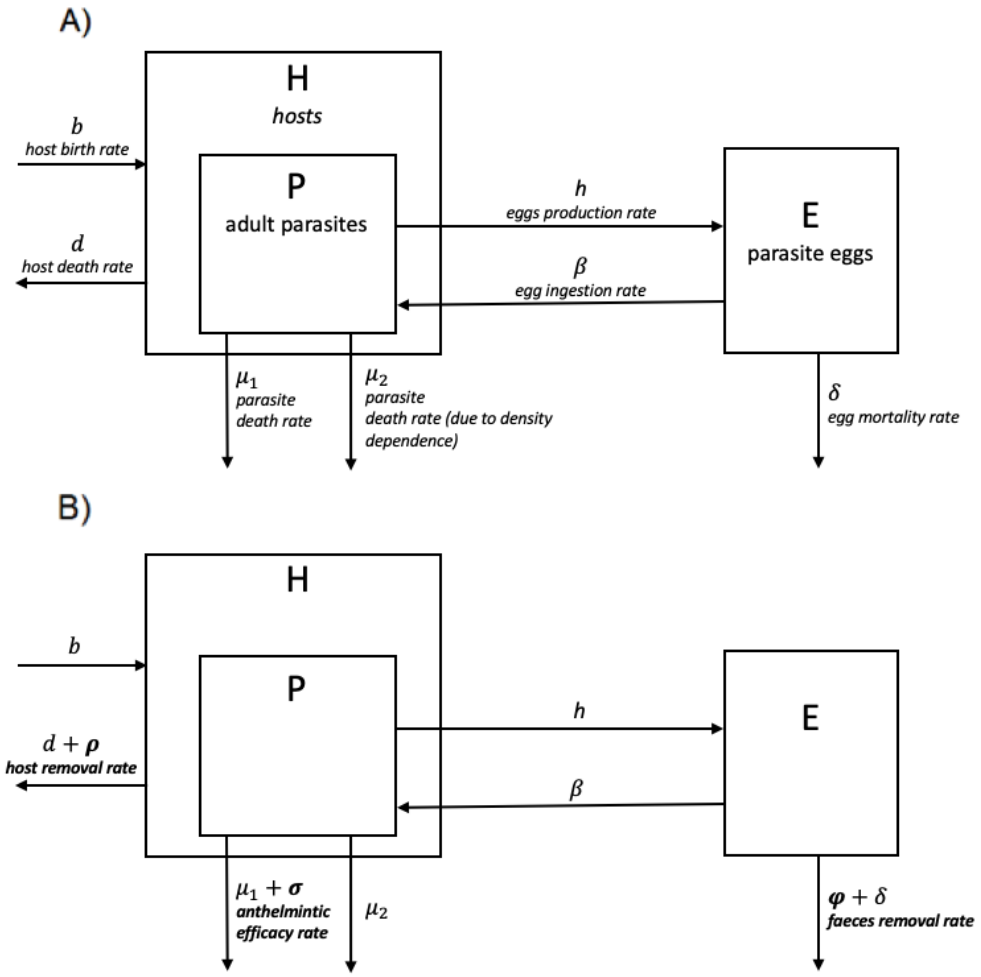

Figure 1. Modelled interactions between raccoons (*Procyon lotor*) and *Baylisascaris procyonis.* (A) Schematic representation of the interaction between *P. lotor*, the adult stage of the parasite *B. procyonis* and its free-living stages, illustrating the biological processes included in the model. (B) Schematic representation of the interaction between *P. lotor* and *B. procyonis*, their biological processes, biological parameters included in the model and the simulated intervention strategies.

In this model, host population size (system 1, equation 1) increases with raccoons' birth rate (*b*) and decreases with death rate (*d*). Density-dependence in host population growth is taken into account by including in the model a fixed carrying capacity of the environment (*K*). Since *B. procyonis* impacts on raccoon health are rarely described and age resistance and/or intestinal immunity with self-cure are considered to be the main

limiting process on *B. procyonis* number in raccoons [21], we assumed that the effect of the parasite on host survival and reproduction at the population level is negligible, and thus host population size is not affected by the parasite. Parasite population (system 1 equation 2) increases through the rate of ingestion (β) of infective eggs by the hosts, and decreases due to the combined effects of parasite death rate (μ_1), host death rate (d), and parasite density-dependent mortality (μ_2), which depends in turn on the aggregated distribution of parasites within the host population [33]. The parameter k affecting parasite density-dependent mortality provides an inverse measure of the extent of parasite aggregation [34]. Under natural conditions, raccoons may acquire infections even through predation of paratenic infected host, such as small mammals, but because of the central role of raccoon latrines in the transmission dynamics of *B. procyonis* [35], here we considered infection only through ingestion of environmental eggs. Finally, egg population size (system 1, equation 3) increases with adult female parasites fecundity rate (h), and decreases through both natural egg mortality (δ), and host ingestion rate (β). Because of the lack of information on the effective proportion of ingested infective larvae that develop to the adult stages within the host, we considered all ingestions of eggs resulting in a successful parasite establishment.

Simulation of intervention strategies:

In order to explore the effects of intervention strategies on *B. procyonis* eggs, the base model introduced above has been modified as described by the following system of equations and by Fig 2B:

$$\begin{cases} \dfrac{dH}{dt} = (b-d)H\left(\dfrac{K-H}{K}\right) - \rho H \\ \dfrac{dP}{dt} = \beta EH - (d+\mu_1+\sigma+\rho)P - \mu_2 H\left(\dfrac{P^2}{H^2}\dfrac{k+1}{k} + \dfrac{P}{H}\right) \\ \dfrac{dL}{dt} = hP - \beta EH - (\delta+\varphi)E \end{cases} \quad (2)$$

Raccoon depopulation affects the system by removing hosts and the parasites they harbour by a quantity that depends on the host removal rate (ρ). Anthelmintic treatment, by killing adult parasites harboured in raccoons by a quantity that depends on the anthelmintic administration rate (σ), only affects the parasite population. Similarly faeces removal, only acts on the egg population by decreasing its size by a quantity that depends on faeces removal rate (φ).

