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## RESEARCH ARTICLE

## Parasitized or non-parasitized, why? A study of factors influencing tick burden in roe deer neonates

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**Data Availability Statement:** All the data and R code required to rerun the analyzes have been included in a R package named tickTF available on Github (<https://github.com/ClementCalenge/tickTF>). The package tickTF is also available on Zenodo at the following URL: [https://zenodo.org/record/5764798#\\_Ya-RxiXjJhE](https://zenodo.org/record/5764798#_Ya-RxiXjJhE) A DOI has been assigned to this package by Zenodo ([10.5281/zenodo.5764798](https://doi.org/10.5281/zenodo.5764798)). However, we think that it will be much easier for the reader to install it through github, as the R software provides functions that easily allow to install it directly from this website.

## Abstract

*Ixodes ricinus*, the most common species of tick in Europe, is known to transmit major pathogens to animals and humans such as *Babesia* spp. or *Borrelia* spp. Its abundance and distribution have been steadily increasing in Europe during recent decades, due to global environmental changes. Indeed, as ticks spend most of their life in the environment, their activity and life cycle are highly dependent on environmental conditions and therefore, on climate or habitat changes. Simultaneously, wild ungulates have expanded their range and increased dramatically in abundance worldwide, in particular roe deer (*Capreolus capreolus*), allowing tick populations to grow and spread. Currently, tick infestation on newborn wild ungulates is poorly documented. However, newborn ungulates are considered more sensitive to tick bites and pathogen transmission because of their immature immune systems. Thus, improving knowledge about the factors influencing tick infestation on newborns is essential to better understand their health risks. This study was conducted at Trois-Fontaines Forest, Champagne-Ardenne, France (1992–2018). Based on a long-term monitoring of roe deer fawns, we used a novel Bayesian model of the infestation of fawns to identify which biotic or abiotic factors were likely to modify the level of infestation by ticks of 965 fawns over time. We show that tick burden increased faster during the first days of life of the fawns and became constant when fawns were five days old and more, which could be explained by the depletion of questing ticks or the turnover of ticks feeding on fawns. Moreover, despite the known positive influence of humidity on tick activity, the tick burdens were weakly related to this parameter. Our results demonstrate that tick infestation was highly variable among years, particularly between 2000–2009. We hypothesize that this results from a modification of habitat caused by Hurricane Lothar.

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**Competing interests:** The authors have declared that no competing interests exist.

## Introduction

Ticks are considered to be one of the most important arthropod vector of diseases in the world [1]. They are of major concern as they can transmit several pathogens to their host during their blood meal, such as viruses, bacteria, or protozoans [2, 3]. They can also have direct negative effects to their host, such as blood loss (known as spoliation effect [4]) or destruction of host tissue by enzymes present in their saliva [5]. Over past decades, these ectoparasites have become an increasing worldwide health burden, threatening both humans and animals [6]. The abundance and distribution of ticks can strongly vary among areas and have considerably increased in recent decades [7–9]. In order to understand the population dynamics and the geographic distribution of ticks, and to predict the risks of transmission of tick-borne pathogens, it is of major concern to determine the factors driving the abundance and spread of these parasites [10].

In Central Europe, *Ixodes ricinus* is the most common hard-tick species. It is of particular concern due to their major role as a vector of many pathogens for both animals and humans, such as *Anaplasma* spp. [11], *Babesia* spp. [12], *Borrelia* spp. [13], and tick-borne encephalitis virus [14]. This three-host tick species take one blood meal on their host per life stage (3 life stages: larva, nymph, and adult), with blood meals lasting for a few days (2–12 days according to their life stage; [15, 16]) before dropping off the host onto the ground where they molt into the next stage or lay eggs and die [17]. As this tick spends most its life free in the environment, its survival, development, and questing activity are highly dependent on the weather conditions and microclimate [18, 19], including temperature and relative humidity or saturation deficit [20, 21]. Ground-based vegetation that retains moisture and provides shadow is therefore highly important for ticks, explaining the abundance of ticks in the leaf litter and low strata vegetation of woodlands and forests [7, 22], particularly deciduous forests containing oak and beech, with an important shrub cover [23, 24].

The life cycle of *I. ricinus* is also highly dependent on the availability of hosts, because of their questing behavior (i.e., the “ambush” technique; [7]). This ectoparasite species is a ubiquitous tick in Europe, which can parasitize a wide range of animal species, including mammals, reptiles, and birds [25]. Larvae and nymphs of *I. ricinus* are commonly found on small animals such as rodents [26], although they can also be seen on larger hosts such as sheep or roe deer [27, 28]. Large vertebrates are the main suitable hosts for blood meals and mating of adult ticks [23]. The infestation consequences on the host will be greater especially if these parasites are found in large quantities [4].

Hosts can develop an immune response against ticks to decrease their fixation time and engorgement, and hence, the spoliation effect and risk of pathogen transmission [29, 30]. Resistance to tick infestation implicates both acquired and innate immunity [31]. However, ticks can circumvent host defenses with active components secreted in their saliva which can induce host immunosuppression and facilitate acquisition of blood meals for ticks and the transmission of the tick-borne pathogens [32]. This is why newborns can be considered more sensitive to tick bites. Indeed, they have a naïve and immature immune system even if they are not totally vulnerable thanks to the transmission of acquired immunity by the colostrum of the mother [33, 34]. In addition, failure in the transfer of humoral immunity from mothers can happen and increase the susceptibility of newborns to tick bites [35, 36].

The cervids, and particularly the roe deer (*Capreolus capreolus*), are considered key hosts for the population dynamics of *I. ricinus* [23]. During recent decades, their population has been increasing in the Northern Hemisphere [37, 38], which, in turn, has led to the increase and geographical spread of tick populations [10, 23, 39–41]. Tick activity is most important in spring and egg laying by female ticks occurs at the same time as the birth period of fawns, i.e.,

around the month of May [42, 43]. Furthermore, fawns of roe deer have a hider behavior (i.e., they hide in the vegetation cover to limit the probability of detection by a predator; [44]) and therefore, have a preference for dense vegetation. Their naïve immune system also makes them an easy target for questing ticks even if their movements are limited during the first days of life [45, 46]. To our knowledge, no study has yet investigated the factors influencing the level of infestation by ticks on roe deer neonates.

