Transmission Potential of Chikungunya Virus and Control Measures: The Case of Italy

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Abstract
During summer 2007 Italy has experienced an epidemic caused by Chikungunya virus – the first large outbreak documented in a temperate climate country – with approximately 161 laboratory confirmed cases concentrated in two bordering villages in North-Eastern Italy comprising 3,968 inhabitants. The seroprevalence was recently estimated to be 10.2%. In this work we provide estimates of the transmission potential of the virus and we assess the efficacy of the measures undertaken by public health authorities to control the epidemic spread. To such aim, we developed a model describing the temporal dynamics of the competent vector, known as Aedes albopictus, explicitly depending on climatic factors, coupled to an epidemic transmission model describing the spread of the epidemic in both humans and mosquitoes. The cumulative number of notified cases predicted by the model was 185 on average (95% CI 117–278), in good agreement with observed data. The probability of observing a major outbreak after the introduction of an infective human case was estimated to be in the range of 32%–76%. We found that the basic reproduction number was in the range of 1.8–6 but it could have been even larger, depending on the density of mosquitoes, which in turn depends on seasonal meteorological effects, besides other local abiotic factors. These results confirm the increasing risk of tropical vector–borne diseases in temperate climate countries, as a consequence of globalization. However, our results show that an epidemic can be controlled by performing a timely intervention, even if the transmission potential of Chikungunya virus is sensibly high.

Introduction
During summer 2007 Italy has experienced the first large outbreak caused by Chikungunya virus (CHIKV) documented in a temperate climate country [1]. CHIKV is an arthropod–borne virus which can be transmitted to humans by Aedes mosquitoes [2], widespread in some tropical regions [3–8]. Aedes albopictus is highly competent for CHIKV [9,10]. In Italy, the presence of this mosquito was first documented in Genoa and Padua (Northern Italy) in the earliest 1990’s [11,12]. Over the following years, Aedes albopictus expanded its distribution and is now well established in Northern and Central Italy [13,14]. Having the potential to colonize the Mediterranean basin [15], the species has been reported from most Mediterranean European countries [16]. Samples of Aedes albopictus from the two villages were found to be positive for CHIKV sequences [1].

Sustained transmission of CHIKV was mainly observed in two neighboring villages in Emilia–Romagna region (North-Eastern Italy), namely Castiglione di Cervia and Castiglione di Ravenna [1], comprising 3,968 inhabitants in a built–up area of about 70 ha. The two villages are separated by a river with relatively stagnant water resulting from the presence of a lock. Houses are typically low (two storeys), surrounded by small gardens with many flowers, plants and flower pots. During the outbreak in the streets, drainage systems were visible, indicating open stagnant water underground [17].

A total of 161 laboratory confirmed cases were reported to the enhanced surveillance system developed in the two villages [1]. Sporadic cases, probably due to travel towards the most affected villages and not leading to sustained transmission, were also observed in other areas of the same region [1].

Moreover, a seroprevalence study, conducted on a random sample of residents in the village with the largest number of reported cases, shows a 10.2% of protected individuals [18]. Specifically, 82% were symptomatic – similar to 72.3% estimated in Mayotte, Indian Ocean [19] – 85% of which satisfied the surveillance case definition, 63% of which were identified by the active surveillance system [18]. Higher prevalences were observed in La Reunion Island and in Mayotte, Indian Ocean, 38.2% and 37.2% respectively [19,20].

The index case was recorded on June 23 2007 (a man who had arrived in Italy from India on June 21, [1]). On August 23, after the identification of CHIKV as the pathogen responsible for the ongoing epidemic, a set of interventions were undertaken to control the epidemic spread [1]: breeding sites and eggs removal on August 23; use of adulticides from August 23 to August 25 (3 days) and antilarval measures. Breeding sites were attempted to be removed in the entire area (house–to–house interventions were performed and community participation was encouraged as well) while insecticide interventions were undertaken within a radius of 100 m of each suspected case’s residence (300 m for clusters of cases).
In this study we investigate the transmission potential of CHIKV in Italy, to provide insight into the possible impact of future outbreaks in temperate climate regions, and the effectiveness of the interventions performed during the outbreak, to provide insight into the epidemic control. To such aim, we developed a model describing the temporal dynamics of the competent vector, known as *Aedes albopictus*, explicitly depending on climatic factors, coupled to an epidemic transmission model describing the spread of the disease in both humans and mosquitoes, which allowed us to reproduce several observed features of the epidemic.

**Methods**

In Italy the competent vector for the transmission of CHIKV is *Aedes albopictus* [1]. CHIKV can spread from human to human through bites of adult female mosquitoes. As the dynamics of the vector depends, among several abiotic factors, on meteorological parameters, a population dynamics model accounting for seasonal temperature variations was used to estimate vector abundance. In particular, temperature plays a very significant role as it affects development and mortality rates of *Aedes albopictus* [10,21], influencing vector abundance and distribution over time [22]. The population dynamics model was then coupled to an epidemic transmission model describing the spread of the epidemic in both humans and mosquitoes [see Fig. 1], allowing the estimate of the crucial parameters of the epidemic (e.g. basic reproduction number, effective reproduction number, probability that a major outbreak of the disease would occur after the introduction of a single infective host) and the assessment of intervention strategies.

