



## Temporal distribution of the annual nymphal stock of *Ixodes ricinus* ticks

M. VASSALLO<sup>1,\*</sup>, R.E.L. PAUL<sup>2</sup> and C. PÉREZ-EID<sup>1</sup>

<sup>1</sup>Unité d'Écologie des Systèmes Vectoriels, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris cedex 15, France

<sup>2</sup>Laboratoire de Biochimie et Biologie Moléculaire des Insectes, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris cedex 15, France

(Received 26 April 2000; accepted 27 September 2000)

**Abstract.** The human risk of contracting Lyme disease or other tick borne diseases transmitted by the tick species *Ixodes ricinus* is broadly linked to the tick nymph density. The study was performed in Rambouillet forest (Yvelines, France), a known focus of Lyme borreliosis, from January 1997 to December 1999. We used a nymph sampling methodology which permitted us to obtain a monthly nymph density index (from 0 to 5). Studying the seasonal nymph and larval activity patterns and estimating the larval developmental duration, we demonstrate the existence of an annual nymphal stock. Secondly, we elucidate how this stock is distributed throughout the year, month by month. Its distribution is principally dependent on two factors: the monthly mean ambient temperature and the proportion of active nymphs which find a host each month. Expected monthly nymph densities derived from a theoretical model describing the temperature-dependent stock distribution gave a good fit to the observed densities, accounting for between 76–86% of the monthly variation in observed nymph densities. Predicting the temporal distribution of nymph activity within a stable Lyme borreliosis focus enables more precise identification of risk periods.

**Key words:** Lyme borreliosis, Lyme disease, *Ixodes ricinus*, tick nymph density, population dynamics

### Introduction

*Ixodes ricinus* is the tick vector in France and the rest of Europe responsible for the transmission of Lyme borreliosis, the most prevalent Northern hemisphere vector-borne disease. The human risk of contracting this disease at a given time and for a given biotope is directly linked to the relative host-questing nymph density (Clover and Lane, 1995; Falco *et al.*, 1999). The forecasting of such a risk requires knowledge of the risk zones (Kitron and Kazmierczak, 1997; Vassallo-Paul *et al.*, submitted) and the risk periods

\* Author for correspondence: Tel.: 33 1 40 61 33 71; Fax: 33 1 40 61 30 89; E-mail: vassallo@pasteur.fr

throughout the year. Annual nymph abundance can vary locally according to the biotope, reflecting the biotic and abiotic factors characterizing the biotope, which determine the survival, development and activity patterns of the tick species. Furthermore, even within a biotope abundance can also vary as a function of annual changes in biotic factors, the most evident being the deer density (Stafford, 1993; Daniels and Fish, 1995).

Understanding variations in the active nymph density requires not only the study of environmental factors but also the life-history dynamics (e.g. the duration of each life-history stage and the transition from larva to nymph). Experimental studies on *I. ricinus* development have shown that the developmental period is, for the most part, temperature-dependent (MacLeod, 1934; Gardiner and Gray, 1986). Thus the developmental period from engorged larvae to emerging nymphs varies according to the climate, whereas the developmental period from active larva to active nymph depends on many factors, such as host availability.

In this study, we develop the concept of an annual nymphal stock, the majority of which are ready to become active before the beginning of the activity-season (i.e. before temperatures are adequate for activity). Secondly, we explain how this stock is distributed throughout the year and hence how it is possible to predict the active nymph density, and thus the human risk of contracting *I. ricinus* borne diseases.

## Material and Methods

### *Study site*

The study site was Rambouillet forest, which is a recognized focus of Lyme disease (Zhioua *et al.*, 1996; Pérez-Eid, 1998). The forest is approximately 60 km south-west of Paris and covers an area of 15,000 hectares. The climate can be defined as oceanic with a weak continental disposition, benefiting from relatively low hygrometry (annual precipitation in the order of 650 mm). The forest is divided into plots, separated by roads and paths, as well as being demarcated by landscapes of different vegetation types. In the south-east (SE) part of the forest, seven areas were delimited according to the landscape diagnosis method (Boiret *et al.*, 1988; Vassallo *et al.*, 2000). In each area thus delimited, the pressure of both abiotic and biotic factors is maximally homogeneous for a given time. According to a previous botanical study (C. Figureau, personal communication) in the forest, these areas are representative of the SE part of the forest.

### *Sampling technique and tick stage studied*

The study initially targeted only the questing nymph stage, which is the stage most pertinent to disease epidemiology (Clover and Lane, 1995; Falco *et al.*, 1999) because it is the most abundant stage, has an anthropophily more marked than the other stages (Gray, 1991) and remains attached to man longer, most probably due to its small size.

