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A dynamic transmission model of eastern equine encephalitis virus

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Abstract

Eastern equine encephalitis virus (EEEV) is one of several arthropod-borne viruses (arboviruses) endemic to the United States. Interactions between arthropod (mosquito) vectors and avian amplification host populations play a significant role in the dynamics of arboviral transmission. Recent data have suggested the hypothesis that an increased rate of successful feeding on young-of-the-year (YOY) birds might play a role in the dynamics of EEEV transmission. To test this hypothesis, we developed a model to explore the effect of the interactions of the vectors and avian host populations on EEEV transmission. Sensitivity analyses conducted using this model revealed eleven parameters that were capable of disproportionately affecting the predicted level of EEEV infection in the vertebrate reservoir and vector populations. Of these, four parameters were related to the interaction of the vector with young-of-the-year birds. Furthermore, adult birds could not substitute for young-of-the-year in initiating and maintaining a predicted enzootic outbreak of EEEV. Taken together, the model predicted that young-of-the-year birds play a key role in establishing and maintaining enzootic outbreaks of EEEV.

Keywords

Arbovirus; Avian; Mosquitom; Stella; Alabama

1. Introduction

Arthropod-borne viruses (or arboviruses) are transmitted primarily between reservoir hosts by blood-feeding arthropods (e.g. insects, ticks and mites) that serve as vectors for these agents. Because arboviruses infect two very different types of hosts (vertebrates and invertebrates) the transmission dynamics are often quite complex. The interaction between the reservoir host species and the arthropod vector is the one of the most important factors in determining whether an arboviral infection can be sustained in a given environment (Black and Moore, 1996). Thus, knowledge of the dynamics of arboviral transmission ultimately requires an in-depth understanding of how the vertebrate reservoir and arthropod vector interact.

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In North America, the most important arboviral infections from a public health perspective are those that cause encephalitis (the arboviral encephalitides). There are five major arboviral encephalitides in North America: eastern equine encephalitis (EEE), Saint Louis encephalitis (SLE), LaCrosse encephalitis (LAC), western equine encephalitis (WEE) and west Nile encephalitis (WN) (Tsai, 1991). The viruses associated with these infections are classified in several different taxonomic families, but they share important features of their life cycles. For example, with the exception of LAC, all are commonly infections of the endemic avifauna. Transmission from bird to bird in the enzootic cycle is generally thought to occur primarily through the action of ornithophilic mosquitoes, i.e. those species that feed primarily or exclusively upon birds (Tsai, 1991).

Of the arboviruses endemic to the United States, EEEV is particularly dangerous. In the southeast, horse infections with EEE virus occur commonly in the summer months, accompanied by sporadic human cases. Infection with EEE virus in both mammalian hosts is highly virulent, with death resulting in about 70% of the symptomatic cases. Most survivors have residual neurological sequelae and health care during acute phase illness and afterwards may result in the expenditure of several million dollars per person (Villari et al., 1995).

Previous studies of EEEV transmission in the southeastern USA have suggested that vector mosquitoes in this region (including *Culiseta melanura*, *Coquillettidia perturbans*, *Aedes vexans* and *Culex erraticus*) targeted particular avian species (Hassan et al., 2003). Furthermore, most blood meals derived from the most commonly fed-upon birds were confined to a rather narrow time frame that coincided with the breeding season of the targeted birds. This suggested that the vector mosquitoes were taking more blood meals from nestlings than from adult birds (Cupp et al., 2004a; Hassan et al., 2003). These findings are consistent with previous observations that have suggested that nestlings and young-of-the-year (YOY) are more often successfully fed upon by arboviral vectors than are adult birds (Blackmore and Dow, 1958). Furthermore, laboratory studies have suggested that nestlings and YOY of some species also develop infectious circulating viremias more rapidly than adult birds and peak viral titers present in nestlings are as high as or higher than those seen in adult birds (McLean et al., 1995). Taken together, these observations suggest that mosquitoes might be feeding intensively on YOY of the chosen host species and that this concentration might increase the intensity of EEEV transmission (i.e. the incidence rate in the vector and amplification host populations).

Here, we report the development of a model for the transmission of EEEV that concentrates upon the interaction of the vector and avian reservoirs. This model contains separate modules for adult birds, YOY and mosquitoes, allowing us to test the hypothesis that feeding on YOY may affect the dynamics of EEEV transmission.

2. Materials and methods

2.1. Development of the model

The model was created using Stella[®] (Wallis et al., 2002), a software package specifically designed for modeling dynamic systems. Stella[®] has been utilized by other researchers to create models of ecological (Angelini and Petrere, 2000; Costanza and Voinov, 2001; Gertseva et al., 2004; Suplicy et al., 2003; Vitale et al., 2003), economic (Costanza and Voinov, 2001), physiological (Narayana et al., 1997) and clinical (Anderson et al., 2002) systems. Our model contains three interacting modules; adult birds, YOY and mosquitoes (Fig. 1). It is structured to run for a single transmission season (200 days in Alabama). The adult bird module models in-migration, mortality, infection and recovery of adults. Natality as a function of time and the size of the adult bird population and mortality, infection and recovery of young birds is captured in the YOY module. The third module captures the population dynamics of the mosquito vector.

EEEV transmission and the growth of the virus are captured through the interactions among modules and the proportion of each population that is infected. The model contains 18 fixed parameters, 7 time-dependent parameters and 4 latency periods. The model uses a second-order Runge–Kutta algorithm (Press, 1992) to simultaneously iterate all 29 parameters in the model on a daily time-step. We used standard population-dynamics models and published equations for the calculation of vectorial capacity (Black and Moore, 1996) as the foundation for this model. Initial values for all parameters in the model were obtained from previously published sources, or from observations collected in 2001 at the Tuskegee National Forest (TNF) study site described in detail below. Starting values for each parameter, and the sources used to determine the values for each of the parameters are provided in Table 1.

Adult birds were modeled to follow a standard susceptible, infected, recovered (SIR) paradigm. Young-of-the-year birds were permitted to follow two paths, based on a time-dependent function. This was done because studies have suggested that the immune system of a nestling is not capable of producing a vigorous response to an antigen until roughly 12 days following hatching (Mast and Goddeeris, 1999). Thus, newly hatched birds were modeled to cycle between susceptible and infected states for the first 12 days of life. After this period, the YOY were modeled to follow the SIR pattern, identical to that used for the adult bird population. Similarly, values for YOY/adult relative attack rate and feeding success on YOY were modeled using YOY-specific parameters for only the first 98 days of life. After this, it was assumed that the YOY would have begun to develop adult plumage and defensive behaviors. Thus, after 98 days, the YOY/adult feeding preference and feeding success for the YOY was adjusted to gradually approach that of the adult bird population, reaching equality with the adult parameters at day 120. This time of transition from YOY to adult birds was based upon extensive observations of breeding bird behavior in the southeastern USA by one of the authors (G. Hill, unpublished data). In accordance with laboratory studies suggesting that once infected, the majority of mosquitoes remain infected for a prolonged period (Howard and Wallis, 1974), we modeled vectors to move uni-directionally from a susceptible to a permanently infectious state.