**Modeling mosquito dynamics**

The main purpose for modeling the dynamics of the vector is to give an approximate estimation of the abundance of female adult mosquitoes during the CHIKV epidemic outbreak in order to get a reasonable value of the ratio of mosquitoes to humans over time, a crucial factor for the calculation of the fundamental parameters of the epidemic and for the assessment of intervention measures. To achieve this goal, a differential equation model, structurally similar to those analyzed in [23–25], was introduced.

The dynamics of the mosquitoes over a land surface of about 70 ha (the extension of the study area) is described by a homogeneous mixing model. Briefly, the model simulates the abundance of the vector in the four life stages of *Aedes albopictus*, namely eggs (*E*), larvae (*L*), pupae (*P*), female adults (*A*), as follows:

\[
\begin{align*}
\dot{E} &= n_E d_A A (1 - \frac{E}{K_E}) - m_E E - d_E E \\
\dot{L} &= d_E E - m_L L - d_L L \\
\dot{P} &= d_L L - m_P P - d_P P \\
\dot{A} &= \frac{1}{2} d_P P - m_A A
\end{align*}
\]

where \(d_E\), \(d_L\), \(d_P\) and \(d_A\) are the temperature dependent developmental rates; \(m_E\), \(m_L\), \(m_P\) and \(m_A\) are the temperature dependent mortality rates; \(n_E\) is the average number of eggs laid in one oviposition; \(K_E\) is the carrying capacity of *E*, the term 1/2 in the fourth equation accounts for the sex ratio (sex ratio is 1:1, as reported in [21]). The four developmental rates correspond to egg hatching (\(d_E\)), pupation (\(d_P\)), adult emergence (\(d_A\)) and gonotrophic cycle (\(d_A\)). Length of the gonotrophic cycles subsequent to the first one and number of eggs laid at each gonotrophic cycles are not significantly different within a range of temperature between 20°C and 35°C [21]. Therefore, we consider only one equation for modeling adults (and not a set of equations, describing transitions through gonotrophic cycles of different length, as in [23] for *Aedes aegypti*) and the number of eggs laid in one oviposition does not depend on temperature.

**Figure 1. Schema of the model.** a Model describing the evolution of the four life stages of the vector (eggs, *E*, larvae, *L*, pupae, *P*, adult females *A*); black boxes and blue arrows), coupled to the epidemic transmission model in vectors (susceptible, *A*, latent, *A*; infectious, *A*; black boxes and red arrows). The dashed blue box and arrow refer to oviposition. b Model describing the epidemic transmission in humans (susceptible, *S*; latent, *E*; infectious asymptomatic, *I*; infectious symptomatic, *R*; recovered, *R*; black circles and red arrows).

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Modeling epidemic transmission

Human host population is assumed to be constant during the epidemic outbreak, given the brief duration of the epidemic compared to the lifespan of humans. We indicate with $N_h = 3968$ the number of humans [1]. As for the epidemic transmission model, hosts are classified as susceptible ($S_h$), latent ($E_h$), infectious symptomatic ($I_h^s$) or asymptomatic ($I_h^a$), and recovered ($R_h$). As only adult female mosquitoes are responsible for virus transmission, adult males are not explicitly represented in the transmission model. Female adult vectors are classified as susceptible ($A_v^s$), latent ($A_v^l$) and infectious ($A_v^i$). A susceptible vector enters the latent class after biting an infectious host at the per capita rate $k_{Th}$, where $k$ represents the biting rate of the vector (i.e., the number of bites to humans per mosquito per day) and $\chi_v$ is the susceptibility to infection of the vectors (i.e., the probability that a mosquito get infected after biting an infectious host). A latent vector enters the infectious class after an average latent period of $1/\omega_v$ days and remains infectious for the rest of its life [26]. Thus, to account for the epidemic transmission process, the 4th Eq. of system (1) is replaced by the following three equations:

\[
\begin{align*}
A &= \frac{1}{2} d_p P - m_A A - \lambda_v A \\
A_v^s &= -m_A A_v^s + \lambda_v A_v^s - \omega_v A_v^i \\
A_v^i &= -m_A A_v^i + \omega_v A_v^i
\end{align*}
\]

where

\[
\lambda_v = k_{Th} (I_h^a + I_h^s)/N_h.
\]

Moreover, as we assume that the infection does not affect oviposition, the first Eq. of system (1) becomes:

\[
E = n_E d_A (A + A_v^s + A_v^i) (1 - E / K_E) - m_E E - d_E E.
\]

A susceptible host enters the latent class following the bite of an infectious vector at the per capita rate $k_{Th}$, where $k$ is the susceptibility to infection of humans. A latent host becomes infectious after an average latent period of $1/\gamma$ days and remains infectious for the rest of its life [26]. Thus, to account for the epidemic transmission process, the 4th Eq. of system (1) is replaced by the following three equations:

\[
\begin{align*}
S_h &= -\lambda_h S_h \\
E_h &= \lambda_h S_h - \omega_h E_h \\
I_h^s &= p_s \omega_h E_h - \gamma I_h^s \\
I_h^a &= (1 - p_s) \omega_h E_h - \gamma I_h^a \\
R_h &= \gamma (I_h^s + I_h^a)
\end{align*}
\]

where

\[
\lambda_h = k_{Th} A / N_h.
\]

In what follows we refer to the full system coupling dynamics of the vector and epidemic transmission process as model $\mathcal{M}$.