It is important to notice that once host population becomes extinct, the parasite population will die out too, reducing the model to the following equation:

$$\frac{dE}{dt} = -\delta E \quad (3)$$

Parameters estimation

Parameters used in the simulations were derived from published data. The mean lifespan of *P. lotor* is assumed as 2.3 years (839.5 days) [36], and the mean number of offspring as 3.8 youngs/female per year (0.01041/day) [35]. Thus, considering a sex ratio of 1:1 [37], daily host birth rate *b* is 0.0052, and daily host mortality *d* is 0.00123/day. Population density of racoons is highly dependent on habitat conditions, with reported values ranging from 1 to 100 raccoons/km^2 in the wild and exceeding 100/km^2 in urban areas [38–40]. For this reason, for both scenarios we arbitrarily chose an environmental carrying capacity (K) of 1000, in order to simulate a plausible population unit of

intervention for both an introduced and a native raccoon population. We estimated *B. procyonis* lifespan based on data available for closely related ascarid nematodes such as *Ascaris lumbriocoides* and *Baylisascaris shroederi.* Both these species have a 1-2 years lifespan (365-730 days) [41,42], thus daily death rate of adult parasites μ_1 was set at 0.001369 individuals/day. We used 0.393 as parameter of parasite aggregation (*k*), in accordance with the measure estimated for the ascarid *Toxocara cati* [43]. The reproductive output of a single *B. procyonis* female in one day (*h*) is reported to be 179,000 eggs/day [21,44] and incorporates egg production rate, scaled by the development time from egg to infecting stage. Egg mortality rate δ was chosen in order to allow a complete extinction of eggs within 5 years [45] when simulating with equation (3) and an initial egg number of 7.97×10^7 eggs (i.e. the number of eggs at the equilibrium of the system). Since the estimation of μ_2 and β from literature data was unfeasible, we arbitrarily identified those values with successive simulations, aiming at the achievement of the equilibria of the system with a mean abundance for *B. procyonis* of 15 parasites/host [21].

Intervention strategy rates

Host removal rate ρ, anthelmintic treatment rate σ and faeces removal rate φ represent the proportion of raccoons, parasites and eggs removed from the system in one day to the total number of raccoons, parasites or eggs present on that day. Rates can vary between 0 and 1, with 0 representing the removal of 0 hosts/parasites/eggs, and 1 represents the removal of the whole raccoon/parasite/egg population (100%). In order to comply with practical needs, when simulating raccoon depopulation in the native scenario we did not consider the possibility of host extinction, whereas in case of an

introduced population we analysed both possibilities: the reduction of raccoon population without extinction, and the extinction of raccoons. Performing an analysis of the equilibria of the system (Supplementary materials S5) based on the actual parameters, the boundary of discriminating between these two situations resulted 3.97×10^{3}, meaning that by removing more than 0.397% hosts/day, the host population goes towards extinction.

The full list of parameter values is given in Table 1, the time unit used is one day.

Sensitivity analysis on ρ, σ and φ:

To evaluate the effect of intervention strategies, we performed a global sensitivity analysis to determine the effect of parameters ρ, σ and φ on the number of environmental eggs in the native population scenario. Since we did not investigate possible combinations of intervention strategies, the sensitivity analysis was carried out by moving one parameter at a time. Parameters σ and φ were left free to vary between their minimum and their maximum (i.e. simulating the elimination of 0-100% population/day) following a uniform distribution. The parameter ρ was left free to vary between 0 and 0.00397, in order to avoid the extinction of hosts and the consequent shift from the system of three populations (system 2) to the system with only the egg population (equation 3). The final mean egg number (averaged over the simulation interval) was used to evaluate the effects of the changes in parameter values. The simulation interval for the sensitivity analysis was considered as 50 years, and simulations were performed 500 times for each intervention strategy. Global sensitivity analysis was carried out using the function "*modCRL*" of the package FME on R software [32].

Table 1. Parameters included in the models.

Parameter	Interpretation	Value	Source
H	Total number of host population	-	-
P	Total number of parasite population	-	-
E	Total number of free-living infective stage population (eggs)	-	-
K	Host population carrying capacity	1000	-
b	Instantaneous birth rate of host (day^{-1})	0.0052	3.8 young/female/year [35]
d	Instantaneous death rate of host due to all causes except parasites (day^{-1})	0.00123	2.3 years lifespan [36]
μ_1	Instantaneous death rate of adult parasite (day^{-1})	0.001369	1-2 years lifespan [41,42]
μ_2	Instantaneous death rate of adult parasite due to density dependent effects (day^{-1})	0.0009	-
h	Instantaneous rate of production of infective parasite eggs ($worm^{-1}day^{-1}$)	179 000	179000 eggs/worm/day [21,44]
β	Instantaneous rate of ingestion of free-living infective eggs ($host^{-1}day^{-1}$)	4.25e-12	-
k	Aggregation parameter of the negative binomial distribution	0.393	[44]
δ	Instantaneous death rate of eggs (day^{-1})	0.015	-
ρ	Host removal rate (day^{-1})	-	-
σ	Anthelmintic efficacy (day^{-1})	-	-
ϕ	Eggs removal rate (day^{-1})	-	-

Results

Dynamics of the system

The base model, in the absence of human intervention and after the introduction of two raccoon individuals with a parasite intensity of 5 *B. procyonis* each, predicts a globally sigmoid growth curve for all the three populations (Fig 2). However, the dynamics of parasite and egg populations in the first four years show a slight decrease in their size followed by a sigmoid growth (Fig 2D and F).

Simulations indicate that the base system reaches the equilibrium around 20 years, when the raccoon population counts 1000 individuals, the parasite population 14,772 parasites and the egg population $1.7x10^{11}$ eggs (Fig 2A, C and E). The 50% of the carrying capacity for the host population (500 raccoons) is reached in about 4 years from the beginning of the simulations, with a total number of 11 parasites and $7.97x10^{7}$ eggs (Fig 2B, D and F).