Based on previous knowledge on ticks, we predicted a higher tick burden (H1) during warm and wet spring conditions as it favors tick activity. We also predicted (H2) an influence of habitat on tick infestation, with more parasitized roe deer fawns living in habitats with important shrub cover and ground-based vegetation which retains humidity; and (H3) an influence of the density of roe deer on tick burden, with the highest values of infestation by ticks during the years when roe deer density, calculated the winter preceding the catches of fawns, is the highest. Indeed, as the intensity of tick burden on deer increases with high host populations densities, we can assume that ticks will be more abundant in the environment and parasitize fawns more [10, 47].

Based on a long-term monitoring of roe deer neonates from 1992 to 2018 in the population of Trois-Fontaines in France, we studied the factors influencing the tick burden of newborn roe deer fawns. This long-term monitoring of the infestation of fawns during their first days of life by the tick, *I. ricinus*, allowed us to study the effects of factors linked to the host (individual and populational factors) on tick risks, but also of variations in spring weather conditions (temperature, humidity, extreme events), and habitat characteristics. We developed a Bayesian model of the tick infestation process, describing how the number of ticks on a roe deer fawn changes with time, accounting for biotic factors (e.g., fawn body mass) as well as abiotic factors (e.g., humidity, temperature) that are likely to affect the rate of infestation of fawns by ticks.

## Materials and methods

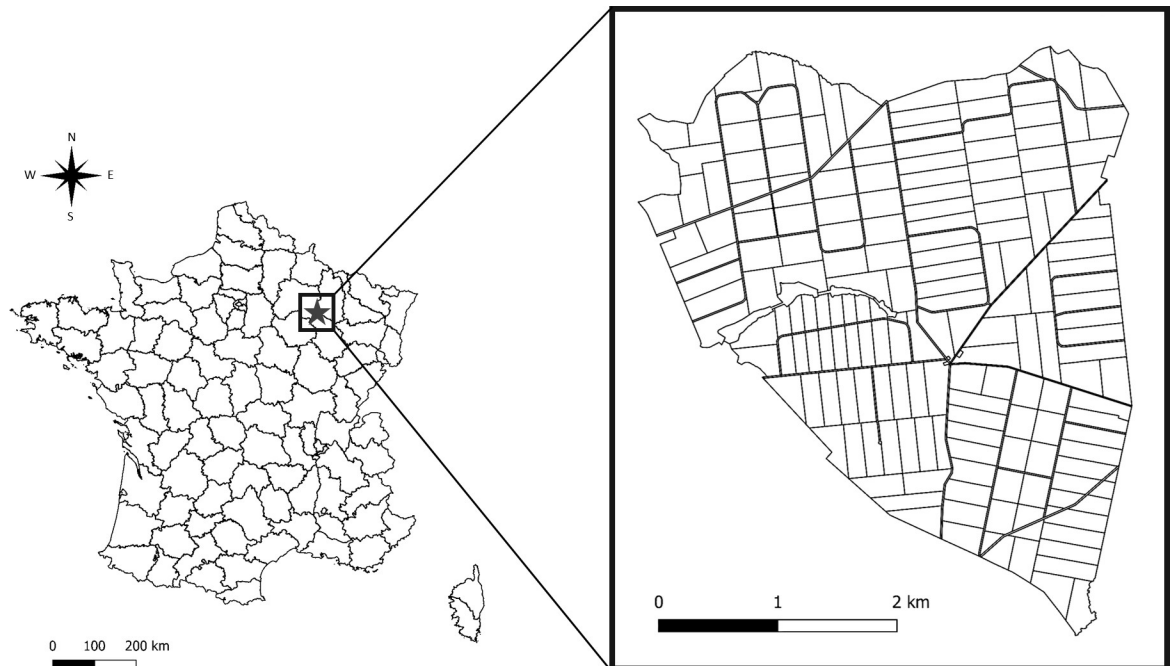
### Ethics statement

In accordance with European and French laws, roe deer captures were carried out minimizing animal stress and handling time (limited to 10 minutes for a fawn), and ensuring animal welfare, as defined in the guidelines for the ethical use of animals in research. All methods were approved by the authorities (French Ministry of Environment). These experiments were performed in accordance with the conditions detailed in the specific accreditation delivered to the Office National de la Chasse by the Préfecture de Paris. Animal captures and experimental procedures were in line with the French Environmental Code (Art. R421-15 to 421-31 and R422-92 to 422-94-1) and duly approved by legislation from the Prefecture of Paris (agreement n° 2009-014, n°. 2013-118, n°2019-02-19-003).

### Data collection

**Study area.** This study was carried out in the Territoire d'Étude et d'Expérimentation (TEE) of Trois-Fontaines, located in north-eastern France (48° 38' N, 4° 54' E) (Fig 1). This is an enclosed forest of 1360 ha with an overstory dominated by oak (*Quercus* spp.) and beech (*Fagus sylvatica*), while the coppice is dominated by hornbeam (*Carpinus betulus*) [48]. The climate is continental, characterized by cold winters (mean daily temperature in January–February was 3.2°C between 1992 and 2018, data from Météo France) and hot but not dry summers (mean daily temperature in July was 19.2°C and total rainfall in July and August was 137.63 mm between 1992 and 2018, data from Météo France).

**Fawn data and tick sampling.** The search for fawns was organized in the TEE of Trois-Fontaines by field workers and volunteers (hunters, foresters, etc.) every year during the



**Fig 1. Geographic location in France (on the left) of the study site composed of 172 forest plots (on the right).** Figure made by authors using QGIS software. Authors have assigned the figure the CC-BY 4.0 license.

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fawning period (April–June) [42]. Fawns detected hiding in a bedding site during their first days of life were captured. Then, in a short handling time (10 min maximum) to avoid too much disturbance of the fawn, we identified each of them with ear-tags and collected information on sex, age (in days), body mass (in kg), and tick burden. Umbilicus characteristics and behavior of fawns during the capture were used to determine their age [49]. For fawns found between 2010–2018, the GPS coordinates of the place of capture were collected.