A downloadable version of the model may be obtained from www.dom.uab.edu/geomed/unnasch/EEEmodel.html. This web site contains the model, a text file describing how to navigate the model's interface and a link to a free engine that may be used to run the model.

2.2. Collection of experimental data

Data collected at swamp-forest study site during the summer of 2001 were used to set some initial parameter values in the model, while similar data collected during the 2002 and 2003 transmission seasons at the same location were used to validate the model's performance. This study site has been described in detail in a previous publication (Cupp et al., 2003). In brief, the study site was located in the Tuskegee National Forest (TNF) in Macon County, Alabama [32°26'00"W, 85°38'44"N]. The site is characterized by a maturing wet forest that is re-growing over depleted farmland abandoned in the early 1900s. Much of the site is seasonally flooded. EEEV was documented in mosquitoes at this site in each year of our survey from 2001 to 2003 (Cupp et al., 2003, 2004b).

Mosquitoes were collected by using portable CDC light-traps baited with CO₂ and by vacuum collection, as previously described (Cupp et al., 2003). Mosquitoes in each collection were sorted by date and species into pools with a maximum size of 50 individuals and tested for the presence of EEEV, as previously described (Cupp et al., 2003). Initial vector mosquito numbers, mosquito population growth rates and mosquito infection rates were estimated based upon these collection data. In conducting these estimates, the number of captured mosquitoes was multiplied by a factor of 1000 to obtain an estimate of the overall sizes of the infected and uninfected vector populations, as mark-release-recapture studies suggest that roughly 0.1% of the mosquito population are sampled using collection methods similar to those used here

(Service, 1993). Bird populations at the site were censused throughout the season by point count and mark recapture techniques, as previously described (Hassan et al., 2003).

2.3. Validation of the model's performance

The performance of the model was evaluated with data collected at the TNF study site in 2002 and 2003. These data were independent of the 2001 data used to parameterize the model. In conducting the validation studies, three output statistics were used for which experimental data were available during all 3 years. These included the number of uninfected mosquitoes, the number of infected mosquitoes and the number of mosquitoes containing a blood meal.

The overall goal of the statistical analysis comparing the model output to the field data was to determine the extent to which the model predicted the behavior exhibited by the observed data. The data can be viewed as a “time sequence or series” of values, and the model is a mathematical attempt to predict the behavior of the underlying natural process over time. Thus, both the model and the observations can be considered time series. In statistical time series analysis, Kendall's Tau statistic (Kendall, 1938) is often used to measure the concordance of a pair of time series. The statistic takes on values between -1 and $+1$, with a large positive value indicating a strong concordance and a small or negative value representing poor concordance. Here, the term “concordance” is meant the degree to which the curves of the model and the data as functions of time agree with each other in the sense that where one curve is increasing or decreasing, so is the other. The statistic is non-parametric in nature, and is not greatly affected by the relative sizes of the observed values.

The model is not subject to sampling error, while the observations from the field are. Recognizing that our observed data was subject to some sampling error, we developed a smoothed curve fit to the data, and have used these values in the analysis as well. The smoothed data was produced using a cubic smoothing spline model with the degree of smoothing being chosen by the generalized cross validation method of Wahba (1990). The analysis consisted of calculating the value of the Kendall's Tau statistic for both the raw data and the smoothed data when separately compared to the output from the model.

2.4. Sensitivity analyses

Sensitivity analyses were conducted on each of the 29 parameters in the model. In conducting these analyses, the value of each parameter was changed several times over a 10-fold range if possible, or over the entire possible range of the parameter in those cases where the possible range of the parameter was less than 10-fold. The effect of these changes on four output statistics indicative of the intensity of viral transmission (peak number of infectious mosquitoes, peak number of infectious adult birds, peak number of infectious YOY and total number of immune adult birds) was then determined. Parameter sensitivity was then estimated by determining the mean percentage change in these output statistics resulting from a 1% change in the input parameter. Input parameters were then categorized as “drivers”, those having a relatively large effect on the output (i.e. an average change of greater than $\pm 2\%$ in at least one of the output statistics resulting from a 1% change in the input parameter) and “non-drivers”, those that had relatively small impacts (i.e. an average change of less than $\pm 2\%$ in all of the output statistics resulting from a 1% change in the input parameter).

3. Results

As the major goal of this study was to develop a model for EEEV transmission that concentrated upon interaction between the avian amplification host and the vector population, and in particular on the role of young-of-the-year (YOY) in this process, the model was designed to include three interacting modules (Fig. 1). The initial values for all of the parameters in the

model were determined as described in Section 2. The model's performance was validated with observations obtained at the TNF study site in 2002 and 2003. As described in Section 2, these data were independent of the 2001 observations used to initially parameterize the model. The model was successful in matching the observed data for both 2002 and 2003 with Tau statistics ranging from 0.258 to 0.552, all of which were highly significant (Fig. 2).

Sensitivity analyses were conducted on each of the 29 parameters in the model as described in Section 2. In conducting the sensitivity analysis, the value of each parameter was changed serially over a 10-fold range (or if the possible range of the parameter was less than 10-fold, over the entire possible range of the parameter). This wide range was analyzed to detect potential threshold effects or chaotic behaviors in the model. In several hundred runs of the model with different combinations of parameter values, no conditions were identified that resulted in chaotic behavior of the model. Similarly, no dramatic threshold-like changes in any of the output statistics resulting from continuous changes in the model's drivers were noted (data not shown).

The results from the sensitivity analysis were then analyzed to estimate the effect of changes in each parameter on each of four output statistics indicative of the intensity of EEEV transmission, as described in Section 2. Based upon these analyses, input parameters were categorized as drivers, (those having a relatively large effect on output values indicative of the intensity of EEEV transmission) or non-drivers (those that had relatively small impacts), as detailed in Section 2. Eleven of the 29 parameters were found to be drivers (Table 2). Among the drivers, the mosquito population growth rate resulted in the most dramatic changes in the output statistics (Table 2). Four of the 11 drivers were parameters affecting YOY, with two of these (YOY/adult feeding preference and feeding success on YOY) relating to the feeding behavior of vector mosquitoes on YOY birds. Three of the drivers were parameters relating to the adult bird population, with one of these (feeding success on adult birds) relating to the interaction between the avian adult and vector populations. The remaining four drivers affected the mosquito population alone.

The effect of the four most sensitive drivers on the prevalence of infection in the avian reservoir community are summarized in Table 3. The most sensitive driver was found to be the growth rate of the mosquito population. Doubling the growth rate throughout the season resulted in infection of all of the avian hosts by day 60 of the simulation. Similarly, doubling the feeding success rate on YOY resulted in an epidemic, which reached a plateau of roughly 85% of the avian population having been infected by the virus by day 112 of the simulation. Similar effects were noted when the starting number of uninfected mosquitoes and the duration of viremia in the YOY population were increased (Table 3).