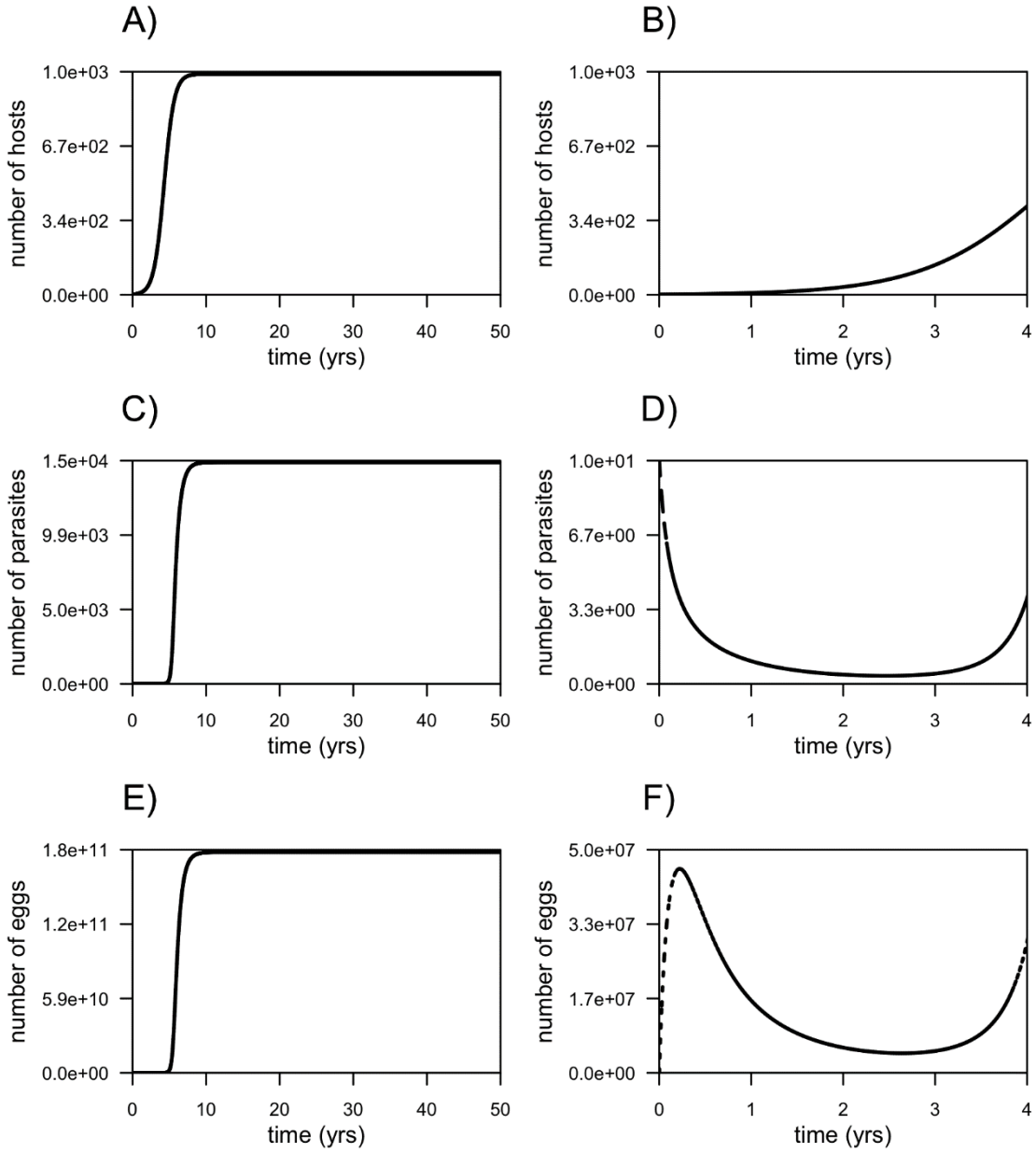

Figure 2. Raccoons-*Baylisascaris procyonis* dynamics: the base model. Temporal dynamics of raccoon population and B. procyonis until the achievement of the steady state of the system (A, C and E); and until the host population reaches 500 individuals (B, D and F).

Sensitivity analysis.

The sensitivity analysis showed that all the three parameters can affect the number of eggs. The parameter which variation has the greatest impact on the egg number is host removal (ρ), followed by parasite removal (σ) and lastly egg removal (φ) (Fig 3).

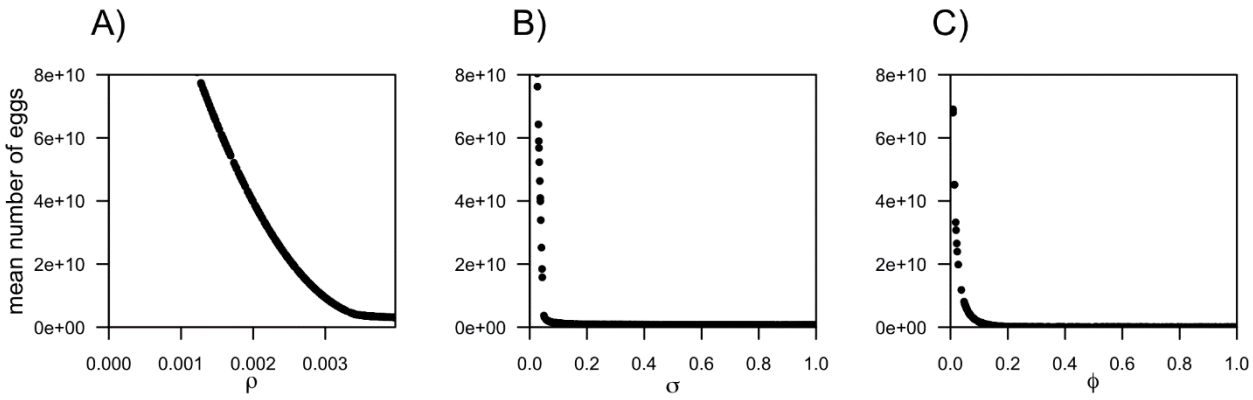

Figure 3. Sensitivity analysis: sensitivity analysis of parameters ρ, σ and φ on the mean egg number.