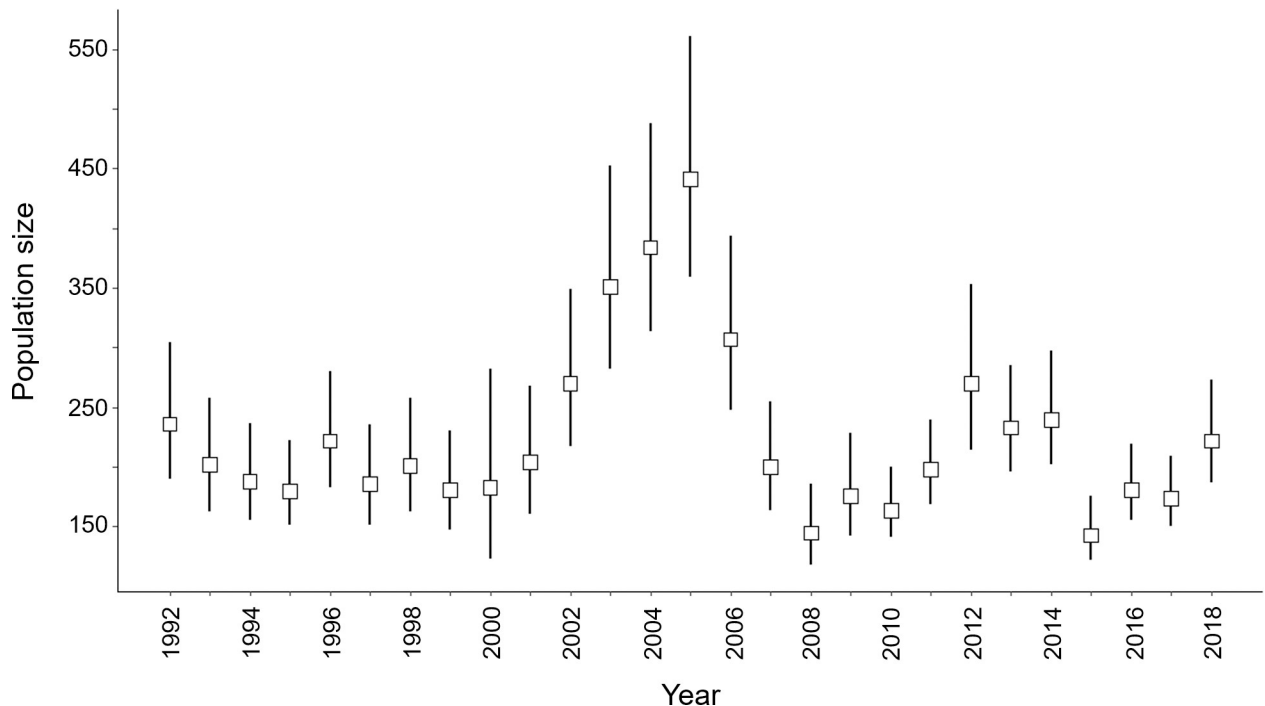
The last minutes of handling the fawn were devoted to assessing the level of tick infestation (all stages: larvae, nymph, adult) by visually and manually inspecting the neck and head of each captured fawn. This measurement was used as a proxy for the fawn's overall infestation. These areas of the body are often the most infested by ticks, easily accessible when handling a fawn, and, as no fur is present in the ears, the probability to visually and manually detect ticks is higher. The detection of the largest ticks—therefore the adult ticks—was much higher than for other stages, due to both their larger size and short handling time of the fawn. However, we know that larvae and nymph stages are also present on roe deer [31]. Therefore, we cannot rule out that these stages were also detected and we supposed that our numbering of ticks covered all three stages.

Based on the number of detected ticks, we attributed to each fawn the following infestation classes: (i) class 1 = < 10 ticks; (ii) class 2 = 10–20 ticks; (iii) class 3 = > 20 ticks. A previous study of the ticks in Trois-Fontaines showed that *I. ricinus* was the only tick species observed on roe deer (G. Bourgoïn, *unpublished data*).

Based on their measured body mass, we calculated the body surface area (bsa) of each fawn using Meeh's formula [50]:

$$bsa = k \times W^{\frac{2}{3}}$$

with k: Meeh constant (k = 10); W: body mass (in kg).



**Fig 2. Yearly estimates (with 95% confidence intervals) of roe deer population density in March individuals > 1 year at TEE of Trois-Fontaines (France) estimated by capture–mark–recapture models (see [51] for further details) for the period 1992–2018.**

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**Data on the population of roe deer and the environment.** The roe deer population in Trois-Fontaines has been intensively monitored for 40 years using capture–mark–recapture methods [51]. Driving nets are used each year in December–March to catch the individuals (10–12 days of capture per year). Using Capture-Mark-Recapture modeling, the number of roe deer older than one year was thus estimated every year in March, which we used to derive a value of density every year (Fig 2) [52].

Weather data for this study were obtained thanks to the SAFRAN system. This is a meso-scale atmospheric analysis system that reconstructs surface atmospheric variables at an hourly timescale on a regular 8 km grid using ground observations and large-scale general circulation model products (Météo France, [53]). This reanalysis is described in Durand et al. [54, 55] and several validations have been performed [53, 56]. In this study, we used the mean values of air temperature (in °C) and relative humidity (in %) of the capture day and four days before this day. Indeed, nymph and larvae of ticks take a blood meal on their host during an average three days, and adults in seven days, before dropping off the host [16]. Assuming fawns were infested by the three tick stages, we therefore considered a mean duration of blood meal of five days, explaining why we took the weather conditions into account during this time. The saturation deficit was considered and calculated according to Randolph and Storey [57]:

$$SD = \left(1 - \frac{RH}{100}\right) \times 4.9463 \times \exp(0.0621 \times T)$$

with SD: the air saturation deficit (in mm Hg); RH: the air relative humidity; T: the air temperature; and where  $\exp(x)$  stands for the exponential of x.

Our study site contained 172 forest plots measuring on average 7.95 ha, delineated by forest trails. In each forest plot during autumn, the dominant plant species and its cover proportion

were determined for the following years: yearly from 1996 to 2005, in 2009, and in 2012 [48, 58]. We used these data to assess the habitat effect on tick infestation. When habitat data were not available for a year: (1) we used habitat data from the closest year (i.e., +/- 2 years max); (2) data after 2014 were not considered, because we did not collect any data on habitat structure after 2012, and we considered that habitat structure had changed too much after two years to be ignored in a study of habitat effect on tick infestation.

Between 1996–1999, forest managers made openings in the habitat (i.e., pasture area) to improve roe deer habitat. Then, in late December 1999, Hurricane Lothar, apparently the strongest hurricane in the region for at least 1000 years, hit the Trois-Fontaines site, disturbing the habitat [59, 60]. This event created several openings and modified the microclimatic conditions of the environment.