The major goal of this model was to explore the effect that parameters affecting the interaction of the amplifying host and vector populations had on EEEV transmission. In particular, we were interested in determining if differences in the interaction of the mosquito vector population with adult birds and YOY might affect viral activity. The sensitivity analysis summarized in Table 2 suggested that this might be the case, as 3 of the 11 drivers were found to affect the interaction of the mosquito vector and the adult and YOY bird populations. The effect of these parameters on the incidence of viral infection in the avian population is summarized in Figs. 3 and 4. An increase in the probability of feeding success of the vector on adult birds or on YOY resulted in an increase in the incidence rate in the avian population (Fig. 3). However, the effect of feeding success on the intensity of infection was greater for YOY than for adult birds (Fig. 3). Similarly, the mosquito YOY/adult relative feeding preference was found to influence the incidence rate, with the incidence rate increasing with increases in the relative preference for YOY over adult birds (Fig. 4).

Of the 11 parameters found to be drivers in the sensitivity analysis, 3 were found to act negatively on the intensity of infection, i.e. increases in the values of these parameters decreased the intensity of infection. Increases in two of these drivers (death rate in the infected and uninfected mosquito populations) intuitively would be predicted to reduce the intensity of infection. Interestingly, however, the model predicted that the starting number of adult birds was also a negative driver in the system. Thus, increases in the starting resident bird population were found to result in disproportionate decreases in the rate of infection incidence in the avian population (Fig. 5).

Results of the sensitivity analysis suggested that both the number of uninfected mosquitoes and the number of uninfected adult birds were drivers in the model. This suggested the hypothesis that the key underlying parameter in determining the rate of transmission might be the ratio of uninfected avian hosts to the mosquito vector. To test this hypothesis, the initial number of infected mosquitoes, uninfected mosquitoes and adult birds were co-varied, maintaining a fixed ratio among at least two of the three parameters at a time. If the hypothesis that the ratio of mosquitoes to birds was the key parameter, co-varying the absolute number of hosts and vectors while maintaining the ratio between them should not affect the overall incidence rate in the avian population. This was found to be the case. Doubling the starting values of all three parameters (the number of infected mosquitoes, the number of uninfected mosquitoes and the number of adult birds) had an insignificant effect on the incidence rate in the avian population (Fig. 6, Panel A). Doubling the starting number of uninfected mosquitoes and adult birds, while keeping the starting number of infected mosquitoes constant resulted in an incidence rate that was roughly half that of the baseline, consistent with the fact that this change resulted in a halving of the starting proportion of infected mosquitoes (Fig. 6, Panel B). In contrast, doubling the starting number of adult birds and infected mosquitoes, while keeping the starting number of uninfected mosquitoes constant resulted in a striking decrease in the predicted incidence rate (Fig. 6, Panel C).

From the above analyses, it was apparent that the relative abundance of the host and mosquito vector was predicted to play an important role in the rate of transmission. In addition, several of the drivers were found to be parameters associated with the YOY and their interactions with the mosquito vector population. However, an analysis of the temporal pattern of the infection outbreaks predicted by the model suggested that the major period of transmission began around day 115 and continued through roughly day 135. The peak in transmission occurred on day 122 in the mosquito population and on day 123 in the bird population (data not shown). This was interesting, because as described above, the YOY were modeled to begin behaving like adult birds in their interaction with the vector at day 99, and the transformation to adult behavior was completed by day 120. Thus, when peak transmission was occurring, the YOY population was behaving more like adults than YOY in their interaction with the mosquito vector. This suggested the hypothesis that the most important factor driving the appearance of the peaks in viral activity was a change in the relative number of birds to mosquitoes at this point in time, and not something that was specific to the YOY. To test this hypothesis, the parameters of the model were adjusted to set the birth rate at zero. This prevented the introduction of any YOY into the system. The parameters were further adjusted to permit in-migration of susceptible adult birds at the same rate and to the same overall number that was seen in the model set to permit production of YOY. The overall effect of these changes resulted in a system where the population growth in the avian population was identical in the two models. The only difference in the two iterations of the model were that in the first case, new birds were introduced by birth and were therefore YOY, while in the second case new birds were introduced by in-migration and were therefore susceptible adults. If the hypothesis that peaks in viral transmission was driven by relative changes in the numbers of birds and mosquitoes was correct, we predicted that the two models would produce identical infection rates in the avian population. Instead, while the model introducing the new birds through birth produced the expected incidence rate,

the model in which birds were introduced by migration produced no infection in the avian population (Fig. 7).

From the data presented above, it could be concluded that the ratio of uninfected mosquitoes to susceptible birds and the intensity of feeding upon YOY were both important parameters affecting the degree of EEEV transmission. This suggested the hypothesis that control measures targeted to reduce mosquito numbers during the period of most intensive feeding upon YOY might result in a more significant effect on EEEV transmission than would control measures applied later in the year. To test this hypothesis, the model was modified to convert the questing mosquito death rate to a time dependent parameter. The death rate was then adjusted to model a single-day 50% dieoff in the questing mosquito population. A death rate of 50% was chosen as it has been suggested that a successful aerial insecticide application will result in the death of roughly 50% of the questing adult mosquitoes in the treated area (Peairs, 2005). The date of the 50% mortality event was varied from day 40 in the simulation through day 122, the day representing the predicted peak in the number of infectious mosquitoes (data not shown). The model predicted that a single day 50% mortality event occurring at day 40 resulted in interruption of viral activity, as measured by the cumulative percentage of birds infected (Fig. 8). Shifting the mortality event to day 80 (a time point roughly 40 days prior to the peak in viral activity in the model run with baseline parameters) resulted in a dramatic reduction but not elimination of viral activity. In contrast, shifting the mortality event to day 122, when the number of infectious mosquitoes was at its peak, had little effect on viral activity (Fig. 8).

4. Discussion

The model reported here was developed to explore the dynamics of EEEV transmission in the context of the interaction of the vector and reservoir host populations, and was parameterized with data collected from a site with endemic EEEV. There have been published reports of several models that have been developed to study arboviral transmission. These models have concentrated upon different aspects of the biology of arboviral transmission, including the effect of climate and habitat on transmission (Letson et al., 1993; Shaman et al., 2002, 2003, 2004) and the importance of the density of the amplification hosts and vectors on arboviral activity (Lord and Day, 2001a; Wonham et al., 2004). Our model most closely resembles models for SLEV and WNV developed by Lord and Day (2001a,b), which also concentrated upon the interaction of the amplification host and mosquito vector and also considered potential differences in adult and YOY birds. Their model and our model are both deterministic state and transition models. However, our model differs from these previous models in some significant ways. First, our model explores the differences in the reservoir capacity of YOY and adult birds in more detail. For example, the model reported here permits one to study the ramifications on viral infection patterns resulting from both differences in searching behavior and relative feeding success of foraging mosquitoes as well as differences in susceptibility and reservoir competence among these different age classes. Secondly, because the model is iterated on a daily time-step, we were able to include in our model explicit discontinuous time dependent functions. Thus, our model enables us to examine the impact on both large-scale seasonal patterns (e.g. differences in the time of nesting initiation and nesting success in birds) and shorter pulse impacts (e.g. mosquito control through spraying or weather impacts). Finally, our model was parameterized using data collected from a study site endemic of EEEV, and is to our knowledge the only model that specifically addresses the ecology of EEEV transmission in the southeastern USA, where, apart from Florida, EEEV is a long-standing but little studied public health threat.