The cloth-lure technique was used throughout according to the methodology we have previously established (Vassallo *et al.*, 2000). This methodology consists of performing 10 m<sup>2</sup> subsamples on the vegetation with a 1 m<sup>2</sup> of towel tissue, at the speed of 50 cm s<sup>-1</sup>. All the subsamples performed in a botanically homogeneous area (Boiret *et al.*, 1988; Vassallo *et al.*, 2000), at a given date and a given period of the day, can be defined as a sample. From April 1997 to December 1999, 130 samples (composed from 5 to 40 subsamples) were regularly carried out in the whole areas. We grouped the samples which were not significantly different and we allocated to each group a density index as described previously (Vassallo *et al.*, 2000). This density index varied from 0 to 5. (0 + < CI<sub>90%</sub> < 1.2, index 1; 1.4 < CI<sub>90%</sub> < 4.5, index 2; 4.5 < CI<sub>90%</sub> < 7.5, index 3; 9.13 < CI<sub>90%</sub> < 17.5, index 4; 14.5 < CI<sub>90%</sub> < 29.5, index 5. CI<sub>90%</sub>, 90% confidence interval (CI) of the mean number of nymphs collected per 10 m<sup>2</sup> subsample)

We also assessed larval stage activity patterns to ascertain their seasonal distribution in Rambouillet forest.

### *Ambient temperature and soil temperature*

Daily mean ambient temperature data were taken from a Météo-France station located in the forest. Monthly ambient temperatures were calculated from these data (average of daily mean ambient temperature). Expected soil temperatures were calculated according to the relationship found in a previous three-year study performed in the forest on 102 paired observations (ambient and soil temperature) carried out during the same period (1997–1999) (Vassallo-Paul *et al.*, submitted).

### *Developmental duration from engorged larva to active nymph*

Published data sets concerning the developmental duration of each stage of the species were taken from the literature. For a constant and high hygrometry (near to saturation), larval pupation duration is broadly correlated to the ambient temperature (MacLeod, 1934) and the relationship increases when using soil temperature (Gardiner and Gray, 1986). We calculated the daily proportion of development completed for engorged larvae according to the daily

expected soil temperature from the start of larval activity in the forest (questing larvae on vegetation). When the sum of the daily proportions reaches unity, larval pupation is complete and hence we can deduce the required number of days prior to nymph emergence ( $n$  = number of days required to obtain a sum = 1). The nymph activation period (from emerging nymph to active nymph) has previously been found to vary from 10 to 570 days (Pomerancev, 1950).

Statistical analyses were performed in SIMSTAT for Windows (Provalis research).

## Results

### *Seasonal activity of nymph and larval stage*

The three nymph seasonal activity curves, pertaining to 1997, 1998 and 1999, show different forms. In 1997 there was a high peak in activity, whereas in 1998 the peak was lower and in 1999 the peak was almost non-existent. We noted that the lower the peak, the longer the nymph activity season. Whereas the nymph activity curves present differences, the larval activity curves are relatively similar for all years and can be described as simple peaks, always occurring in mid-May (Figure 1).

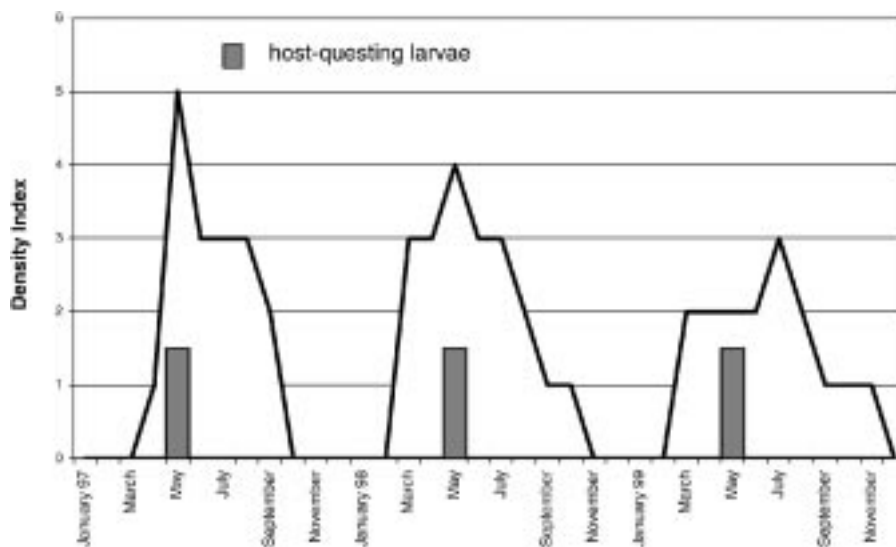


Figure 1. Monthly density index of host-questing *Ixodes ricinus* nymphs in Rambouillet forest from January 1997 to December 1999. The time during which larvae presented host-questing behaviour is indicated by the histograms.