### Basic reproduction number

The basic reproduction number $R_0$ of host–vector infectious diseases is the number of secondary infections that arise when a single infective host is introduced into a fully susceptible host population through pathogen transmission by the vector [27]. The average number of hosts directly infected by the introduction of a single infective vector into a fully susceptible host population is given by the transmission probability $k_{Th}$ multiplied by the adult mosquito infectious lifespan (that is, the entire lifespan) $1/m_A$.

\[
R_{0h}^{IV} = k_{Th} N_h / \gamma.
\]

The average number of vectors directly infected by the introduction of a single infective host into a fully susceptible vector population is given by the transmission probability $k_{Th}$ multiplied by the initial number of mosquitoes per human $N_v / N_h$ ($N_v = A + A_v^s + A_v^i$ is the sum of all female adult mosquitoes, regardless of the epidemic status) that survive the latent period (probability: $\omega_v / (\omega_v + m_A)$), multiplied by the human infectious period $1/\gamma$.

\[
R_0 = R_{0h}^{IV} = k_{Th}^2 N_v / \gamma N_h \omega_v / (\omega_v + m_A).
\]

### Model parametrization

On the basis of data presented in [21], we estimated the length of the developmental stages (egg hatching, larval and pupal development) and of the gonotrophic cycle as a function of temperature. To estimate, for instance, the length of the egg hatching period we used the following procedure: let $e_T$ be the length of the egg hatching period for temperatures $T \in T = \{15,20,25,30,35\}$, as reported in [21]. We assume that $e_T = l_g(T; P) + \sigma_T$, where $l_g(T; P)$ is a parametric function of the temperature $T$ ($P$ indicate the set of parameters) in a suitable set of functions, comprising exponential and parabolic functions, and $\sigma_T$ is a random sample of a 0 mean normal distribution with unknown variance $\sigma^2$. The square error $Err$ between predicted and observed length of the egg hatching period is defined as $Err = \sum (e_T - l_g(T; P))^2$. Parameters $P$ were estimated by minimizing $Err$. The variance $\sigma^2$ was computed as the average of the estimated residuals of the model (i.e., the average of the quadratic differences of $(e_T - l_g(T; P))^2$).
between the observed data and the best model fit $l_E(T; P)$. The uncertainty of the parameters was estimated by using a technique similar to that used in [33]. Specifically, we simulated 1000 different $(e_t)_{T∈T^*}$, obtained by perturbing the best-fit $l_E(T; P)$ by adding a simulated error sampled from the normal distribution $N(0, σ^2)$ and we repeated the optimization procedure described above. Finally, the rate of eggs hatching is defined as $d_E(T; P) = 1/l_E(T; P)$. The same technique was used to estimate the length of larval and pupal development and the length of the gonotrophic cycle. Results are reported in Table 1. Fig. 2b shows a comparison between observed and modeled data.

We estimated mortality rates of eggs, larvae and pupae as a function of temperature on the basis of data on the survival rates and modeled data. For a fixed value of temperature $T$, $T_0$ were directly available from [21]. The procedure described above for estimating the length of the developmental stages was used to estimate the mortality rates of all stages as a function of air mean temperature. Since 2008 a monitoring activity has been carried out in order to estimate the water temperature (a key parameter in the developmental stages) of breeding sites. In fact, most of $Aedes albopictus$ life stages develop in aquatic micro-environment. Specifically, a linear regression model was used to estimate the daily mean water temperature as a function of daily mean, maximum and minimum air temperature and daily mean air relative humidity (see Fig. 2a). This allowed us to get estimates of the water temperature for the 2007 season in order to get a more truthful calculation of developmental rates for eggs hatching and immature stages (larvae and pupae), impossible to obtain otherwise.

### Implementation and parameters optimization

To account for the stochastic nature of the processes regulating both dynamics of mosquito and epidemic transmission, we used a discrete–time stochastic version of model $M$, with time-step

### Table 1. Length of the developmental stages.

| Development cycle | $σ^2$ | $P_1$ (95% CI) | $P_2$ (95% CI) | $P_3$ (95% CI) |
|-------------------|------|---------------|---------------|---------------|
| $l_E(T) = p_1−p_2 \exp\left(-(T−20)/p_3\right)$ | 1.03 | 6.9 (5.7, 9.9) | 4.0 (1.8, 7.2) | 4.1 (0.2, 17.2) |
| $l_L(T) = p_1 T^2 + p_2 T + p_3$ | 1.65 | 0.12 (0.08, 0.15) | −6.6 (−8.3, −4.8) | 98 (76.6, 118.8) |
| $l_P(T) = p_1 T^2 + p_2 T + p_3$ | 0.44 | 0.027 (0.018, 0.038) | −1.7 (−2.2, −1.26) | 277 (22.2, 327) |
| $l_T(T) = p_1 T^2 + p_2 T + p_3$ | 0.11 | 0.046 (0.038, 0.049) | −2.77 (−2.96, −2.36) | 45.3 (39.7, 47.8) |

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\( \Delta t = 0.1 \) days. The time step was chosen short enough to obtain, on average, results comparable with those obtained by simulating the deterministic version of the model. Infections (either in vectors or hosts) occur with probability

\[ p = 1 - \exp\{-\lambda \Delta t\} \]

where \( \lambda \) is the force of infection (see for instance [38–40]). For instance, to simulate the number of new infections in mosquitoes at a given time of the simulation, we sample from a binomial distribution with probability \( p \), where \( l \) is given in Eq. (3), and sample size \( A \), where \( A \) is the number of susceptible mosquitoes. Other transitions, e.g. through different life stages of the mosquito or through different epidemic classes, occur at the rate \( r \Delta t \) where \( r \) is the suitable rate (e.g. inverse of infectious period, inverse of the eggs hatching period, etc.).