A major goal in the model's design was allow us to test the hypothesis that preferential feeding upon YOY might play an important role in the development of EEE enzootic outbreaks. The

results presented above suggest that preferential feeding upon YOY may significantly promote the development of avian enzootics of EEEV. Four of the 11 parameters categorized as drivers were associated in some way with YOY, or the interaction of YOY with the vector mosquito population. Furthermore, replacement of YOY with adult birds resulted in a dramatic reduction in the intensity of viral transmission, demonstrating that some quality of the YOY was an important driver in the model. This is perhaps not surprising, as the YOY were modeled to be more susceptible to both viral infection and mosquito attack than were the adult birds. However, all of the parameters specific to the YOY were modeled to be time-dependent, allowing the YOY to become identical to the adult birds in their interaction with the mosquito population as the season progressed. The transformation to adult bird parameters was complete by day 120. However, the peak period of viral transmission was predicted to begin around day 115 and continued through day 135, with the peak intensity of infection occurring on days 122 and 123. Thus, the peak in transmission intensity occurred after the YOY were modeled to behave as adult birds in their interaction with the vector mosquito population. This could not be explained as an artifact of the extrinsic incubation period in the mosquito population or the pre-patent period in the avian reservoir, as these were set at 3 days and 24 h, respectively, in the model. These results, when taken together, suggest that some event or events involving the interaction of the vector and the YOY that occurred prior to the peak in transmission was responsible for driving the subsequent peak in viral activity. These results are in concordance with previous models of arboviral infections that have attempted to correlate meteorological data with the development of arboviral outbreaks in Florida, which suggested that meteorological conditions that lead to the concentration of avian hosts and mosquito vectors increased the probability of the development of an outbreak of transmission (Shaman et al., 2002). These predictions all suggested that it might be possible that mosquito control measures directed at this critical point may be an effective means of controlling outbreaks. The simulations presented above provide support for this hypothesis. A simulated 1-day 50% mortality event in the questing vector population that occurred at day 40 proved to be more effective in reducing peak viral transmission at day 122 than did equivalent events occurring at either days 80 or 122. These results suggest that mosquito control measures applied early in the year may prove to be more effective at controlling transmission of EEEV later in the year than are control efforts applied either just prior to, or during the peak of viral activity.

In the sensitivity studies, small changes in the mosquito population growth rate were found to result in dramatic changes in the intensity of infection, both in the mosquito and avian populations. This observation is concordant with observations of natural enzootics of EEEV that have suggested that outbreaks of EEEV are associated with an increased density of the mosquito vector (Pagac et al., 1992), and with predictions from recent models of SLEV (Lord and Day, 2001b) and WNV (Lord and Day, 2001a). Intuitively, one would predict that an increase in the numbers of the mosquito vector would result in an increase in the intensity of viral transmission. Surprisingly, however, the model also predicted that the starting number of adult birds was a sensitive parameter, with the intensity of infection decreasing with increases in the number of birds. Both of these predictions are similar to those made by a recent model of WNV transmission in North America, which predicted that mosquito control measures would decrease the chances of an outbreak of WNV, but bird control efforts would increase rather than decrease the chances of an outbreak (Wonham et al., 2004). Together, all of these models suggest that the ratio of mosquito vectors to avian hosts, and therefore the biting intensity on the avian reservoir, may be a significant factor in determining whether an outbreak will develop and be sustained.

This model was primarily developed to explore the dynamics of EEEV transmission in the southeastern US. However, many of the arboviruses endemic to North America share most of the salient features of their life cycles in common. This model might therefore be adapted to study the role of YOY in the transmission of other arbovirus in the US, such as WEEV,

SLEV and WNV. For example, recent studies of the feeding patterns of the *Culex pipiens* complex, the major vector for WNV in the USA, have demonstrated that a small number of avian species predominate in the blood meals identified in this species (Apperson et al., 2004). These data suggest that the feeding behaviors documented for the vectors of EEEV at the TNF study site may be shared by the major vector of WNV in the USA.

Although developed from data collected from field observations in 2001, the model's predictions were found to correspond well to the data collected in 2002 and 2003, which were independent of the 2001 data used to develop the model. The model run with baseline values for the parameters predicts a major peak in viral activity lasting for roughly 20 days, beginning around day 115 and lasting through day 135. As the model was designed to replicate field collection data from the beginning of April through the middle of October, this peak of viral activity represents a period from the latter part of July through mid-August. As presented in Fig. 2, this prediction corresponded well to the temporal pattern of viral activity seen at the TNF study site in 2002 and 2003. Furthermore, this pattern appears to be typical for EEEV activity in Alabama in general, as records of the Alabama Department of Agriculture and Industries' Veterinary Diagnostic Laboratory in Auburn, Alabama indicate that the majority of horse cases reported in the state occur during late July and August (Dr. George D'Andrea, personal communication). The fact that the model's predictions closely matched observations collected independently from the data used to parameterize the model suggests that the model's performance is robust. However, the observations used to validate the model were collected on a limited number of parameters. These all related to the mosquito vector population, including the number of infected mosquitoes, the number of uninfected mosquitoes and the number of mosquitoes having taken a blood meal. Thus, it was not possible to evaluate the model's performance at predicting viral activity in the avian reservoir. Additional data on infection in the avian reservoir population will be needed to validate the performance of the model in predicting the dynamics of infection in that part of the life cycle. Because viremias in infectious birds are transient, it is impractical to attempt to quantify the actual number of infectious birds at any given time during the transmission season. However, it may be possible to estimate the total number of birds infected with the virus by serially measuring circulating antibodies to EEEV in the sera of birds captured at the site (Chiles and Reisen, 1998). Studies to obtain such data at the TNF study site are currently planned.