*Developmental period from engorged larva to active nymph*

Linear regression between temperature (data from 10°C to 35°C) and developmental period (for a given hygrometry from 90% to 95%) using previously published data sets from MacLeod (1934) showed that both variables are strongly linked (regression equation:  $D = 153.4 + (-4.04 \times T)$ ; where  $D$  is the developmental period and  $T$  is temperature;  $R = -0.97$ ,  $R^2 = 94\%$ ,  $P = 0.009$ ). Expected daily soil temperatures were calculated from the daily ambient temperatures according to the following regression equation:  $T_s = 3.6 + (0.503 \times T_a)$  ( $T_s$ , soil temperature;  $T_a$ , ambient temperature,  $n = 102$ ,  $R = 0.86$ ,  $R^2 = 0.74$ ,  $P < 0.0001$ ) and were used for calculating the daily proportion of larval development completed.

Larval activity was found to be high but of short duration, occurring in mid-May and persisting for a very short time. From mid-May, we calculated the daily proportion of larval development from the expected soil temperatures providing estimated periods of developmental completion of 118, 125 and 132 days (respectively to 1997, 1998 and 1999), which means that the first emerging nymphs are expected in mid-September or later. The activation period observed under laboratory conditions varies from 10 days to 540 days (Pomerancev, 1950) and MacLeod (1932) showed that the nymph activation duration was 20 days at 22°C. The expected soil temperatures calculated from the observed daily ambient temperature from mid-September to the beginning of the refractory developmental temperatures (< 5–7°C) were 10°C at their maximum (average 4.5°C). Thus we assumed that it was impossible for a nymph emerging in September to become active before the end of the current year.

*The annual nymph stock and its temporal distribution*

In the beginning of the year, we assume that the majority of the nymphs waiting for adequate temperatures for their host-questing activity originate from the previous year larvae. We estimated this stock ( $N$ ) by calculating the area under the curve for each year (Figure 1, 1997–1999) by the trapeze method giving respectively 20, 21 and 19 area units. No significant differences were found between the three stock values (Kruskal-Wallis,  $P = 0.89$ ).

We elaborated a distribution model of this stock:

$$D_m = \left[ N - \left( \sum_{i=1}^{m-1} D_i \times (1 - e^{-hb}) \right) \right] \times a_{Tm}$$

where  $D_m$  is the nymph density at month  $M$ ;  $N$ , the annual nymph stock;  $D_i$ , the density at month  $i$ ;  $1 - e^{-hb}$ , the proportion of the nymph stock

successfully finding a host, where  $h$  is the host density and  $b$  is the encounter probability constant (cf. Nicholson's area of discovery, Varley *et al.*, 1973);  $a_{T_m}$ , proportion of the nymph stock which was active as a function of the ambient temperature. We assume that host-finding by nymphs is a random process depending on host abundance and an encounter probability constant. Values of this host-finding constant and of the nymph temperature-activation relationship ( $a_{T_m}$ ) were chosen to maximize the fit of the expected (derived from the model) to the observed densities. The best fit was found to occur when nymph activation was exponentially related to temperature for the temperature range of between 7°C and 40°C (threshold temperatures for activity and survival, respectively (MacLeod, 1934; Lees, 1948)) and when 67% of active nymphs successfully found a host each month.

We calculated for each month during 1997–1999 the expected monthly density ( $D_m$ ) by taking the mean value of the stocks (= 20 units). Linear regression analyses were performed on the variables 'expected monthly density' and 'observed monthly density', giving highly significant determination coefficients of 78% ( $P = 0.0001$ ), 86% ( $P < 0.0001$ ), 77% ( $P = 0.0002$ ) and 77% ( $P < 0.0001$ ), respectively for 1997, 1998, 1999 and 1997–1999 periods.