### Table 2. Mortality rates.

| stage | \( a^2 \) | \( p_1 \) (95% CI) | \( p_2 \) (95% CI) | \( p_3 \) (95% CI) |
|-------|---------|----------------|----------------|----------------|
| \( m_L(T) = p_1 + p_2 \exp(T - p_3) \) | 0.003   | 0.031 (0.028,0.04) | 95820 (2954,98553) | 50.4 (46.9,50.6) |
| \( m_P(T) = p_1 + p_2 \exp(T - p_3) \) | 0.005   | 0.021 (0.018,0.026) | 37 (14,8,57) | 36.8 (35,8,37,2) |
| \( m_A(T) = p_1 + p_2 \exp(T - p_3) \) | 0.012   | 0.029 (0.017,0.041) | 848 (20,6,973) | 43.4 (39,7,43,5) |
| \( m_E(T) = p_1 + p_2 \exp(T - p_3) \) | 0.092   | 506 (43,4,925) | 506 (43,4,925) | 27.3 (18,3,30,8) |

\( r \) is the suitable rate (e.g. inverse of infectious period, inverse of the eggs hatching period, etc.).
Transmission Potential of Chikungunya Virus

Table 3. Epidemic parameters.

| Parameter | Description                        | Value  | Reference |
|-----------|------------------------------------|--------|-----------|
| 1/\(\alpha_h\) | Latent period in humans | 2–4 days | [3,58–60] |
| 1/\(\gamma\)  | Infectious period in humans      | 2–7 days | [58,59,61] |
| \(I_h\)       | Human susceptibility to infections | 50%–80% | [58]      |
| \(p_t\)      | Symptomatic ratio                | 82%    | [18]      |
| \(p_n\)      | Notification ratio               | 54%    | [18]      |
| 1/\(\alpha_m\) | Latent period in mosquitoes      | 2–3 days | [26,58]   |
| \(I_m\)       | Mosquito susceptibility to infections | 70%–100% | [62]      |

All model parameters were calibrated to minimize the score function \(F = \sqrt{\sum (y_t - z_t)^2}\), where \(y_t\) is the observed daily number of notified cases at time \(t\) and \(z_t\) is the daily number of notified cases as predicted by the model at time \(t\) (times \(t\) represent days before the intervention). Model \(M\) outputs the daily incidence of symptomatic cases, \(I_s(t)\), and asymptomatic cases, \(I_a(t)\). Notified cases \(z_t\) at time \(t\) were estimated by sampling from a binomial distribution of size \(n = I_s(t)\) and probability \(p = p_n\), where \(p_n\) is the notification ratio \(p_n = 0.54\) [18].

Latin Hypercube Sampling (LHS) allows an efficient sampling of the parameter space which requires a smaller sample size than simple sampling to achieve the same accuracy [41]. LHS was used to build \(n_Q = 1000\) sets of parameters \(Q \equiv \{q_1, \ldots, q_{n_Q}\}\) (uniform sampling was used to determine the \(n_Q\) values of each model parameter) and thus, through model simulations, \(n_Q\) time series of predicted notified cases \(\{z_t(q)\}_{q \in Q}\). The optimal parameter set \(q^*\) was chosen as the one minimizing the score function, i.e. \(q^* = \arg \min_{q \in Q} F(q)\). We repeated the above described procedure 100 times. This allowed us to estimate distributions of model parameters and, consequently, of the other quantities of interest (e.g. \(R_0\), probability of major outbreak, attack rate). The index case was recorded on June 23 2007 (a man who had arrived in Italy from India on June 21, [1]) and thus we initialized all simulations with 1 infected individual on June 23. Results of the optimization procedure are shown in Fig. S1. Fig. S2 shows that 100 simulations are sufficient to obtain meaningful distributions of parameters and quantities of interest.

Results and Discussion

In summer 2007, an outbreak of chikungunya fever affected the Italian provinces of Ravenna, Cesena-Forli, Rimini and Bologna [1,42–44]. Health authorities identified 214 laboratory-confirmed cases with date of onset from July 15 to September 28 2007. Most cases (161) occurred in the two neighboring villages of Castiglione di Cervia and Castiglione di Ravenna, but five smaller clusters of local transmission were also detected in five towns in the same region (i.e., Cervia, Cesena, Ravenna, Rimini, and Bologna) which are located 9 to 75 km from the initially affected villages [1,42–44], see Fig. 3.

Model \(M\) was parametrized to describe epidemic spread only in Castiglione di Cervia and Castiglione di Ravenna. Daily estimates of the number of vectors over time \(N_v\) were obtained by the vector dynamics model. The ratio of mosquitoes to humans was estimated to be in the range of 10–35 during the peak mosquito activity (Fig. S3 show the predicted dynamics of the vector for different numbers of breeding sites \(B\)). By fitting model \(M\) to notification data up to August 23 (the day before intervention) and by assuming \(B = 200\) ha\(^{-1}\) we estimated \(k\) to be 0.09 days\(^{-1}\) (95% CI 0.05–0.16 days\(^{-1}\), see Fig. 4b). We recall that the explored range for \(k\) through the LHS procedure was [0,1]. Good fit to data were obtained for values in the entire range explored for all the other model parameters (see Fig. S4).