Although the model presented here contains 29 parameters, it still does not address many of the intricacies found in the ecology of EEEV transmission in the southeastern US. For example, the model is now constructed such that the vector mosquitoes feed exclusively upon avian hosts. This feeding behavior is characteristic of ornithophilic species such as *C. melanura*, the primary vector for EEEV in foci in the Northeastern states and along the Atlantic Coast. (Nasci and Edman, 1981). *C. melanura* is also present at the TNF study site (albeit in limited numbers) and has historically exhibited the highest rate of infection of any species at this focus (Cupp et al., 2003). However, by far the most common mosquito species found at the TNF study site (Cupp et al., 2003), and elsewhere in the southeast (Cupp et al., 2004a) is *C. erraticus*. In all 3 years, the greatest overall number of EEEV positive pools at TNF have been from *C. erraticus* (Cupp et al., 2004a). It is therefore likely that *C. erraticus* plays an important role in transmission of EEEV in the southeastern US. However, *C. erraticus* is a relatively catholic feeder, selecting avian hosts roughly 50% of the time (Hassan et al., 2003). Thus, modeling the dynamics of transmission of an enzootic cycle vectored by *C. erraticus* will require modifications of the model to permit the choice of blood meals from non-avian sources.

A second simplification present in the current model involves the mechanism of over-wintering or re-introduction of EEEV in the southeastern US. In the results presented above, the model was run with EEEV initially introduced by infected mosquitoes. This assumption will be biologically correct if birds are the only reservoir host for EEEV and the virus over-winters in

a vector mosquito found at the site. However, recent data have suggested that reptiles and/or amphibians may play a role as a reservoir for EEEV and may also serve as potential overwintering hosts for EEEV (Cupp et al., 2004b). If this is found to be correct, it will be necessary to modify the current model to contain an additional viral reservoir apart from the avian amplification host.

Currently, the model assumes that a mosquito, once becoming infectious, remains so for life. However, some laboratory studies suggest mosquitoes may revert to a non-infectious state after a prolonged period. For example, studies by Howard and Wallis (1974) have suggested that 58% of the *C. melanura* remain infectious 20 days following infection with EEEV. However, 20 days represents a very long life span for a mosquito, and in the current model, only 12% of the infectious mosquitoes are modeled to live this long. Reducing the number of infected mosquitoes to adjust for the possibility that some might revert to a non-infectious state after a prolonged period had little effect on the predicted outcome (data not shown). Thus, it is unlikely that altering the model to permit some proportion of the infectious mosquitoes to revert to a non-infectious state will change the predictions made by the model to a great extent.

Another simplification relates to the potential role that interrupted feeding by the mosquito vector may play in viral transmission. Studies involving an exotic caged bird species (the European Starling, *Sturnus vulgaris*) suggested that interrupted feeding by *C. melanura* might be very common, with rates approaching 40% (Hodgson et al., 2001). However, analysis of over 500 blood meals from mosquitoes collected at the TNF study site demonstrated that only 2% of the blood meals in all EEEV vector species were from more than one host, and only 4% of the blood meals from *C. melanura* were from multiple hosts (TR Unnasch, unpublished data). These data suggest that while interrupted feeding upon captive European Starlings was quite common, this observation did not extend to the behavior of mosquitoes feeding upon free-living birds at the TNF study site. Thus, interrupted feeding is unlikely to play an important role in the dynamics of EEEV transmission in the situation modeled in the current work.

A final simplification of the current model concerns the extrinsic incubation period and time necessary to digest a blood meal in the mosquitoes. Currently, both values are set at 3 days, which correspond to values derived from laboratory studies (Sardelis et al., 2002; Scott and Burrage, 1984; Scott et al., 1984). However, other studies have suggested that both of these values may be influenced by the ambient temperature, which can vary widely across the transmission season (Chamberlain and Sudia, 1955). Thus, modifying the model to convert these values to time dependent parameters might improve the accuracy of the model.

As it is now parameterized, the model reflects the interaction of *C. melanura* with the entire avian population. However, the model may be easily modified to investigate the role that other vector species (and indeed individual bird species) play in the overall transmission of EEEV. Using the model in this way, it may be possible to predict the vector-amplification host pairs that are the most important in maintaining and amplifying EEEV transmission. Furthermore, the results from the model suggest that events resulting in significant short-term mortality in the mosquito population early in the season may significantly affect viral amplification later in the season. Thus, it is possible the environmental factors that result in significant early season mortality (such as a late freeze or a spring drought) may have an effect on EEEV transmission later in the summer. Experiments to address these possibilities are currently underway.