### Model of the infestation process

First, based on the GPS location of 308 fawn bedding sites, we explored the spatial autocorrelation of infestation levels. More precisely, we used the bivariate K function [61] to assess the mean number of fawns infested by ticks within a given radius from a fawn not infested by ticks, for different radii comprised between 0–2.5 km. We tested the significance of a clustering of ticks at various scales by comparing this observed function to a set of bivariate K functions calculated using the same dataset after randomly allocating the infestation status to the fawn locations (i.e., random labeling [61]). We carried out this analysis with the package `spatstat` [62].

Then, we designed a Bayesian model describing the infestation process of the roe deer fawns by ticks during the first eight days of their lives, to test the effect of environmental variables on the infestation rate. All the codes and data used for this modeling approach are available in an R package named `tickTF` [63], (Digital Object Identifier: 10.5281/zenodo.5764798), available on Github at the URL: <https://github.com/ClementCalenge/tickTF>. It can be installed in R with the package `devtools` [64], using the function `devtools::install_github("ClementCalenge/tickTF", ref = "main")`. The package `tickTF` includes a vignette describing how the user can easily reproduce the model fit (available with the command `vignette("tickTF")` once the package has been installed). This vignette is also available as supplementary material of this paper (S1 Appendix), and describes the complete mathematical development of the model, the implementation of the model using the R package `NIMBLE` [65], the complementary analyses, and model checks.

In this section, we give a short description of the model, and we refer the reader to the supplementary material for a more complete development.

We modeled the infestation process by a Poisson process (e.g., Taylor and Karlin [66]). In other words, the number  $N_j$  of ticks present on a given fawn  $j$  is supposed to follow a Poisson distribution with mean  $\Lambda_j$ :

$$N_j \sim P(\Lambda_j)$$

The expected number of ticks on fawn  $j$  is supposed to be the result of a process of accumulation of ticks, i.e., is equal to the integration over time  $t$  of an instantaneous risk function  $\lambda_j(t)$ :

$$\Lambda_j = \int_0^{a_j} \lambda_j(t) dt$$

where  $a_j$  is the age of fawn  $j$  at the time of capture. We modeled this instantaneous risk of infection of a fawn having  $t$  days old with:

$$\lambda_j(t) = \zeta_j \times \mu(\mathbf{x}_j) \times s_j(t) \times \exp(\gamma_v \times I(t > 5)) \quad (1)$$

The instantaneous rate of infestation of a given fawn was therefore the product of an instantaneous rate of infestation per unit of body size area  $\mu(\mathbf{x}_j)$ —which itself was supposed to depend on environmental variables characterizing the capture  $\mathbf{x}_j$  (see below), of the body size  $s_j(t)$  of the fawn  $j$  at times  $t$ , and of a random individual effect  $\zeta_j$ . These random effects followed a gamma distribution  $G(1/\varphi, \varphi)$ , with  $\varphi$  a dispersion parameter to be estimated, and account for the fact that different fawns have different sensibilities to ticks.

As we said before, we considered a mean duration of blood meal of five days. Then, after five days, the ticks that infested the fawn on its first day of life start to drop off the host, which is expected to lead to a decrease in the observed infestation rate, as the number of ticks infesting a fawn may be partly compensated by the number of ticks quitting the animal. Thus, the instantaneous rate of infection is supposed to be different for fawns aged up to five days old and for older fawns (this rate is multiplied by  $\exp(\gamma_u)$  after that)—in this equation,  $I(t>5)$  takes the value one when the fawn is older than five days old and zero otherwise.

To fit this model, we developed a submodel for the instantaneous rate of infestation per unit of body size area  $\mu(\mathbf{x}_j)$ —which was independent of age—and another submodel for the changes of body surface area  $s_j(t)$  of fawn  $j$  with time—which varied with age.

For the latter, we used the data collected on captured fawns to model the relationship between the age at capture  $a_j$  and body surface area at capture  $s_j(a_j)$ . Our data suggested that the following quadratic regression model was reasonable to describe the growth of roe deer fawn during the first eight days of life (see supplementary material):

$$s_j(t) = \beta_0 + \beta_1 \times t + \beta_2 \times t^2 + \epsilon_j$$

where  $\epsilon_j$  is a gaussian residual with zero mean and standard deviation  $\sigma_s$ .

Moreover, we proposed the following loglinear submodel for the instantaneous rate of infestation per unit of body size area:

$$\log \mu(\mathbf{x}_j) = \alpha_0 + \delta_{u(j)} + \sum_{k=1}^p \gamma_k x_{jk} \tag{2}$$

where  $\alpha_0$  is the intercept,  $\delta_{u(j)}$  is a gaussian random effect characterizing the year  $u(j)$  of capture of fawn  $j$ , and  $\gamma_k$  are coefficients characterizing the effect of environmental variables  $x_{jk}$ . Note that preliminary versions of our model revealed that the variance of the year random effects  $\delta_{u(j)}$  varied a lot across periods. We distinguished three periods based on the dynamics of the forest structure: (i) period 1 corresponded to the period before Hurricane Lothar (between 1992–1999); (ii) period 2 corresponded to the 10 years following this hurricane (between 2000–2009); (iii) period 3 corresponded to the later years (2010–2018). We estimated one variance parameter  $\sigma_u^{(p)}$  of the year random effects for each period  $p$ . During the step of model building, we tested the effect of several variables  $x_{jk}$  on the infestation rate: (i) humidity, (ii) temperature, (iii) saturation deficit, (iv) roe deer density, and (v) habitat type. We kept only the most influential variables in the model.

One difficulty with this model is that we did not know the exact number  $N_j$  of ticks on fawn  $j$  but rather the tick-burden class. However, given  $A_j$  we could calculate the probability of all the possible values of  $N_j$  within a given class (since  $N_j$  followed a Poisson distribution), and therefore, we could calculate the probability of each burden class by summing the probabilities of all values of  $N_j$  in this class (see supplementary material for more details).

We estimated the posterior distribution of the vector of parameters of the model, i.e.,  $\theta = (\beta_0, \beta_1, \beta_2, \phi, \sigma_s, \{\sigma_u^{(p)}\}, \{\delta_{u,j}\}, \alpha_0, \gamma_v, \{\gamma_k\})$ . We fitted our model using Markov Chain Monte Carlo (MCMC) using the package NIMBLE ([65] see supplementary material for technical details) for the R software, using four chains of 50 000 iterations after a burn-in period of 1000



samples. To save some memory space, we thinned the chains by selecting one sample every 20 iterations. We checked the mixing properties of the chains both visually and using the diagnostic of Gelman and Rubin [67]. We checked the goodness of fit by using the approach recommended by Gelman and Meng [68]. Each MCMC iteration generated a sampled value  $\theta^{(r)}$  of the parameter vector  $\theta$ . For each simulated value  $\theta^{(r)}$ , we simulated a virtual dataset and calculated a summary statistic on it; we then compared the observed statistics with the distribution of simulated values. We used several summary statistics: the number of fawns in each of the three tick-burden classes in total, for each possible age, and in each year. In all cases, the observed values were within the limits of the 95% credible interval.