Figure 3. Study area. Geographical position of the two most affected villages (Castiglione di Cervia and Castiglione di Ravenna, in red), and municipalities where clusters of local transmission were observed (grey areas represent municipalities, blue points represent the geographic position of the main towns within municipalities).

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The dashed black line identifies the threshold value of the ratio of vectors to humans and vectors from humans to mosquitoes and from mosquitoes to humans, and change substantially by varying the number of breeding sites. The average value of $N_v$ for vectors should be considered that $R_0$ depends on the number of biting rate of $R_B$, as the biting rate controls transmission and its uncertainty from Eq. (8). Besides parameters precisely defined, the model. We estimated $R_0$ for the initial phase of the epidemic), as predicted by the vector dynamics and considering seasonal variations in transmission, it is difficult to precisely define $R_0$. Therefore, we computed $R_0$ by considering the average value of $N_v$ from June 21 to July 26 2007 (i.e. the initial phase of the epidemic), as predicted by the vector dynamics model. We estimated $R_0$ to be 3.3 on average (95% CI 1.8–6, see Fig. 4c). The estimated biting rate increases by decreasing the number of breeding sites and, consequently, estimates of $R_0$ do not change substantially by varying the number of breeding sites $B = 50 \text{ ha}^{-1}$: $k = 0.18 \text{ days}^{-1}$, 95% CI 0.1–0.26 days$^{-1}$, $R_0 = 3.2$, 95% CI 1.9–5.5; $B = 100 \text{ ha}^{-1}$: $k = 0.13 \text{ days}^{-1}$, 95% CI 0.08–0.2 days$^{-1}$, $R_0 = 3.2$, 95% CI 1.9–5.2; $B = 150 \text{ ha}^{-1}$: $k = 0.1 \text{ days}^{-1}$, 95% CI 0.06–0.15 days$^{-1}$, $R_0 = 3.4$, 95% CI 2.1–5, see Fig. 4b and Fig. 4e). Fig. 4d shows that $R_0$ is below the critical threshold for all values of $B$ and thus the epidemic is mainly determined by $R_{HV}$, i.e. by transmission from humans to vectors. As for the effective reproduction number $R_e$ (i.e. the average number of secondary cases generated per primary case at a given time), which accounts for both depletion of susceptible individuals and mosquito dynamics, its value over time is shown in Fig. 4c and Fig. 4f (by considering or not interventions). It emerges that $R_e$, which does not change much by varying the number of breeding sites, can vary substantially over time as an effect of mosquito density. This suggests that $R_0$ could have been even larger, depending on the time of epidemic seeding.

Recently, it has been demonstrated using mathematical modeling in the context of dengue that it is possible to generate outbreaks even in cases when $R_0 < 1$ provided that the vector-to-human component of $R_0$ is greater than one and that a certain number of infected vectors are introduced into the affected population [45]. However, it has been demonstrated that the index case was a man of Indian origin from Kerala living in Castiglione di Cervia, without history of traveling during the previous year [1]. He only reported contact with a relative of his, who had arrived in Italy on June 21 2007 from Kerala, India (a region of India affected by the CHIKV epidemic), and visited him twice from July 22 to 25 and 28 to 29 2007. The index case was a man of Indian origin from Kerala living in Castiglione di Cervia, without history of traveling during the previous year [1]. He only reported contact with a relative of his, who had arrived in Italy on June 21 2007 from Kerala, India (a region of India affected by the CHIKV epidemic), and visited him twice from July 22 to 25 and 28 to 29 2007.

Figure 4. Potential transmission of CHIKV. a Distribution (2.5%, 25%, 50%, 75% and 97.5% percentiles) of the probability of observing a major outbreak for different numbers of breeding sites. b As a but for the biting rate. c Average effective reproduction number (black line, scale on the left) and 95% CI (grey area), and average density of mosquitoes (green line, scale on the right) over time by assuming $B = 200 \text{ ha}^{-1}$ and no interventions. The uncertainty of $k$ depends on the uncertainty of all model parameters. Unfortunately, no field data are available for the study area to validate these results. In [37], plausible values for the biting rate and the ratio of mosquitoes to humans in Europe are considered to be $k = 0.25 \text{ days}^{-1}$ and $N_v/N_h = 20$ (based on published and non published data, e.g. [10]). However, we acknowledge that it might be misleading to compare these results with others carried out in other localities. In fact, abundance and biting rate of Aedes Albopictus are strongly affected by abiotic factors, both climatic and environmental (e.g. presence of other hosts).