Acknowledgements

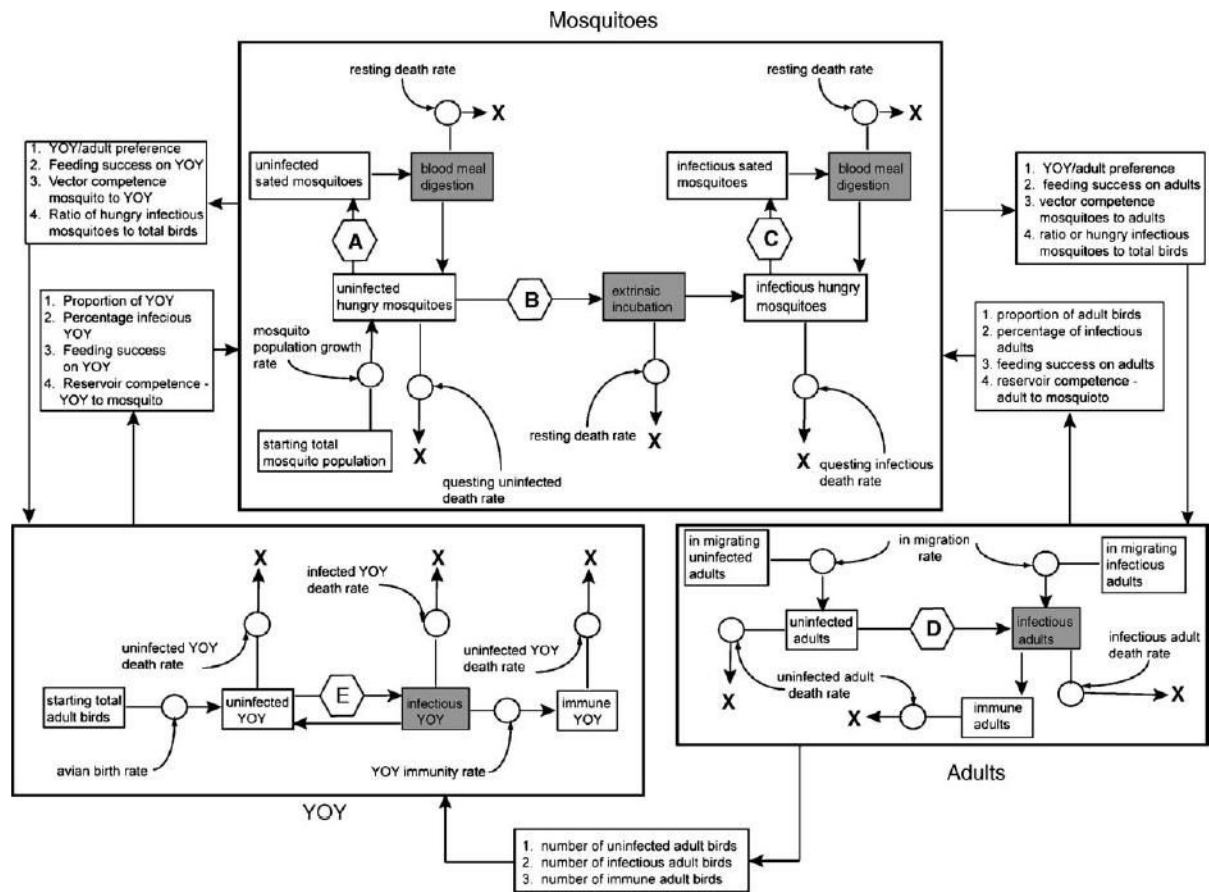
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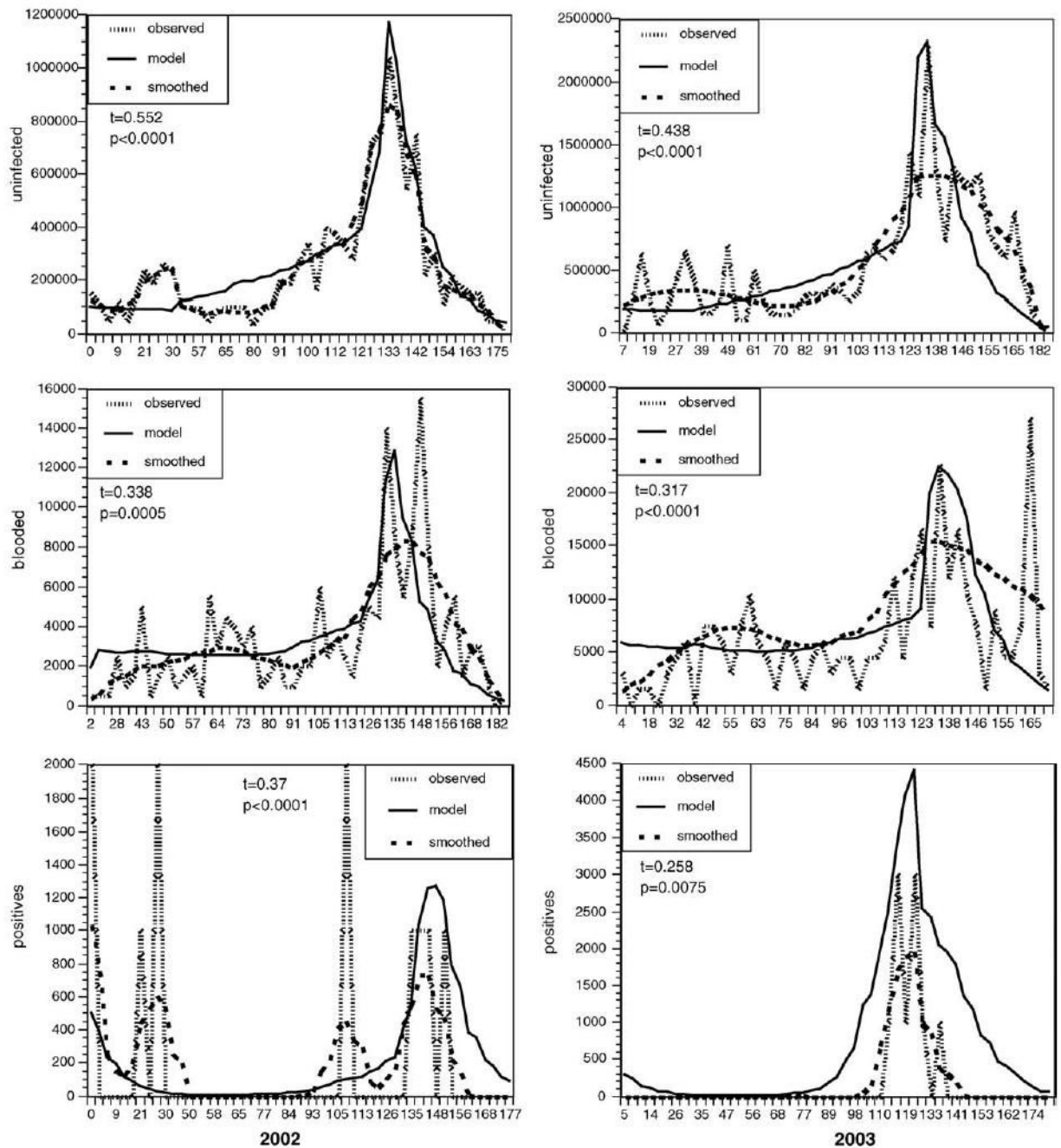
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**Fig. 1.**

A schematic diagram of the model: large boxes highlight the three modules described in the text. Variables that affect the interaction of the three modules are listed in the small boxes interrupting the arrows that connect the modules. The arrows connecting the modules indicate the direction of the interactions among the modules. Within each of the modules, the white boxes indicate stocks of the avian reservoir and vector mosquito species in different states (e.g. uninfected or infectious). Grey boxes indicate conveyors, in which animals moving from one stock to another must rest for a defined period of time before moving to the next stock. The bold Xs indicate death of animals in a given stock, resulting in removal from subsequent iterations of the model. Arrows indicate the direction that animals may move from stock to stock. Arrows interrupted by a circle indicate that movement from one stock to another stock is governed by a single variable. This variable is indicated by the curved arrow pointed to the circle in question. Arrows interrupted by hexagons symbolize that movement between the stocks indicated is controlled by multiple variables, most of which affect the interactions between the modules. The variables affecting the transitions in each of these are as follows: (Hexagon A) number of uninfected adult birds (susceptible and immune), number of uninfected YOY (susceptible, recovered and immune), feeding success on YOY, feeding success on adult birds and YOY/adult relative feeding preference. (Hexagon B) Number of infectious adult birds, number of infectious YOY, feeding success on YOY, feeding success on adult birds, YOY/adult relative feeding preference, YOY reservoir competence and adult bird reservoir competence. (Hexagon C) Total number of adult birds, total number of YOY, feeding success on YOY, feeding success on adult birds and YOY/adult relative feeding preference. (Hexagon D) Number of infectious mosquitoes, number of susceptible adult birds, YOY/adult relative

feeding preference, feeding success on adult birds and vector competence of mosquitoes to adult birds. (Hexagon E) Number of infectious mosquitoes, number of susceptible YOY, YOY/adult relative feeding preference, feeding success on YOY and vector competence of mosquitoes to YOY. A complete list of initial parameter values employed in the model may be found in Table 1.

**Fig. 2.**

Validation of the model: model validation was conducted as described in detail in the text. Comparisons of the model output to the data collected in 2002 are shown in the left panels and comparisons to the 2003 data in the right panels. Values for the Tau statistic and the corresponding p -values from comparisons of the model output and smoothed data are provided in each panel.