Intervention strategies in the "native population scenario"

Raccoon depopulation. Without bringing the host population to extinction (i.e. host removal between 0 and 0.397% hosts/day), a removal of less than 0.36% raccoons/day leads to a progressive reduction, but not to the elimination, of the egg population (Fig 4A and B). When removing more than 0.36% raccoons/day, the elimination of the egg population is achieved and the higher is the coverage, the faster is their elimination (Fig 4B). Coverages between 0.39 and 0.397% raccoons/day lead to the elimination of the egg population in about 20-22 years (Fig 4B).

Anthelmintic treatment. Any treatment coverage that removes more than 5.5% parasites/day leads to the egg population elimination in less than 50 years (Fig 4C). Coverages that remove more than 7.5% parasites/day lead the egg population to zero in

less than 20 years, and to reach the elimination of eggs in less than ten years it is necessary to use coverages that remove more than 10% parasites/day. The elimination of eggs can be achieved between 5 and 6 years with coverages higher than 30% parasites/day (Fig 4D).

Environmental faeces removal. Treatment coverages that remove more than 50% eggs/day lead to the elimination of the egg population in less than 50 years, but the elimination of eggs through faeces removal can never be reached in less than 19 years (Fig 4F).

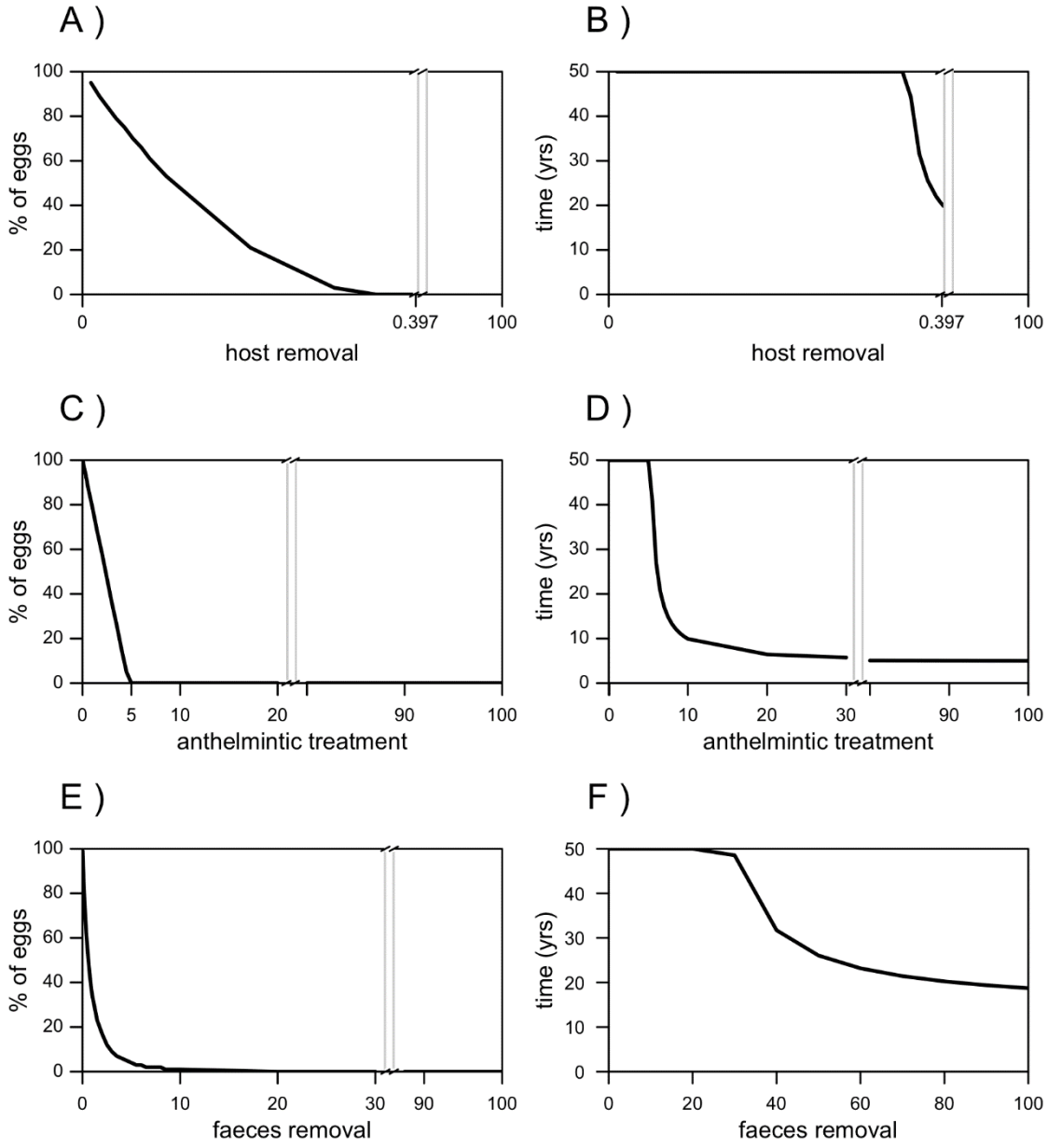

Figure 4. Performance of intervention strategies against *Baylisascaris procyonis*: the native population scenario. Efficacy (expressed as percentage of persisting eggs) and efficiency (expressed as time needed to reach the steady state of the system) of host removal (A and B), anthelminthic treatment (C and D) and faeces removal (E and F).

Intervention strategies in the "introduced population scenario"

Raccoon depopulation. Without bringing the host population to extinction (i.e. by removing less than 0.397% hosts/day), we need to remove more than 0.36% hosts/day to achieve the elimination of the egg population in less than 50 years (Fig 5A), and the time needed to achieve it, is included between 19 years and 43 years (Fig 5B). Within this range of coverages (from 0 to 0.397% hosts/day), the efficiency of the treatment increases linearly, with a progressive reduction in the time needed to eliminate eggs up to a minimum of 19 years. Assuming that the complete extinction of raccoons is instead an allowed outcome, the more hosts/day we remove, the faster we can reach the equilibrium, with a minimum of 3 years needed for egg elimination when removing 100% hosts in one day. Removing between 0.397 and 0.5% hosts/day we need more than 10 years to eliminate the egg population, whereas when removing more than 0.5% hosts/day less than 10 years are required. (Fig 5B).