## Results

We present the spatial distribution of the captured fawns at Trois-Fontaines in Fig 3. This distribution was quite uniform in space, and the tick infestation status seemed to be randomly distributed.

This was confirmed by the analysis of the bivariate K function (Fig 4). We could not identify any departure from a random distribution of the infestation status in space, at all scales.

In total, we collected data of tick infestation on 1043 fawns, assuming that all three tick stages were present. The age of individuals was estimated between 1–20 days (mean  $\pm$  SD =  $4.01 \pm 3.05$ ; min = 1, max = 20) during the 1992–2018 period. Only fawns aged between 1–8 days were kept for our analysis, as older fawns were rare in our data (8% of the total dataset). For the following analyses, we only used data from the 965 fawns less than eight days old (mean =  $3.35 \pm 2.02$ ) when captured (Fig 5). Among them, 696 fawns had < 10 ticks (26 fawns/year on average, SD = 11), 167 had 10–19 ticks (7 fawns/year, SD = 3), and 102 had  $\geq 20$  ticks (4 fawns/year, SD = 3).

The model including an effect of the variables “> 5 days old”, “humidity”, and random effect of the “year” was considered as the best model for the infestation rate of fawns. We did not highlight any effect of temperature (coefficient = 0.00 [−0.04; 0.04]<sub>95%CI</sub>) and roe deer density (coefficient = 0.002 [−0.001; 0.005]<sub>95%CI</sub>) on the instantaneous infestation rate per unit of body surface area. Moreover, all the coefficients associated with habitat types were characterized by 95% CI including 0—they were all characterized by a point estimate comprised between −0.1 and 0.1 and by a standard error equal to 0.11, see supplementary material. The model incorporating the saturation deficit had a higher WAIC (WAIC = 1375.12, SE = 1.58) than the model with the effect of humidity only (WAIC = 1373.71, SE = 1.57). All the estimated parameters of the final model are presented in Table 1.

During the first few days of life, fawns acquire ticks as they grow. Note that the coefficient  $\gamma_0$ , characterizing the rate of infestation for older fawns, was particularly low: this indicates that the instantaneous risk of infestation becomes equal to zero after the first five days of life (Fig 6).

The effect of humidity seemed only marginally significant (coefficient = 0.010 [−0.006; 0.025]<sub>95%CI</sub>). When we compared the model including the effect of humidity with a model excluding it, with the Watanabe–Akaike Information Criterion (WAIC; [69]), we could not detect any significant difference between the two models (model with humidity: WAIC = 1373.8, SE = 1.57; model without humidity: WAIC = 1374.6, SE = 1.57), so that the two models were equally likely according to this criterion. We therefore decided to keep humidity in the model.

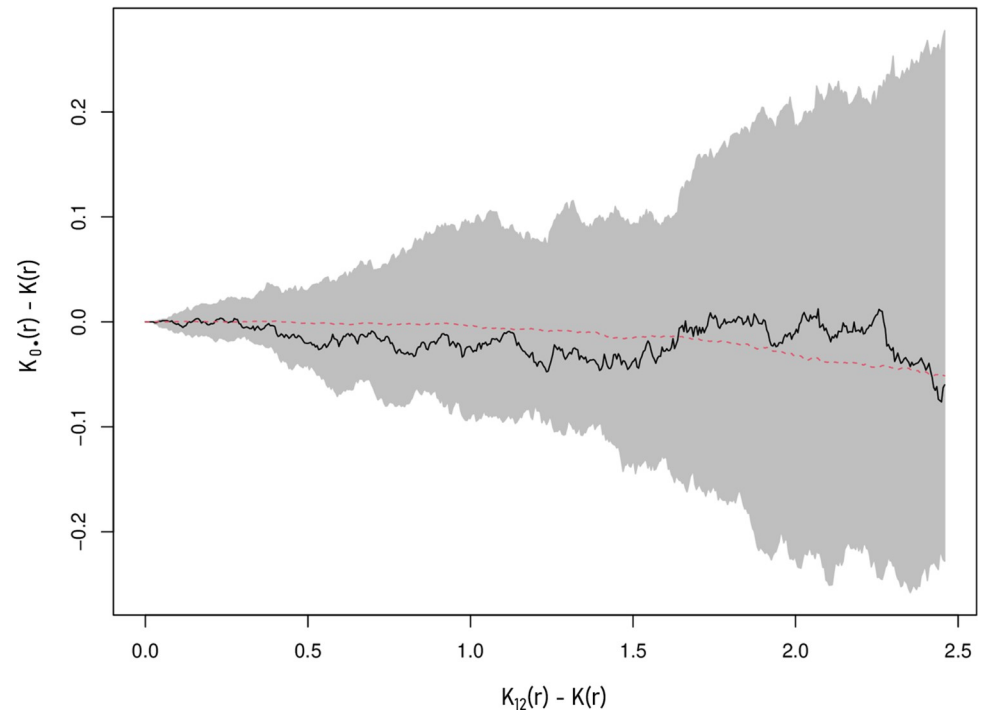
The mean infestation rate was highly variable between years during the 10 years following Hurricane Lothar. Note that this between-year variation was particularly low from 2010 to 2018, with the period prior to Hurricane Lothar being intermediate (Fig 7).



**Fig 3. Distribution of fawns at the study site for the period 2010–2018.** The level of infestation is indicated according to the three tick-burden classes (light gray points = less than 10 ticks; dark gray triangles = between 10–20 ticks; black squares = more than 20 ticks). Figure made by authors using QGIS software. Authors have assigned the figure the CC-BY 4.0 license.

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A substantial amount of variation in our data remain unexplained. Indeed, the coefficient  $\phi$ , characterizing the gamma distribution of the individual random effects, was lower than one. The parameter  $\phi$  is inversely related to the variance of the gamma distribution (with a mean = 1 and a variance =  $1/\phi$ ). A small value of  $\phi$  indicates that the unexplained between-individual random differences in sensibilities are large. And indeed, the Spearman correlation coefficient between the observed dataset and the datasets simulated under our model to assess the goodness of fit of the model was rather small ( $\rho = 0.17$  [0.1; 0.24]<sub>95%CI</sub>), confirming that there was still a lot of unexplained variability in our data.