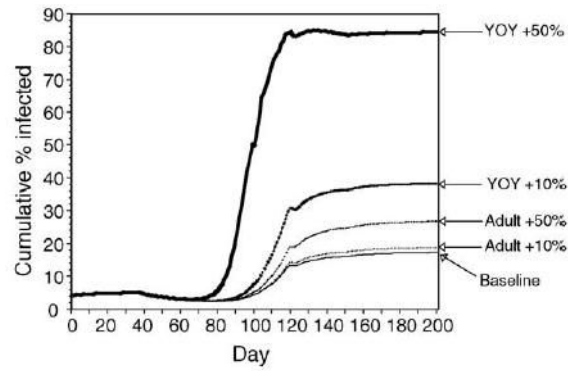
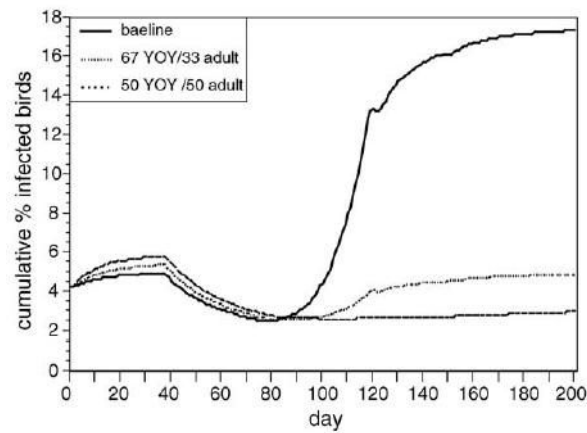


Fig. 3.

Predicted effect of changes in feeding success on the prevalence of infection in the avian population: the initial values for mosquito feeding success on adult birds and YOY were increased by 10 and 50% from their baseline values (0.01 for attacks on adult birds and 0.05 for attacks on YOY). The feeding success on YOY was a time dependent parameter, which was set to become equal to the adult success rate by day 120, as described in the text.

**Fig. 4.**

Predicted effect of changes in the mosquito YOY/adult relative feeding preference in the on the prevalence of infection in the avian population: values for the mosquito YOY/adult relative feeding preference were varied and the predicted effect on the cumulative percentage of infection in the avian population explored. The mosquito YOY/adult relative feeding preference was varied from 80/20 (i.e. a mosquito was four times as likely to choose to attempt to feed upon a YOY as an adult) to 50/50 (an equal probability of choosing a YOY or adult bird). In all simulations, the relative feeding preference was a time dependent parameter, where the preference for adults and YOY was equalized by day 120, as described in the text.

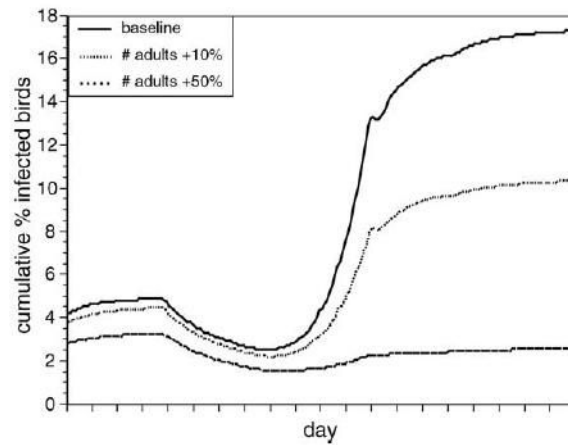
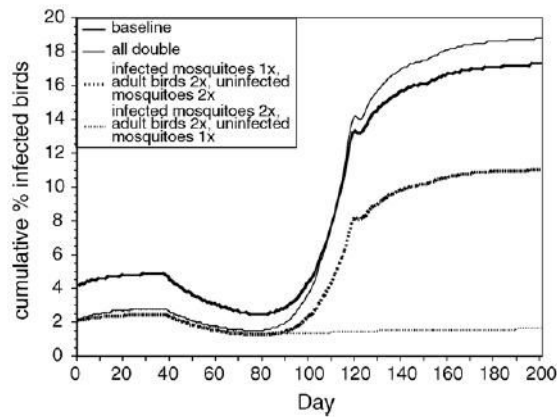


Fig. 5.

Predicted effect of changes in the starting number of susceptible adult birds on the prevalence of infection in the bird population: the starting number of susceptible adult birds was increased by 10 and 50% from the baseline value given in Table 1, and the predicted effect on the cumulative percentage of infected birds was explored.

**Fig. 6.**

Predicted effect of changes in the starting number of uninfected mosquitoes, infectious mosquitoes and adult birds on the prevalence of infection in the bird population: three parameters were co-varied and the effect of these changes upon the cumulative percentage of infection in the bird population explored. In the first simulation, the starting number of infectious mosquitoes, the starting number of susceptible adult birds and the starting number of uninfected mosquitoes were all doubled, keeping the proportion of all three populations identical. In the second simulation, the starting number of infected mosquitoes was kept constant, while the starting number of susceptible adult birds and uninfected mosquitoes were doubled. This kept the ratio of susceptible adult birds to uninfected mosquitoes constant, while halving the ratio of infected mosquitoes to uninfected mosquitoes and halving the ratio of infected mosquitoes to susceptible adult birds. In the third simulation, the starting number of infected mosquitoes and susceptible adult birds were doubled, while keeping the starting number of uninfected mosquitoes constant. This kept the ratio of infected mosquitoes to susceptible adult birds constant, while doubling the ratio of infected mosquitoes to uninfected mosquitoes and halving the ratio of uninfected mosquitoes to susceptible adult birds.

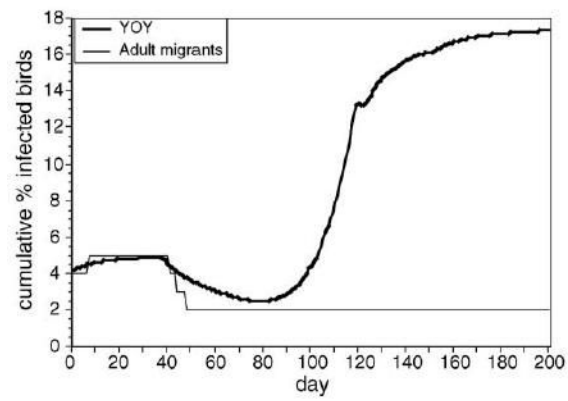


Fig. 7.

Predicted effect of substituting in-migrating susceptible adult birds for YOY on the prevalence of infection in the bird population: YOY were substituted with susceptible in-migrating adult birds following the procedure described in the text.

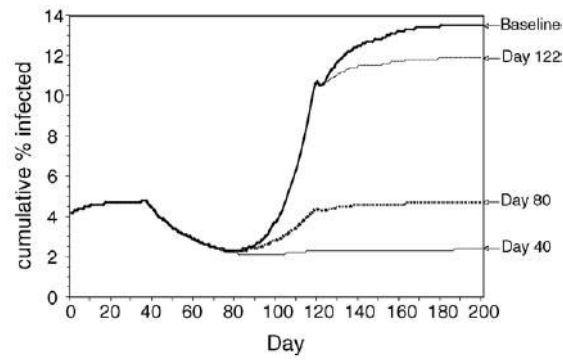


Fig. 8.