Anthelmintic treatment. By using an anthelmintic drug treatment, we can achieve the elimination of the egg population in less than 50 years only when applying coverages that remove more than 5.5% parasites/day (Fig 5C and D). At such rates, the higher are the drug treatment rates, the faster is the elimination of eggs (Fig 5D). To eliminate eggs in less than 20 years, it is necessary to use a coverage that removes more than 6% parasites/day, and to do it in less than 10 years, a coverage that removes more than 7.5% parasites/day. Rates that remove more than 30% parasites/day take 3-4 years to reach the elimination of eggs (Fig 5D).

Environmental faeces removal. Using faeces removal as a control strategy, the elimination of the environmental egg population is achieved in less than 50 years with

removal coverages that remove more than 30% eggs/day, and in less than 20 years with coverages that remove more than 50% eggs/day (Fig 5F).

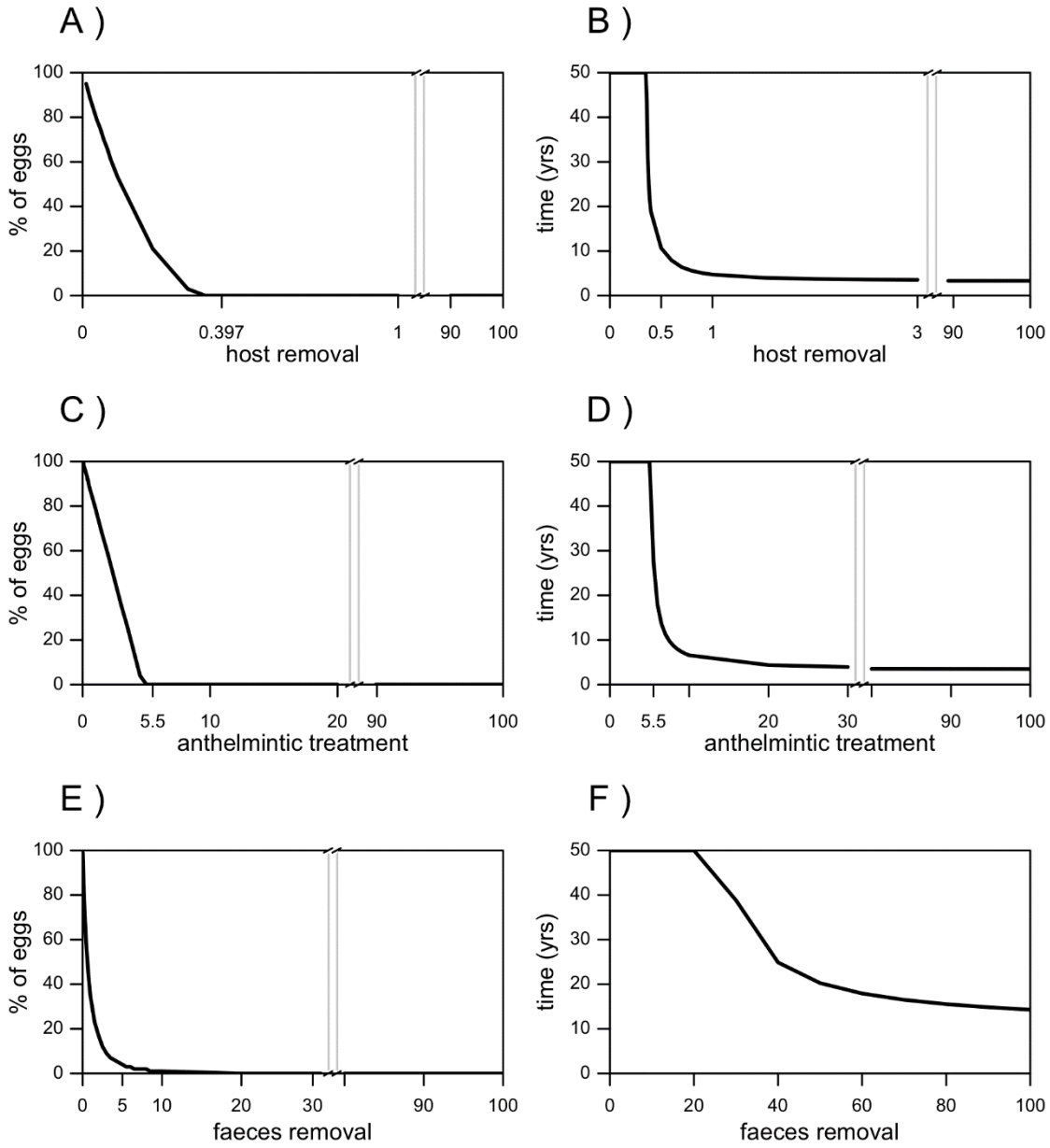

Figure 5. Performance of intervention strategies against *Baylisascaris procyonis*: the introduced population scenario. Efficacy (expressed as percentage of persisting eggs) and efficiency (expressed as time needed to reach the steady state of the system) of host removal (A and B), anthelminthic treatment (C and D) and faeces removal (E and F).

Comparison of intervention strategies

The sensitivity analysis shows that all the examined intervention strategies can eliminate the *B. procyonis* egg population (Fig 3). Additionally, the comparison of the efficacy of the three intervention strategies (Table 2) shows that all the three techniques can eliminate the egg population within a 50-years time frame. However, their efficiency varies largely depending on the simulated treatment coverage: raccoon depopulation requires the lowest coverage both to reach egg elimination and to allow it in the shortest time, while faeces removal requires the highest treatment coverage. Anthelmintic treatment has an intermediate efficiency.