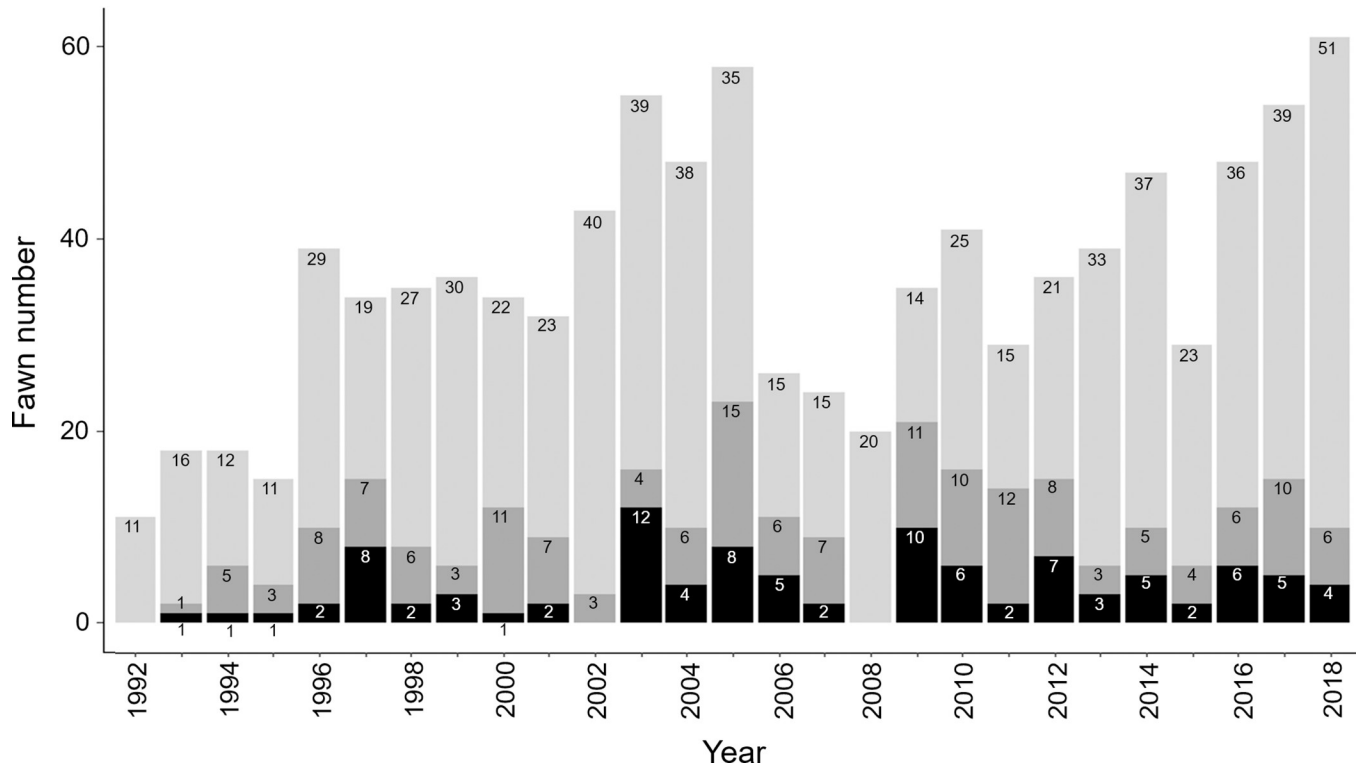
**Fig 4. Test of the clustering of tick-infested fawns at various scales using the bivariate function  $K$ .** For clarity, we present, for various radii  $r$ , the function  $K_{12}(r) - K(r)$  where  $K_{12}(r)$  is the bivariate  $K$  function and  $K(r)$  is the univariate  $K$  function; this difference is lower than zero when there are, on average, less infested fawns within a radius  $r$  of a non-infested fawn than expected under a random spatial distribution of statuses (i.e., clustering of infested ticks). The black curve corresponds to the observed function. The gray area and dotted red curve correspond respectively to the envelope and mean value of the 100 functions simulated under the random labeling assumption (see text).

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## Discussion

The number of ticks on fawns below the age of eight days was frequently low. Only 28% of total observations ( $n = 965$ ) had 10 ticks and more, and 72% of observations had less than nine ticks (Fig 5). The hiding strategy of roe deer fawns in their first days of life may be effective in avoiding parasites such as ticks. Indeed, to increase their chance of survival, they stay hidden in the vegetation to minimize the risk of predation [46]. As a result, they have low mobility in the first days of life, leading to a low probability of touching the vegetation where ticks are questing. From the sixth day of life, our study suggests that the level of tick burden becomes constant (Fig 6). This could be explained by the depletion of questing ticks in the habitat of fawns and the turnover of ticks feeding on fawns. Indeed, as ticks take their blood meal on hosts for a few days (i.e., on average 3 days for nymphs and larvae and 7 days for adults; [16]) before falling to the ground, where they molt or lay eggs [15], the number of ticks gained after this age is probably counterbalanced by the number of ticks that drop off the host.

Factors linked to environmental conditions therefore seem to partly drive the between-individual heterogeneity of infestation by ticks. As expected, the humidity rate was positively related to the tick number on fawns. Based on our model, a 20% increase of humidity leads to an increase of 1.2 ticks on fawns on average (i.e., a humidity coefficient equal to 0.01 which is equivalent to a mean number of ticks multiplied by  $\exp(0.01 \times 20) = 1.2$ ). This result is in agreement with the positive influence of the relative humidity on *I. ricinus* survival and activity, which can lead to a greater presence of questing ticks in the environment [21, 70]. Ticks need at least 70–80% humidity to survive off-host [7]. In this study, the humidity effect was



**Fig 5. Number of fawns studied per year in the Trois-Fontaines Forest between 1992–2018.** The three gray scales correspond to the three tick-burden classes (light gray = less than 10 ticks; dark gray = between 10–20 ticks; black = more than 20 ticks).