Predicted effect of introducing a single mass mortality event in the mosquito population on the prevalence of infection in the bird population: the death rate in the mosquito population was adjusted to include a single day with a mortality rate of 50%, simulating a single aerial insecticide application, as described in the text. The hypothetical control event was modeled to occur at days 40, 80 or 122 (the peak of viral infection in the mosquito population).

Table 1

Initial values of parameters

Parameter	Initial value	Source for value
Avian birth rate	TDP^{a1} : 1–36 0% day^{-1} , 37–78 3% day^{-1} , 79–115 1% day^{-1} , 116–150 0.5% day^{-1} , 151–200 0% day^{-1}	TNF 2001 observations (Unnasch, T.R., Hill, G.E. and Cupp, E.W., unpublished)
YOY immunity rate ^b	TDP: 0–12 days post hatch 0, >12 days post hatch 1.0	(Fair and Myers, 2002; Mast and Goddeeris, 1999)
Uninfected/immune adult bird death rate	0.1% day^{-1}	(Wallace and Mahan, 1975)
Infected adult bird death rate	0.5% day^{-1}	(Stamm, 1966)
Uninfected YOY death rate	TDP: 1–200 0.2% day^{-1}	(Yapp, 1970)
Infected YOY death rate	10% day^{-1}	(Guy et al., 1993)
Starting number of immune YOY	0	TNF 2001 observations (Unnasch, T.R., Hill, G.E. and Cupp, E.W., unpublished)
Starting number of uninfected YOY	0	TNF 2001 observations (Unnasch, T.R., Hill, G.E. and Cupp, E.W., unpublished)
Starting number of susceptible adult birds	1150	TNF 2001 observations (Unnasch, T.R., Hill, G.E. and Cupp, E.W., unpublished)
Starting number of immune adult birds	50	(Bigler et al., 1976; Durden et al., 1997)
In-migrating infected adult birds	0	TNF 2001 observations (Unnasch, T.R., Hill, G.E. and Cupp, E.W., unpublished)
In-migrating uninfected adult birds	0	TNF 2001 observations (Unnasch, T.R., Hill, G.E. and Cupp, E.W., unpublished)
In-migration rate (birds)	TDP: 1–200 0% day^{-1}	TNF 2001 observations (Unnasch, T.R., Hill, G.E. and Cupp, E.W., unpublished)
Time adult birds remain infectious	1 Day	(Komar et al., 1999)
Time YOY remain infectious	3 Days	(Guy et al., 1993)
Mosquito growth rate	TDP: 1–31 7% day^{-1} , 32–118 9% day^{-1} , 119–125 12 → 20% day^{-1} , 126–131 20% day^{-1} , 132–139 1% day^{-1} , 140–200 0% day^{-1}	(Cupp et al., 2003)
Resting mosquito death rate	1% day^{-1}	(Johnson, 1998)
Questing uninfected mosquito death rate	7.5% day^{-1}	(Johnson, 1998; Mokgweetsinyana, 1987; Nasci and Edman, 1984)
Questing infected mosquito death rate	10% day^{-1}	(Scott and Lorenz, 1998)
Starting number of questing infectious mosquitoes	500	(TNF 2001 observations (Unnasch, T.R., Hill, G.E. and Cupp, E.W., unpublished), Cupp et al., 2003)
Starting number of questing uninfected mosquitoes	200000	(TNF 2001 observations (Unnasch, T.R., Hill, G.E. and Cupp, E.W., unpublished), Cupp et al., 2003)
Time needed to digest a blood meal	3 Days	(Service, 1968)
Extrinsic incubation period (mosquito latency)	3 Days	(Sardelis et al., 2002; Scott et al., 1984; Scott and Burrage, 1984)
YOY/adult relative feeding preference	TDP: 1–98 0.8, 99–200 0.5	(Blackmore and Dow, 1958)
Feeding success on adult birds	1%	(Blackmore and Dow, 1958)
Feeding success of YOY	TDP: 1–115 5%, 116–120 5% → 1%, 121–200 1%	(Blackmore and Dow, 1958)
Reservoir competence (infectious bird to mosquito)	1.0	(Scott et al., 1984)
Vector competence adult birds (infectious mosquito to bird)	0.75	(Komar et al., 1999)
Vector competence YOY (infectious mosquito to bird)	1.0	(Howard and Wallis, 1974)

^aTDP, time dependent parameter. Values of time dependent parameters vary in a non-linear manner during the course of the simulation. The values for each iteration (day) in the simulation are given. An arrow (→) indicates that the value was adjusted from the left value to the right value over the period indicated.

^bYOY, young-of-the-year.

Table 2

Input parameters characterized as drivers

Driver	Output statistic				
	Peak number of infectious mosquitoes	Total number of immune adults	Peak number of infectious adults	Peak number of infectious YOY ^a	
Mosquito growth rate	9856.21	18.95	55.75	26.76	
Feeding success on YOY	20.4	7.52	15.53	11.86	
Starting number of uninfected mosquitoes	11.08	4.83	7.87	5.88	
Duration of viremia in YOY	6.43	2.82	3.8	5.06	
Uninfected mosquito death Rate	-3.76	-2.94	-3.69	-4.2	
YOY/adult relative feeding preference	2.92	1.92	2.46	3	
Infected mosquito death rate	-2.62	-1.94	-2.55	-2.63	
YOY reservoir competence	2.48	1.75	2.42	2.64	
Adult reservoir competence	2.5	1.76	2.44	2.47	
Starting number of uninfected adult birds	-2.32	-1.59	-2.24	-2.23	
Feeding success on adults	1.03	1.57	2.2	0.81	

Sensitivity analyses were conducted by varying each parameter over a 10-fold range (or over the entire range of the parameter in cases where the possible range was less than 10-fold) and examining the effect on four output statistics indicative of the level of viral activity, as described in Section 2. The parameters found to disproportionately affect the level of viral activity were designated as drivers, as described in the text. The effect of each driver on each of the output statistics is presented here. Values represent the percent change in the output statistics listed resulting from a 1% relative increase in the value of the driver, averaged over the total range of the sensitivity analysis.

^aYOY, young-of the-year.

Table 3

Effect of the four most sensitive drivers on predicted infection in the avian population

Driver	Increase (%)	Plateau % avian infection	Day plateau reached
None	Baseline	13	118
Mosquito population growth rate	+10	81	119
	+50	100	92
	+100	100	73
	+10	28	120
Feeding success YOY	+50	75	118
	+100	85	117
	+10	22	137
Starting # uninfected mosquitoes	+50	65	138
	+100	86	128
	+10	14	118
	+50	39	118
Duration of viremia in YOY	+100	72	118