Estimates of the biting rate and its uncertainty allowed us to estimate $R_0$ and its uncertainty from Eq. (8). Besides parameters more strictly related to the infectious process, $R_0$ is an increasing function of the square of $k$, as the biting rate controls transmission from humans to mosquitoes and from mosquitoes to humans, and the ratio of vectors to humans $N_v/N_h$ (see Fig. S5). However, it should be considered that Eq. (8) depends on the number of vectors $N_v$ which substantially varies over time as a result of seasonal meteorological factors. Thus, as for models explicitly considering seasonal variations in transmission, it is difficult to precisely define $R_0$. We estimated $R_0$ during the index case, the average number of infected vectors is introduced into the affected population [45]. However, it was demonstrated that the case was a man of Indian origin from Kerala living in Castiglione di Cervia, without history of traveling during the previous year [1]. He only reported contact with a relative of his, who had arrived in Italy on June 21 2007 from Kerala, India (a region of India affected by the CHIKV epidemic), and visited him twice from July 22 to 25 and 28 to 29 2007.

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in Castiglione di Cervia village on June 23, while feverish. Therefore, having the human index case being identified, we can reasonably exclude the contemporaneous introduction of infected vectors in the two villages. Moreover, our estimates show that \( R_0 \) is well below the critical threshold.

As several cases were reported in Italy among travelers returning from endemic areas [46] (only one, however, in the study area; additional imported cases throughout the duration of the outbreaks were not detected), the question arises why no previous outbreaks of CHIKV occurred in other Italian regions. By assuming \( B = 200 \text{ ha}^{-1} \), we estimated the probability \( p \) (see Eq. 9) that a major outbreak of the disease would occur after the introduction of a single infective host to be 0.59 (95% CI 0.35–0.76, see Fig. 4a). Estimates do not change substantially by varying the number of breeding sites \( B = 50 \text{ ha}^{-1} \) : \( p = 0.51 \), 95% CI 0.29–0.7, see Fig. 4a; \( B = 100 \text{ ha}^{-1} \) : \( p = 0.55 \), 95% CI 0.32–0.71, see Fig. 4a; \( B = 150 \text{ ha}^{-1} \) : \( p = 0.59 \), 95% CI 0.4–0.73, see Fig. 4a). For values of the biting rate \( k \) in a given range, results indicate that major outbreaks are possible only for large enough values of \( N_h/N_v \) (see Fig. S5) and, by assuming the same density of mosquitoes, epidemic outbreaks are more likely in rural areas with respect to urban areas – as the human population density is much lower in the former. This could explain why cities like Cesena (96,000 inhabitants), Rimini (141,000 inhabitants) and Bologna (377,000 inhabitants) and Ravenna (157,000 inhabitants) located in the same region of the two most affected villages did not experience any epidemic outbreak, though sporadic CHIKV cases were recorded in the same period [1,42–44]. These results support the hypothesis that outbreaks of Chikungunya virus in those temperate climate countries characterized by high density of *Aedes albopictus* are probable after the importation of an index case from abroad.

The potential epidemic trajectory in the absence of interventions by assuming \( B = 200 \text{ ha}^{-1} \) is shown in Fig. 5a. The resulting cumulative attack rate (i.e., the percentage of symptomatic cases in the population at the end of the epidemic) was estimated to be 73.4% of the population (95% CI 57.8%–81.3%, see also Fig. 5b). Results do not change much by varying \( B \) \( B = 50 \text{ ha}^{-1} \) : 74%, 95% CI 55.3%–81.6%, see also Fig. 5b; \( B = 100 \text{ ha}^{-1} \) : 73.9%, 95% CI 55.8%–81.5%; \( B = 150 \text{ ha}^{-1} \) : 75%, 95% CI 57.3%–81.3%). Much lower prevalence values have been estimated in La Reunion Island and in Mayotte, namely 38.2% and 37.2% respectively. However, these estimates are hardly comparable with our model predictions as these territories have benefited from high resource allocation to mitigate the epidemic [19].

As for the undertaken interventions, breeding sites and eggs were removed on August 23 2007, larvicides were used on August 23 (effect lasting 30 days), and adulticides were used from August 23 to August 25 2007. Through model simulations, we evaluated the effects of strategies mimicking the timing of the actual interventions undertaken in Italy. As for the effects in terms of reduction of breeding sites, eggs, larvae and adults, likely values

![Figure 5. Baseline simulations and reference interventions.](https://www.plosone.org/figures acompanying text)
are: 20% to 60% as for reduction breeding sites and eggs, 80% to 95% as for reduction of larvae and 50% to 95% as for reduction of adults. We discuss first results obtained by assuming 40% as for reduction of breeding sites and eggs, 90% as for reduction of larvae and 95% as for reduction of adults. The effects of such an intervention are shown in Fig. 5c. The resulting cumulative attack rate, by assuming \( B = 200 \) ha\(^{-1}\), was estimated to be 8.7% (95% CI 5.6%–12.7%, see Fig. 5d), in good agreement with the observed value, namely 8.4%, computed by multiplying the overall observed prevalence, 10.2% \([18]\), by the probability of developing clinical symptoms, 0.82 \([18]\).

Red: distribution (2.5%, 25%, 50%, 75% and 97.5% percentiles) of the cumulative attack rate (only symptomatic cases are considered) by assuming the same intervention as in Fig. 5c and Fig. 5d. The resulting cumulative attack rate, by assuming no interventions (baseline scenario) as in Fig. 5a and Fig. 5b, and \( B = 200 \) ha\(^{-1}\). As shown in Fig. 6a, reduction of eggs and breeding sites could be effective only by hypothesizing a massive intervention (cumulative attack rate is reduced on average from 73% to 55% by reducing eggs and breeding sites of 60%); adulticides do not contribute much to reducing the overall number of cases (cumulative attack rate is reduced on average from 73% to 60%); larvicides contribute to a substantial reduction of the overall number of cases (cumulative attack rate is reduced on average from 73% to 40%); In fact, larvicides are effective for a prolonged period of time and thus can contribute to decrease systematically the number of adults for a long period of time and, consequently, to substantially reduce the overall attack rate. Quite the contrary, adulticides were used for a very limited period of time (3 days) and thus their effect is limited due to the rapid increase of adults suddenly after the intervention. Overall, these results suggest that only a combined intervention, as the one performed during the outbreak, can result in a drastic decrease of the number of cases.