Table 2. Comparison of years needed to eliminate *Baylisascaris procyonis* egg population by applying different intervention strategies (host removal, anthelminthic treatment and faeces removal) with different treatment coverages (i.e. percentage of hosts/parasites/eggs removed per day), on both native and introduced raccoon (*Procyon lotor*) host populations.

Treatment coverage (%)	Native population			Introduced population		
	Time (yrs) to reach equilibrium through:			Time (yrs) to reach equilibrium through:		
	host removal[a]	anthelmintic treatment	faeces removal	host removal	anthelmintic treatment	faeces removal
0.01	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]
0.05	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]
0.1	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]
0.2	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]
0.3	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]
0.35	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]	no egg elimination[c]
0.36	44	no egg elimination[c]	no egg elimination[c]	43[b]	no egg elimination[c]	no egg elimination[c]
0.37	30	no egg elimination[c]	no egg elimination[c]	31[b]	no egg elimination[c]	no egg elimination[c]
0.39	22	no egg elimination[c]	no egg elimination[c]	21[b]	no egg elimination[c]	no egg elimination[c]
0.396	20	no egg elimination[c]	no egg elimination[c]	20[b]	no egg elimination[c]	no egg elimination[c]
0.397	20	no egg elimination[c]	no egg elimination[c]	19[b]	no egg elimination[c]	no egg elimination[c]
0.4	-	no egg elimination[c]	no egg elimination[c]	19	no egg elimination[c]	no egg elimination[c]
0.5	-	no egg elimination[c]	no egg elimination[c]	11	no egg elimination[c]	no egg elimination[c]
0.8	-	no egg elimination[c]	no egg elimination[c]	6	no egg elimination[c]	no egg elimination[c]
1	-	no egg elimination[c]	no egg elimination[c]	5	no egg elimination[c]	no egg elimination[c]
3	-	no egg elimination[c]	no egg elimination[c]	3	no egg elimination[c]	no egg elimination[c]
5	-	no egg elimination[c]	no egg elimination[c]	3	no egg elimination[c]	no egg elimination[c]
5.5	-	41	no egg elimination[c]	3	28	no egg elimination[c]
7.5	-	15	no egg elimination[c]	3	10	no egg elimination[c]
10	-	10	no egg elimination[c]	3	7	no egg elimination[c]
30	-	6	49	3	4	39
50	-	5	26	3	4	20
80	-	5	20	3	3	16
100	-	5	19	3	3	14

[a] treatment coverages causing hosts' extinction has not been investigated

[b] no host extinction

[c] elimination of the egg population/equilibrium of the system is not reached within 50 years

Discussion

In the present study, by modelling host-parasite interactions, we analysed the efficacy and the efficiency of alternative intervention strategies to control the environmental persistence of a zoonotic macroparasite infective stage. The analyses showed that both efficacy and efficiency of the intervention primarily depend on the treatment coverage, and only secondary on the chosen treatment and scenario.

The lack of background information when dealing with neglected emerging diseases represents a challenge when assessing control measures, in particular for wildlife originated diseases, since monitoring of wildlife species is often limited [8,11]. The use of mathematical modelling can be helpful to understand the dynamics underlying infectious diseases, providing a tool for an *a priori* evaluation of such dynamics and interactions, and widely contributing to the design of control programs of diverse infections, without the need of demanding empirical studies [18,34,46,47]. For instance, the use of mathematical models in the development of control programs for measles, pertussis or rubella produced useful predictions concerning the level of vaccination coverage required to eradicate them, helping in the determination of the relative merits of different policies for the control of these infections [45,48]. Despite this, mathematical models are still largely underexploited in the planning of public health policies and disease control strategies [20].

Our work aimed at supporting the use of mathematical models as a pre-intervention approach to assess the effectiveness of different control strategies against emerging diseases. By simulating the effect of different intervention strategies on parasite

population dynamics, through their direct inclusion into the system, this approach provides results that can serve as a base for the *a priori* evaluation of an intervention strategy program.

While we used raccoons and their zoonotic helminth *B. procyonis* as a model system to identify the most effective intervention strategy to reduce parasitic environmental contamination, our simulations can be easily generalized to different macroparasitic diseases, including most diseases caused by ascarids and other soil-transmitted helminths. We chose to use a pre-existing and already validated model to highlight how even the application of simple and existing models can provide useful information about systems. This holds especially true when parameters and biological processes are hard to estimate due to a lack of field data, and any modification of base models can be both challenging to perform and less informative than simpler models. Moreover, the use of a validated model can extend the use of mathematical modelling to a wider spectrum of research areas, not limited to mathematicians and field experts.

With respect to the specific simulations performed on the raccoon-*B. procyonis* system, in both the native and introduction scenario all the treatments were potentially effective in reaching the elimination of the egg population within 50 years, but both their efficacy and efficiency varied greatly depending on the applied treatment coverage. The performed sensitivity analysis indicated host removal as the most effective strategy, while faeces removal had the lowest impact on the number of eggs. In agreement with this, the analysis of the intervention strategies via model simulations showed that faeces removal is the less efficient intervention strategy, as both host removal and anthelmintic treatment were faster in eliminating the egg population. However, anthelmintic

treatment requires a higher effort than host removal to reach an effective result. Ultimately, host depopulation is thus the treatment that requires the lowest treatment coverage to provide the elimination of eggs in both native and introduction scenarios and, together with anthelmintic treatment, shows also faster results. However, the time needed to reach the elimination of eggs depends on the simulated scenario. This result highlights the importance of taking into account differences between scenarios when choosing the intervention strategy to apply, although choosing an appropriate treatment coverage remains the most important step to achieve the elimination of the egg population. Moreover, the great difference in the efficiency of treatments resulting from even a slight change in coverages, as it happens between 0.37 and 0.39% hosts/day for host removal or between 5.5 and 7.5% parasites/day for anthelmintic treatment, demonstrates the importance of an *a priori* evaluation of the effects of intervention strategies.

Currently, raccoon depopulation and anthelmintic treatment are indeed the most frequently applied intervention strategies to reduce *B. procyonis* environmental contamination, and many authors suggested them as the most effective techniques [21,26,30,31]. However, a formal framework to objectively assess their efficacy and efficiency was lacking. With our model, we provide a quantitative analysis of both the efficacy and efficiency of these strategies, providing indications about the effort needed to reach the desired result without wasting time and economic resources.