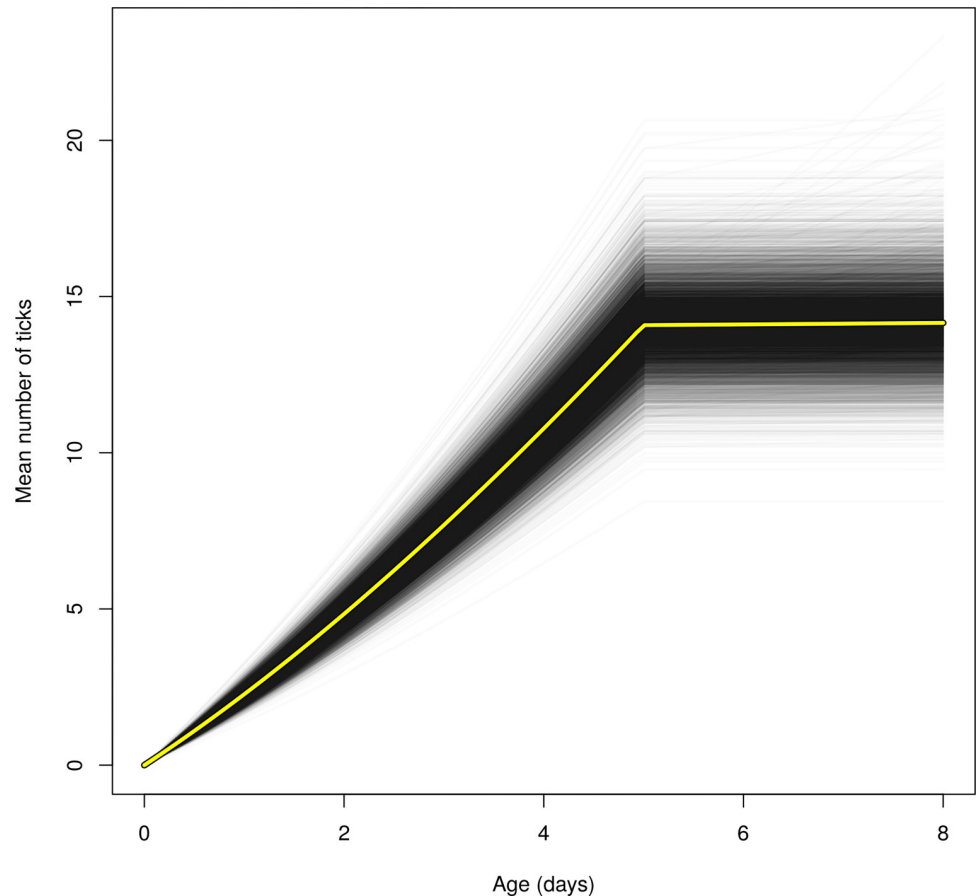
<https://doi.org/10.1371/journal.pone.0262973.g005>

only marginally significant. Our model showed that a 20% increase in humidity (which is large, since humidity varied between 57–90% in our area) leads to an increase of 20% in the number of ticks. Since there was on average between 10–15 ticks per animal (Fig 6), a 20% increase in the number of ticks corresponds to only 2–3 more ticks on average. This may

**Table 1. Estimated top parameters for the final model of the infestation of fawns by ticks in the Trois-Fontaines Forest.** For each parameter, we give the notation used in the text, a short description, the point estimate (corresponding to the mean of the posterior distribution), and the 95% credible interval.

Description	Notation	Est.	95% CI
<b>Intercept in infestation rate log-linear model</b>	$\alpha_0$	2.853	2.68; 3.02
<b>Parameters of the growth model</b>	$\beta_0$	0.176	0.174; 0.178
	$\beta_1$	0.014	0.013; 0.014
	$\beta_2$	-0.001	-0.001; -0.001
	$\sigma_s$	0.021	0.02; 0.022
<b>Effect of &gt; 5 days old</b>	$\gamma_v$	-17.7	-46.6; -2.6
<b>Dispersion parameter of individuals random effects on the infection rate</b>	$\varphi$	0.68	0.53; 0.85
<b>Effect of humidity in infestation rate log-linear model</b>	$\gamma_k$	0.01	-0.006; 0.025
<b>Standard deviation <math>\sigma_u^{(p)}</math> of the years random effects in infestation rate log-linear model for each period p</b>	$\sigma_u^{(1992-1999)}$	0.438	0.044; 1.141
	$\sigma_u^{(2000-2009)}$	0.822	0.422; 1.543
	$\sigma_u^{(2010-2018)}$	0.165	0.011; 0.462

<https://doi.org/10.1371/journal.pone.0262973.t001>



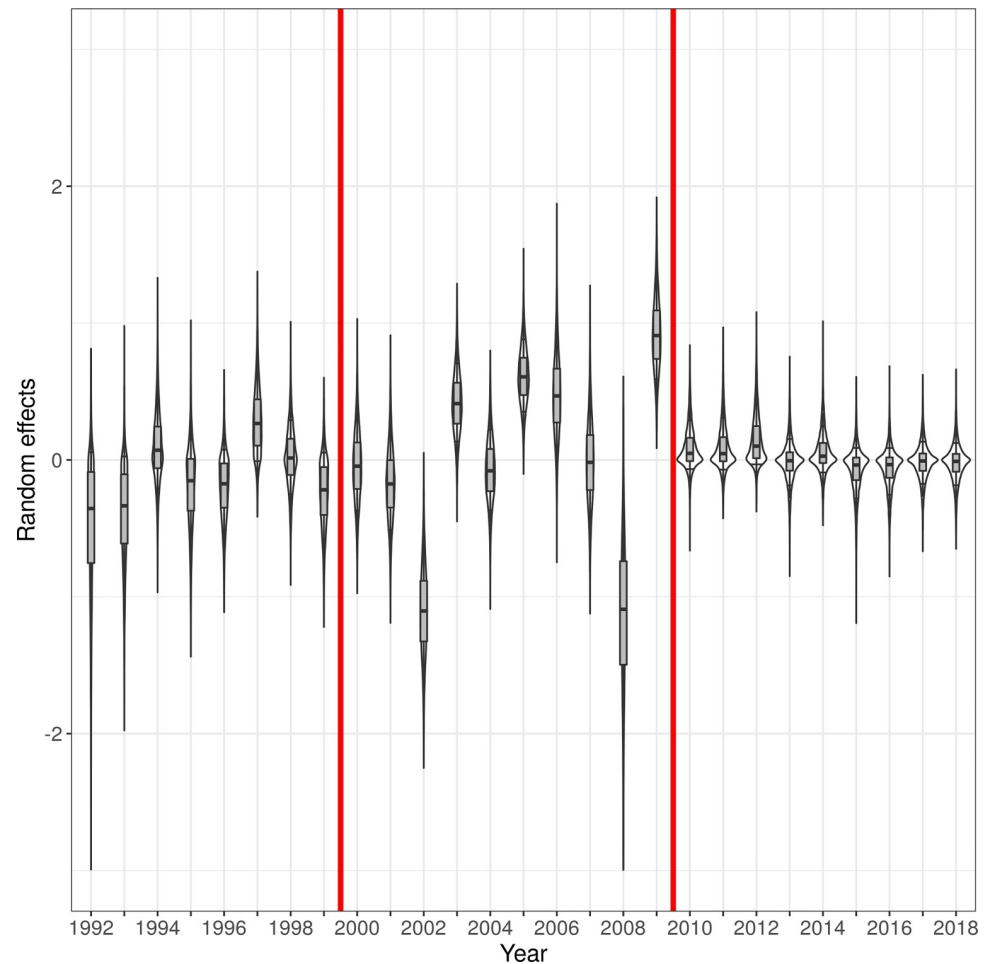
**Fig 6. Mean number of ticks expected under our final model (see text) on an average sized fawn of a given age in the Trois-Fontaines Forest during an “average” year (i.e., a fictitious year characterized by a random effect equal to 0).** Each thin black curve corresponds to the expected number of ticks obtained for one MCMC iteration (we had 10000 iterations displayed on this plot). The yellow curve corresponds to the mean curve.