Five smaller clusters of local transmission were detected in five towns in the same region (i.e., Ravenna, Cervia, Cesena, Rimini, and Bologna). Cervia and Ravenna are the main towns of the municipalities where the two most affected villages (Castiglione di Cervia and Castiglione di Ravenna) are located. The two affected villages account for the 2.1% of the population of the municipalities of Cervia (27,000 inhabitants) and Ravenna (157,000 inhabitants). By analyzing commuting data of the Emilia–Romagna region \([47]\), we found that the number of individuals traveling daily to Cervia and Ravenna for work or study is 8,787 (from 249 different municipalities), and the number

Figure 6. Sensitivity analysis. a Red: distribution (2.5%, 25%, 50%, 75% and 97.5% percentiles) of the cumulative attack rate (only symptomatic cases are considered) by assuming no interventions (baseline scenario) as in Fig. 5a and Fig. 5b, and \( B = 200 \) ha\(^{-1}\). Green: as in the baseline scenario but for reductions of breeding sites and eggs. Blue: as in the baseline scenario but for reductions of larvae. The horizontal dashed red line represents the observed attack rate (symptomatic cases, obtained by multiplying the observed prevalence, 10.2% \([18]\), by the probability of developing clinical symptoms, 0.82 \([18]\)). b Red: distribution (2.5%, 25%, 50%, 75% and 97.5% percentiles) of the cumulative attack rate (only symptomatic cases are considered) by assuming the same intervention as in Fig. 5c and Fig. 5d (reference scenario), namely reduction of 40% as for breeding sites and eggs, 90% as for larvae and 95% as for adults, and \( B = 200 \) ha\(^{-1}\). Green: as in the reference scenario but for different reductions of breeding sites and eggs. Blue: as in the reference scenario but for different reductions of adults. The horizontal dashed red line represents the observed percentage of symptomatic cases as resulting from survey data \([18]\). doi:10.1371/journal.pone.0018860.g006
of persons traveling daily from Cervia and Ravenna to other municipalities is 10,861 (towards 139 different municipalities). The exact number of commuters for Castiglione di Cervia and Castiglione di Ravenna is unknown but it should not exceed 2.1% of the overall number of commuters. However, the probability of traveling from/to a certain municipality should be similar to that observed for the two municipalities as a whole and we found that clusters of local transmission were recorded in municipalities well connected with the municipalities of Ravenna and Cervia (see Table 4). For at least four of the five clusters, population movement (i.e., persons who visited the area that was primarily affected or persons from the primarily affected area who visited one of the four towns) can be reasonably assumed to have been the main determinant of local transmission. Another possible explanation is passive vector mobility (e.g., infected mosquitoes transported by car from the initial cluster), since the flight range (active mobility) is usually considered to be less than 1 km [35,48,49].

Our results suggest that the transmission potential of CHIKV in Italy was similar to the one observed in tropical regions where Chikungunya fever is widespread (e.g., Reunion Island, where the best estimate for the initial $R_0$ was 3.7 [3]). Specifically, we estimated $R_0$ to be in the range of 1.8–6. However, being the reproduction number strongly dependent on the density of mosquitoes, which in turn varies a lot over time as a consequence of seasonal meteorological effects, different (even larger) values of $R_0$ could be observed in future outbreaks, depending on the time of epidemic seeding. In [3], by adapting a method originally introduced in [50] for human–to–human infections, $R_0$ was estimated from the generation interval probability distribution function and the number of gonotrophic cycles of the mosquito. This method can not be applied in our study, as the undertaken control measures have contributed to alter the gonotrophic cycles of the mosquito in an indeterminable manner. We found that the probability of observing a major outbreak after the introduction of an index case depends on the ratio of mosquitoes to humans and was estimated to be in the range of 32%–76%. These results confirm the high risk to Europe of tropical vector–borne diseases as a consequence of globalization, which has been modifying the mobility of humans and vectors. Climate changes could have been playing a role, as the geographical limits of mosquito-borne diseases can be influenced by climate [51,52], but this is still debated [53–55].

Moreover, our analysis strongly support the efficacy of the disinfestation strategy performed during the Italian outbreak, which drastically contributed to reduce the cumulative attack rate (of about 88%), though the application of self-protection preventive measures (insect repellents and window screens) could also have played a role [18]. Therefore, even if the transmission potential of Chikungunya virus could be sensibly high also in temperate climate countries, the epidemic can be controlled by performing timely interventions.