However, it must be underlined that the choice of the most appropriate intervention strategy cannot overlook the need of an accurate analysis of its logistical feasibility under field conditions. A mathematical model can provide information to identify the most

efficient method, but its application also needs the participation of field scientists and technicians, to evaluate the logistical feasibility and applicability of the intervention strategies through a cost-benefit analysis.

However, for a more complete outline of an intervention strategy, combinations of different treatments, or of discontinuous treatments (such as a monthly administration of anthelmintic baits) should be simulated. A continuous daily treatment, as we simulated, allowed for a more explicit comparison between intervention strategies, but when assessing an intervention strategy, it is advisable to include a realistic time frame between two consecutive treatments. Finally, in addition to the insights provided by the model on *B. procyonis* control, the base model simulating raccoons-*B.procyonis* dynamics without any human intervention provides interesting information about the system as well. For instance, the simulation of the base model in the introduction scenario shows that the adult parasite population growth is markedly slower than the growth of both host and egg populations. Indeed, based on our estimates, while both host and egg population sizes increase very fast soon after simulating the introduction of raccoons, the number of adult parasites initially decreases. This relevant difference in the dynamics of the three populations highlights the need of a deeper analysis of the dynamics of the system at the early stages of introduction. When dealing with recently established raccoon populations, an early detection of *B. procyonis* is indeed fundamental to limit environmental contamination and reduce the infection risk for humans. However, the low parasite abundance during the first stages of raccoon invasion suggested by our simulations could hinder *B. procyonis* detection and must therefore be taken into account when implementing surveillance plans. This unexpected dynamic of the parasite

population could depend on the initial low number of hosts eliminating eggs, which will in turn determine a small egg population and, overall, a low parasite transmission rate. The inclusion of a stochastic model to predict more accurately the early stages of raccoon introduction, and the inclusion of specific field data can represent an implementation of the study, allowing for a more precise estimate of biological and epidemiological parameters, resulting in a more detailed and realistic simulation of population dynamics and in the inclusion of diverse mortality or transmission rates for adult parasites and eggs. Finally, we focussed our study on the effects of the intervention strategies on the system, but a wider sensitivity analysis including rates representing parameters other than ρ, σ and φ could be performed to further investigate which biological processes affect the base system the most. In conclusion, our simulations suggest host depopulation as the most efficient strategy to control environmental contamination by *B. procyonis* eggs, but they also highlight that, no matter the chosen technique, the treatment coverage is the most important parameter determining the effectiveness of control strategies. This work highlights the potential benefits of applying mathematical modelling in epidemiology and public health management, showing their efficiency as a tool to analyse disease dynamics and implement time- and cost-effective intervention strategies, even when a complete knowledge about the system is lacking and an empirical approach is unpractical.

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Supplementary materials S5

System for analytical computation of equilibria

$$\begin{cases} \widehat{H} = K\dfrac{(b-d-\rho)}{(b-d)} \\ \widehat{P} = (\dfrac{\widehat{H}\beta h}{\widehat{H}\beta+\varphi\delta} - (\sigma+\mu_1+d+\rho+\mu_2))(\dfrac{\widehat{H}k}{\mu_2(k+1)}) \\ \widehat{E} = \dfrac{h\widehat{P}}{\widehat{H}\beta+\varphi+\delta} \end{cases} \quad (1)$$

Where $\widehat{\mathrm{H}}$, $\widehat{\mathrm{P}}$ and $\widehat{\mathrm{E}}$ respectively represent the number of hosts, parasites and eggs at equilibrium.

CHAPTER 6

Conclusions and Perspectives

Conclusions and Perspectives

With the present work we investigated through mathematical modelling two wildlife-borne infections, West Nile virus (WNV) and *baylisascariasis*, with the double aim of exploring the mechanisms promoting infection spread and assessing efficacy of available intervention strategies to reduce human infection risk.

Infectious diseases have always been, and still are, one of the main threat for human beings, having a great impact on both global health and worldwide economies. Animals in particular are an important source of infection for human beings, representing 60.3% of all emerging diseases. Several zoonoses can involve multiple species in their transmission cycles thus making them complex to be investigated, and consequently impairing our full comprehension of mechanisms underlying their spread. Thus, despite of the importance of preventing human infections, the interactions among populations involved in infection cycles and the possible obstacles in data collection, can hamper their survey and control. Mathematical modelling applied to epidemiological studies, in contrast to the classical epidemiology, allows a deeper understanding of mechanisms underlying infection spread and interactions among populations, thus providing us with tools to explore processes underlying infection dynamics. For this reason, we here applied mathematical modelling to explore West Nile disease (WND) and *baylisascariasis*, two emerging infectious zoonoses that still present knowledge gaps related to the lack of knowledge of their transmission mechanisms.

WND is a mosquito-borne infection caused by West Nile virus (WNV), emerging and re-emerging in several countries, Italy included. It involves a wide range of bird species as

hosts, thus impairing our possibility to fully comprehend, and then prevent, its spread. In chapter 2 and 3 we then addressed at some of the existing knowledge gaps.

With chapter 2, we explored through a sensitivity analysis those epidemiological mechanisms, represented by model parameters, that have the major impact on infection dynamics. We showed that among them, birds recovery rate and mosquito biting rate have the major impact on infection spread. These results highlight the need to concentrate further investigations to better define among all species-specific rates, birds' recovery rate and mosquito biting rate, which highly influence infection spread, while others as birds susceptibility and their competence to infection deserve less attention due to their negligible influence on infection spread. This result therefore allows us to prioritise investigations into the most impactful parameters and mechanism, thus achieving the greatest improvement in our knowledge of WNV dynamics through the least number of experiments and with the minimal effort.