## Discussion

The human risk of contracting Lyme borreliosis is strongly linked to *I. ricinus* nymph activity. Forecasting temporal risk patterns therefore depends on the ability to predict active nymph densities. In this study, we used the cloth-lure technique sampling methodology, previously established to measure the relative density of nymphs host-questing on vegetation (Vassallo *et al.*, 2000), permitting us to obtain valid and representative nymph-density data for Rambouillet forest. Nymph seasonal activity patterns show annual differences: in 1997 there was a high peak of nymph activity but did not spread late into the year despite temperatures adequate for activity and survival during this period (October–November, 1997). In 1999 there was the inverse pattern of nymph activity, with no peak but spread late into the year and the 1998 activity profile was intermediate. Although a behavioural diapause mechanism could explain the activity pattern in 1997 (no autumnal nymph activity despite permissive temperatures), the 1999 nymph-activity profile which occurred up to late November does not corroborate this idea. In addition, despite these broad differences in nymph-activity profiles, total nymph activity (area under the activity curve) was equivalent for all three years, suggesting a stable annual nymph population density. The absence of nymph activity later in the year (e.g. 1997), despite permissive temperatures, suggests that the nymph

population exists as a stock consisting of mature nymphs at the beginning of the year ready to become active with sufficiently clement temperatures. This implies that the nymphal stock originates from the larval population of the previous year which assumes that (1) such larvae are unable to become active nymphs in the same year and (2) active nymphs produced in one year, by contrast, do not over-winter to the next.

To verify these assumptions, we assessed the larval annual activity profile using the same cloth-lure technique. The technique was designed to measure active nymphs, which are randomly distributed, rather than larvae, which have an aggregated distribution and thus the larval harvest data is hence given as a qualitative result (presence–absence). Larval activity was found to be extremely concentrated, in mid-May for all three years. This estimated larval activity could have been biased by the herbaceous strata, because much larval activity occurs low in the vegetation. However, three of the seven areas where we harvested the larvae did not contain any herbaceous strata irrespective of the time of the year (areas only composed of the *Epicea exelsa* group). In addition, a previous study performed in the same part of the forest showed that rodents were parasitised with *Ixodes ricinus* larvae maximally during late spring (Pichon, 1997), which is in concordance with the larval activity profile found by the tissue-lure sampling. Thus we conclude from this observation: firstly, hosts must be sufficiently numerous to permit rapid host-finding by the entire larval cohort; secondly, eggs which were laid the previous year have passed the winter and larval emergence is relatively synchronous, when temperature is sufficiently high.

To assess whether emerged larvae could become active nymphs within the same year, we calculated the daily proportion of larval development completed from mid-May using the relationship found with published data (MacLeod, 1934) between temperature and larval pupation duration for a given and high hygrometry. We assume that the development from engorged larva to emerging nymph in Rambouillet forest followed the same relationship because all the measurements of the hygrometry at the soil level, performed in a previous study (Vassallo *et al.*, 2000), were greater than 75% irrespective of the season. Very little is published concerning the nymph activation period (from emerged to active nymph), hence making it impossible to build a relationship between temperature and activation duration as carried out for the larval pupation. According to the soil temperatures (where the development takes place) and the MacLeod observation (1932) concerning the nymph activation period (20 days at 22°C), the nymphs can not become active before the end of the current year. If it was the case, it would not result in a high active nymph density (because (1) nymph activity is proportional to ambient temperature and (2) this ‘new’ autumnal nymph stock would be

small), and hence not result in a significant deletion of the stock for the next year. It was thus considered impossible that a larva emerging in May, could become an active nymph in the same year. Furthermore, we reasoned that active nymphs do not pass the winter and continue their host-questing the following year for two reasons: (1) nymph activity stopped before the end of the adequate temperatures (e.g. October in 1997) and (2) if the hosts are sufficiently numerous for larvae, they are probably sufficiently numerous for nymphs, as suggested by the high estimated proportion of active nymphs successfully finding a host each month (67%).

Significant animal variation in vertebrate host population densities could significantly alter tick population densities and hence confound any temperature nymph activity relationship. The absence of any significant differences in the annual nymphal stock (area under activity profile curve) suggests a stable tick population density. Furthermore, the following facts strongly suggest that the absolute host number (all vertebrates mixed) did not change during the period studied, and that therefore the hosts are not a bias in the development of our model: (1) The spatial distribution of the nymph stage has been found always at total random irrespective of the area or the period of the year, (2) the stability of deer density in the forest (the absolute number did not change between 1990 and 1995), (3) there is a great variety of vertebrate hosts living in a forest and (4) the nymphs are not discriminating in their choice of host.