The proposed model has several limitations. We assume exponential distribution for all parameters of model $M$ related to the length of the different periods (e.g., latency, infectiousness, etc.), though it would be preferable to use multiple classes within each group to give more realistic gamma distributed lifetimes (see for instance [56]). We assume density-dependent growth only in eggs, though other density-dependent regulating processes should be considered for other lifestages of the mosquitoes, e.g., larvae and pupae [25]. These modeling choices are due to the lack of data for parametrizing the model. The lack of information on the actual number of breeding sites – it could be assessed only by performing a field study – prevent us to give precise estimates on the density of mosquitoes over time in the study area. However, we would note that our estimates of $R_0$, attack rates and probability of major outbreak are robust with respect to assumptions on the number of breeding sites. Moreover, the temporal dynamics of the vector is qualitatively well captured by model $M$ (though not in terms of absolute abundance) and this allowed us to clarify whether or not the sharp decrease in the number of cases observed after the intervention was due to the intervention itself or to the spontaneous reduction of adults due to decrease of temperature. Definitely, a key lesson learnt from the analysis of the Chikungunya outbreak in Italy is the necessity to improve tools for obtaining reliable, though costly, estimates of the vector density (see for instance [57]) – thus bypassing the necessity of developing ad-hoc models. The proposed model, describing the temporal dynamics of Aedes albopictus, provides a valid alternative in the absence of reliable field data.

**Supporting Information**

Figure S1 Parameters optimization. a Green points represent the values of the score function $F$ plotted versus the number of notified cases as predicted by the model (with $B=200$ ha$^{-1}$) before intervention for $Q$ different values of the model parameters as obtained by the LHS procedure. Red point represents the minimum of $F$. Black points represent the values of the score function $F$ plotted versus the number of notified cases as predicted by the model before intervention as obtained by repeating 100 times the optimization procedure. The inset shows the minimum of $F$ for the 100 replicates (red points). The blue vertical line represent the number of notified cases reported to the surveillance system before intervention, namely 132. b As a but for $B=50$ ha$^{-1}$. (TIF)

Figure S2 Results for increasing number of simulations. a Mean (red points), median (blue points) and 95% CI (shaded grey area) of $R_0$ for increasing number of simulations with $B=200$ ha$^{-1}$ in the absence of interventions (baseline scenario). b As a but for probability of observing a major outbreak. c As a but for the cumulative number of symptomatic cases. d As a but for the biting rate. (TIF)

Figure S3 Temporal dynamics of the mosquito. a Average density (number per ha) of adult female mosquitoes over time as predicted by the model by assuming $B=200$ ha$^{-1}$ (green line) and 95% CI (grey area). b As a but for $B=150$ ha$^{-1}$. c As a but for $B=100$ ha$^{-1}$. d As a but for $B=50$ ha$^{-1}$. (TIF)

**Table 4. Commuting patterns.**

| municipality | origin (rank) | destination (rank) | cases [63] |
|--------------|---------------|--------------------|-------------|
| Cesena       | 18% (2)       | 10% (3)            | 15          |
| Rimini       | 2.9% (10)     | 3.1% (10)          | 6           |
| Bologna      | 9.5% (3)      | 1.6% (13)          | 5           |
| Cervia       | -             | -                  | 19          |
| Ravenna      | -             | -                  | 9           |

Probability of origin and destination (and rank over all possible origins/destinations) of individuals commuting daily for work or school from/to other municipalities were local clusters of transmission were observed. The two most affected villages are located in the municipalities of Cervia and Ravenna. doi:10.1371/journal.pone.0018860.0004
**Figure S4** Range of the optimal parameters values. Distribution of the model parameters (2.5%, 25%, 50%, 75% and 97.5% percentiles) after LHS optimization. Numbers below and over the boxplot represent the explored range of values.

**Figure S5** Epidemic threshold and probability of major outbreak. **a** Epidemic threshold \( R_0 \) in relation to biting rate \( k \) and ratio of mosquitoes to humans \( N_h/N_b \). The black line represents the average threshold condition and the shaded blue area represents 95% CI, as resulting from uncertainty of model parameters. The red rectangle identifies the likely range of the two parameters in the two Italian villages affected by CHIKV. **b** Probability of observing a major outbreak as a function of the ratio of mosquitoes to humans \( N_h/N_b \) for two extreme values of the biting rate \( k \), namely \( k = 0.1 \) days\(^{-1} \) in red (solid line black represents the average probability and the shaded area represents 95% CI) and \( k = 0.2 \) days\(^{-1} \) in blue.

**Figure S6** Baseline simulations and reference interventions. **a** Average daily number of symptomatic notified cases as predicted by the model in the absence of interventions (baseline scenario, blue line, scale on the left) and 95% CI (grey area) by the model. **b** Epidemic threshold and probability of major outbreak: \( R_0 \) in relation to biting rate \( k \) and ratio of mosquitoes to humans \( N_h/N_b \). The black line represents the average threshold condition and the shaded blue area represents 95% CI, as resulting from uncertainty of model parameters. The red rectangle identifies the likely range of the two parameters in the two Italian villages affected by CHIKV. **b** Probability of observing a major outbreak as a function of the ratio of mosquitoes to humans \( N_h/N_b \) for two extreme values of the biting rate \( k \), namely \( k = 0.1 \) days\(^{-1} \) in red (solid line black represents the average probability and the shaded area represents 95% CI) and \( k = 0.2 \) days\(^{-1} \) in blue.

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**Author Contributions**

Conceived and designed the experiments: SM GM CR MA. Performed the experiments: PP RV MA SM. Analyzed the data: PP RV GM CR SM. Wrote the paper: SM MA PP CR RV GM.
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