In chapter 3, we explored through theoretical simulations the role in WNV spread of different population dynamics of birds. We showed that the only variable affecting WNV spread is the avian abundance from June on. Moreover, this effect only affects the infection prevalence into the mosquito population, and not the timings of infection spread. With this analysis, we also posed the attention on the importance of understanding if mosquito biting rate is driven primarily by birds' abundance or by mosquito feeding preference. Indeed, results obtained rely on the choice to include in model simulations only one competent avian species on which mosquitoes feed on. It implies the choice that mosquito biting rate depends by mosquito preference towards certain species and not by species relative abundance, highlighting the need for further

investigations. With these two chapters, we also highlighted that the involvement of diverse bird species in WNV spread and maintenance is crucial. Both epidemiological and demographic characteristics of birds can heavily influence WNV spread, thus highlighting the direction for deepen our knowledge about the role of different bird species to understand and predict it. In particular, emerged the urgence to assess new studies to explore the recovery rate of birds and mosquito biting rate on different avian species to identify the most suitable species to spread WNV in our study area. Moreover, we highlighted the need of investigating if mosquito biting is affected by avian species abundance or not. These studies stimulate the integration with further theoretical modelling of birds' dynamics and statistical analyses on recorded birds abundance, in order to have a clearer understanding of the role of species involvement in WNV spread and of birds dynamics role in infection mechanisms.

The following chapters 4 and 5 exploit the potential of mathematical modelling to answer to "what if?" question and explore possible intervention strategies to reduce human infection risk.

In chapter 4 we investigate six different intervention strategies to reduce the risk for human beings to be infected by WNV. We estimated a base infection risk for human beings, based on the number of infectious circulating mosquitoes, and then calculated the reduction of that risk following the application of six intervention strategies. The tested strategies are: i) the elimination of the overwintering mosquito population, ii) a larvicide treatment, iii) adulticide treatment, iv) the reduction of mosquito breeding sites v) the active bird culling and vi) the reduction of birds' breeding sites. The mathematical simulations showed that the most efficient interventions are those reducing vector

abundance if compared with intervention on the avian population, and especially are: the reduction of mosquitoes breeding sites and the active elimination of eggs and larvae. One of the strengths of this approach is to provide a quantitative analysis of the efficacy of interventions preventing demanding and time consuming field experiments.

In chapter 5 we focused on interventions against *baylisascariasis*. *Baylisascariasis* is a zoonosis caused by the accidental ingestion of eggs of *B.procyonis*, an helminth that has raccoons (*Procyon lotor*) as its definitive host. Due to the recent spread of raccoons in several new areas, Italy included, *baylisascariasis* can now be considered an emerging disease in several countries of both Europe and Asia. Despite of that, an elective control strategy is not available to reduce the risk of human infection which develop into severe syndrome. We both assessed the efficacy (i.e. capacity of an intervention strategy to reduce the number of infective stage into the environment) and the efficiency (i.e. the time needed) of intervention strategies directed to the host population (e.g. active racoons culling), the parasite population (e.g. anthelmintic treatment of raccoons) and infective stages population (e.g. faeces removal from environment). We showed that host removal is the best intervention to fast reducing the risk caused by *B. procyonis*. Moreover, we showed that treatment coverage chosen highly affects obtained results, highlighting the importance of a careful assessment of interventions.

With these two chapters then, we show that mathematical modelling can be of aid in practical assessment of intervention strategies, especially against emerging infectious disease, like WN and *baylisascariasis* are. For both these chapters, an additional deepening of efficacy of intervention strategy could be performed by also including combinations of interventions or specific intervention intensities.

The results obtained in these chapters, highlight the importance of including mechanistic relationship of systems in epidemiological investigations. Their inclusion indeed can help us to understand the extent to which an event can influence another consequential event and allow us to explore theoretical scenarios, like the use of intervention strategies, preventing or sustaining field or laboratory experiments. This possibility has a great potential in investigating emerging infectious diseases especially but can also be beneficial when a strategy is already available and a comparison or testing with other interventions is needed. With this work we then further show that mathematical modelling can provide us with an efficient and adaptable tool to support epidemiological studies, providing solid and objective results on which to base public health choices.

However, despite their potential, the application of mathematical modelling to guide public health choices is still limited, and they tend to be relegated to a niche of theoretical works. The reason why of this relegation can be attributed to different causes. To build models that can be efficiently applied to epidemiological studies, a strict collaboration and interaction among different expertise is necessary, but models' complexity do not plays in its favour. Their intrinsic complexity indeed tends to make them hard to be managed and understood except for insiders, thus limiting the number of people prone to use this tool. This can lead them to be relegated to their niche and gradually loose contact with concrete problems to solve. Moreover, the expectation when using mathematical modelling might be to have a perfect prediction, or analysis, of phenomena, that instead is not possible. All models are simplification of reality, and for this reason none of them can capture all facets of a dynamic process, but each model can catch some aspect of the investigated system thus helping us to deepen our knowledge

about that specific aspect. On the one hand then it is necessary a closest, less pretentious, and more aware, approach of public health institutions to mathematical modelling. On the other hand, the future perspective for mathematical modelling is to become more and more entrenched with field and laboratory experiments in order to be more applicable and increasingly involved in the choices of public health.

These studies emphasize the urgence of a closer communication between research and public health institutions in to develop this, for now theoretical, tool and use it in practice to choose the most suitable intervention to be performed, especially at light of the current intense intervention plan ongoing for both infections. To pursue these future aims, the cooperation of professionals in modelling, analysis and data collection, but also of different expertise including ornithologists, entomologists and public health institutions, is fundamental, but makes these perspectives challenging. Despite of that the results obtained provide us with objective proves of the potential of these investigations to help in better understand, and then potentially reduce, WNV and *baylisascariasis* spread.

In conclusion, the potential of mathematical modelling is undeniable. They allow a deeper comprehension of dynamics of infectious diseases by including spreading mechanisms and non-linearity of interactions among individuals and subgroups of populations. For this reason, their support to epidemiological studies can be of help for public health choices. On the other hand, it is fundamental for modellers to stay connected with concrete needs of public health and not to get lost in self-referential lucubration. The future development for mathematical modelling is a closer cooperation of different expertise in order to make them used to serve health institutions.