In our study site, the tick population appears stable and the lack of spatio-temporal variation in *Borrelia burgdorferi* prevalence rates in the tick population suggests that Rambouillet is a stable focus of Lyme borreliosis transmission (Pichon *et al.*, 1998; M. Vassallo-Paul, personal communication). Such stability facilitated the development of the notion of the nymphal stock, enabling predictable nymph activity patterns reflecting the temperature-dependent temporal distribution of the stock. In essence, the higher the temperature during the initial activation period, the faster the stock depletion and hence nymph activity is not spread later into the year. Conversely, cooler temperatures early on lead to an extended activity profile with no or a reduced peak. Thus the effects of host abundance and temperature on both the larval and nymph populations result in a polarization of the active nymph population into discrete annual cohorts, namely a stock. Predicting the temporal distribution of the nymphal stock using ambient temperature will in principle enable prediction of the temporal human risk of acquiring Lyme borreliosis in regions of stable transmission. To what extent such a predictive relationship is robust will depend on the stability of the transmission system and remains to be seen in other Lyme disease foci.

## Acknowledgements

We thank J.P. Widmer from ONF (Office National des Forêts) for granting us free access to Rambouillet forest. This work was supported by a grant from the Fondation Mérieux (Lyon, France) to M. Vassallo.

## References

- Clover, J.R. and Lane, R.S. 1995. Evidence implicating nymphal *Ixodes pacificus* (Acari: ixodidae) in the epidemiology of Lyme disease in California. *Am. J. Trop. Med. Hyg.* 53(3): 237–240.
- Daniels, T.J. and Fish, D. 1995. Effect of deer exclusion on the abundance of immature *Ixodes scapularis* (Acari: Ixodidae) parasitizing small and medium-sized mammals. *J. Med. Entomol.* 32(1): 5–11.
- Falco, R.C., McKenna, D.F., Daniels, T.J., Nadelman, R.B., Nowakowski, J., Fish, D. and Wormser, G.P. 1999. Temporal relation between *Ixodes scapularis* abundance and risk for Lyme disease associated with erythema migrans. *Am. J. Epidemiol.* 149(8): 771–776.
- Gardiner, W.P. and Gray, J.S. 1986. A computer simulation of the effect of specific environmental factors on the development of the sheep tick *Ixodes ricinus* L. *Vet. Parasitol.* 19: 133–144.
- Gray, J.S. 1991. The development and seasonal activity of the tick *Ixodes ricinus*: a vector of Lyme borreliosis. *Rev. Med. Vet. Entomol.* 79(6): 323–333.
- Kitron, U. and Kazmierczak, J.J. 1997. Spatial analysis of the distribution of Lyme disease in Wisconsin. *Am. J. Epidemiol.* 145(6): 558–566.
- Lees, A.D. 1948. The sensory physiology of the sheep tick, *Ixodes ricinus* L. *J. Exp. Biol.* 25: 145–207.
- MacLeod, J. 1932. The bionomics of *Ixodes ricinus* L., the “sheep tick” of Scotland. *Parasitology* 24: 382–400.
- MacLeod, J. 1934. *Ixodes ricinus* in relation to its physical environment. The influence of climate on development. *Parasitology* 26: 282–305.
- Pérez-Eid, C., Pichon, B., Zhioua, E., Tremel, N., Villeret, R., Deruaz, D., Mousson, L., Vassallo, M. and Ferquel, E. 1998. Lyme borreliosis, emergent disease linked with the environment. *Bull. Acad. Natl. Med.* 182(2): 267–283.
- Pichon, B., Mousson, L., Figureau, C., Rodhain, F. and Pérez-Eid, C. 1998. Density of deer in relation to the prevalence of *Borrelia burgdorferi sensu lato* in *Ixodes ricinus* nymphs in Rambouillet forest, France. *Exp. Appl. Acarol.* 23(3): 267–275.
- Pomerancev, B.I. 1950. Fauna of U.S.S.R. Arachnida. Vol. IV, No. 2. Ixodid ticks (Ixodidae). The American Institute of Biological Sciences, Washington, 199 pp.
- Stafford, K.C. 1993. Reduced abundance of *Ixodes scapularis* (Acari: Ixodidae) with exclusion of deer by electric fencing. *J. Med. Entomol.* 30(6): 986–996.
- Vassallo, M., Pichon, B., Cabaret, J., Figureau, C. and Pérez-Eid, C. 2000. Sampling methodology for the questing nymph stages of *Ixodes ricinus* L. (Acari: Ixodidae), the principal vector of Lyme disease in Europe. *J. Med. Entomol.* 37 (in press).
- Varley, G.C., Gradwell, G.R. and Hassell, M.P. 1973. Insect Population Ecology. An Analytical Approach. Blackwell Scientific Publications, Oxford.
- Zhioua, E., Postic, D., Rodhain, F. and Pérez-Eid, C. 1996. Infection of *Ixodes ricinus* (Acari: Ixodidae) by *Borrelia burgdorferi* in Ile de France. *J. Med. Entomol.* 33: 694–697.