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explain our difficulty in clearly identifying this effect with our three broad classes of tick burden.

Tick development and questing activity are strongly temperature dependent [17, 43]. Ticks do not tolerate dry and hot conditions due to the risk of desiccation, and below 1 °C, they are not active and most of the time goes into wintering [21, 43, 71]. Thus, climatic conditions are decisive for the survival, development, and activity of *I. ricinus*, and this depends on geographic locations and habitat characteristics. We therefore expected to detect an effect of temperature on infestation rate. However, no relationship was observed, which could be explained by the lack of power caused by the absence of a precise measure of the number of ticks on fawns.

We were unable to identify any effect of the habitat structure on the infestation rate of fawns in our study, and could not validate our second hypothesis. However, it is known that several factors can impact tick abundance and distribution, including habitat structure [17]. For instance, forest vegetation provides a more stable microclimate compared to open habitats, with less extreme variation in climate and mortality risk for ticks, especially when the overstory and the shrub cover are important [72, 73]. In addition, ground vegetation retains moisture and provides shade, which are very important for tick survival and their water balance. This explains why *I. ricinus* are mainly found in leaf litter and low layers of vegetation in forests



**Fig 7. Posterior distribution of the (random) effects  $\delta_{u(t)}$  of the year on the instantaneous infestation rate per unit of body size area of fawns by ticks in the Trois-Fontaines Forest.** The vertical red lines delimit the three periods: (i) period 1 corresponded to the period before Hurricane Lothar (between 1992–1999); (ii) period 2 corresponded to the 10 years following this hurricane (between 2000–2009); (iii) period 3 corresponded to the later years (2010–2018). For a given year, the posterior distribution is represented by a boxplot superposed on a violin plot (i.e., for a given random effect, a kernel smoothing of the MCMC simulations of a random year effect was rotated and put on both sides of a vertical line, mirroring each other).

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[7, 22, 39]. In this study, our inability to isolate any effect of the habitat structure was probably due to the inappropriate scale of habitat measurement: we mapped the vegetation at the resolution of the forest plot, which was probably too large to identify such an effect. Actually, the characteristics at the microhabitat level, i.e., whether the vegetation structure within a few meters around the fawn provides the fawn a thermal protection [74] and low light penetration [75], would be more accurate to see differences of infestation rate in terms of vegetation structure. In addition to the influence of microhabitat and microclimate on tick density, considering local density and movements of hosts could be interesting to better explain the heterogeneity of tick abundance at a fine spatial scale. In fact, ticks drop off from the host to the ground after engorgement to molt or lay eggs, creating a local “nest” of several questing larvae. They have limited abilities to move spatially on their own on the ground, and depend on the presence of hosts to complete their life cycle. Therefore, the density and movements of hosts can be strong drivers of the local density of ticks in the environment [10].

The mean level of tick burden on fawns did not vary according to the decade of our study and it exhibited low interannual variability during the periods 1992–1999 and 2011–2018 (Fig 7). However, there was a stronger interannual variability over the period 2000–2010. We first thought that this high interannual variability in infestation rate could have been caused by variations in roe deer density [76], since deer are major hosts of *I. ricinus* and a high variation in roe deer density occurred in Trois-Fontaines during the 2000–2010 period. However, contrary to our initial hypothesis, we did not find a link between this factor and the level of tick infestation. As stated by Carpi et al. [77], the tick infestation of roe deer is not necessarily dependent of the roe deer density. Tick populations can be maintained with a small density of deer, and ticks in the environment do not automatically increase with the abundance of deer [40, 78]. Furthermore, during its life cycle, *I. ricinus* can parasitize a large number of vertebrates including mammals (e.g., fox, wild boar, rabbit, squirrel) or even birds (e.g., blackbird, pheasant, harrier) [25]. Thus, to better understand the effect of host density, we should, in theory, consider the density of all possible hosts of this tick species, which is difficult to monitor. Another explanation for this interannual variability in tick infestation could be linked to Hurricane Lothar, which took place in December 1999. This event has considerably changed the forest structure, by creating numerous opening patches, modifying forest dynamics, and habitat use by roe deer [60]. Prior to the hurricane, the Trois-Fontaines Forest appeared homogeneous with no strongly different habitat types. After the hurricane, microhabitats and microclimatic conditions became more spatially heterogeneous [79]. Thus, fawns captured between 2000–2010 were probably found in habitats with very different characteristics and/or density of hosts, which influenced the estimated infestation rate of individuals by ticks.

In summary, the level of tick burden became constant when fawns were five days old or more. Humidity was significant but no effects of temperature, vegetation structure, or roe deer density were found on the tick burden of fawns. However, we noticed a strong heterogeneity of tick burden between years suggesting other variables could be involved, such as local density of various host species or habitat characteristics of fawns (i.e., bedding sites), which may be important for tick activity. Studies at finer scales need to be carried out to understand why some fawns are more parasitized than others. This would require additional data on ticks in the environment, at different times of the year, which would allow a more precise study to improve our understanding of tick infection in ungulate newborns.

## Supporting information

**S1 Appendix. Additional mathematical demonstrations, R code to fit the model and complementary analyses.**  
(PDF)

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