**Autonomous Abnormal Behaviour Detection in Intelligence Surveillance and Reconnaissance Applications**

**Range expansion of *Ixodes ricinus* to higher altitude, and co-infestation of small rodents with *Dermacentor marginatus* in the Northern Apennines, Italy.**

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**Abstract.**

Immature ticks (*Ixodes ricinus* and *Dermacentor marginatus*) were collected from small rodents (*Apodemus* spp. and *Myodes glareolus*), in the Northern Apennines, Italy, at an altitude up to 1650 m above sea level (a.s.l.), from 2009 through 2012. While *D. marginatus* had been found at the same location in studies carried out in 1994, *I. ricinus* was very rare or absent. Prevalence (95% confidence interval) of infestation by *I. ricinus* larvae on *Apodemus* spp. was 54.4% (47.5, 61.2), and it was greater than prevalence of *D. marginatus* larvae on the same hosts (23.3%, 17.8, 29.5). The mean (standard deviation) numbers of *I. ricinus* and *D. marginatus* larvae per individual *Apodemus* spp. were similar: 2.3 (4.1) and 2.1 (9.8), respectively. The monthly infestation pattern of the two tick species on *Apodemus* spp. were different. *I. ricinus* larvae were more frequent in June and September, than in July-August. *I. ricinus* nymphs were generally rare, and were most frequently found in July. The prevalence of *D. marginatus* larvae peaked in July-August, whereas nymphs were mostly active in August-September. Increasing population densities of roe deer (*Capreolus capreolus*), and increasing temperatures, in the last decades, in the Apennine area might have contributed to the observed range expansion of *I. ricinus*.

**Keywords**

*Ixodes ricinus*, *Dermacentor marginatus*, *Apodemus* spp., Apennines, Italy.

## Introduction

In the last decades, the geographic range of *Ixodes ricinus*—the most important vector of tick borne zoonoses (TBZ) in Europe—expanded to previously free areas, including greater latitudes in Northern Europe, as well as greater altitudes in the mountains of Central and Southern Europe (Kirby et al., 2004; Materna et al., 2008; Danielová et al., 2006; Jore et al., 2011; Jaenson et al., 2012; Léger et al., 2012). Immature *I. ricinus* are commonly found on small rodents, which may play important roles in the transmission of agents of TBZ. Rodents can also be used as indicators of the presence of the tick and of transmitted agents at a given location (Mannelli et al., 2012a; Pérez et al., 2012; Mihalca and Sándor, 2013).

*Dermacentor marginatus* was the dominant tick species collected on small rodents (*Apodemus* spp. and *Myodes glareolus*) trapped between 1100 and 1400 m a.s.l., in the Northern Apennines, in 1994. In the same study, *I. ricinus* was only found in one out of 128 examined rodents. Furthermore, no host-seeking *I. ricinus* was found by dragging approximately 50 km distance in the same area (Mannelli et al., 1997). In the present article, we report on the analysis of infestation of small rodents by ixodid ticks at the same Apennine location, from 2009 through 2012. The collection of *I. ricinus* from small rodents allowed us to investigate on an increased altitudinal limit for the tick, and to describe the monthly pattern of activity of immature stages, during Summer, in the newly colonized area.

## Materials and methods

### Study area.

The study was carried out on the Tuscan side of the Tuscan-Emilian Apennine National Park, in the province of Lucca, Italy (44° 12' N, 10° 22' E). Climate is characterised by relatively cold winters, and cool summers. In the period 1996-2010, monthly, mean temperature was 1.9 °C in January (minimum), and 17.7 °C in July and August (maximum) (Regione Toscana, 2013a, 2013b). August 2003 was the warmest month on record, with an average temperature of 21.3 °C. Annual rain precipitations are among the greatest in Italy, and showed much variation (between 1350 and 2150 mm) in the 1996-2010 period. Snow cover normally lasts from December through March-April.

Wild ungulates were reintroduced during the 1960's and 1970's. In 2011, the red deer (*Cervus elaphus*) and mouflon (*Ovis orientalis musimon*) populations were estimated at 9.2 and 12.3 head/100 ha. The roe deer was once rare (Ciucci, 1994), but it underwent a remarkable population increase in the last two decades and, in 2011, population density was estimated at 13.2 head/100 ha (Bongi Paolo, unpublished report). The wild boar (*Sus scrofa*) is abundant, although census data are not available.

**Small rodent trapping.**

Small rodent trapping was carried out, from 2009 through 2012, during trapping sessions of two or three days, approximately one month apart, from June to September. In 2009, no trapping was carried out in June, whereas repeated trapping sessions were carried out during the other months. No trapping was carried out in September 2011. During the first three years (2009-2011), trapping was carried out in three 3 x 10 grids (30 traps, 10 m apart), for a total of 2520 trap nights. Sites were chosen, within 2 km from the park premises, based upon convenience of access, and at locations where rodents were previously captured in 1994 (Mannelli et al., 1997). Site A, at 1220 m above the sea level (a.s.l.), was characterized by a mixed wood with common hazels (*Corylus avellana*), Turkey oaks (*Quercus cerris*), wild apples (*Malus sylvestris*), and shrubs (*Spartium junceum*, *Rosa canina*). Site B (1140 m a.s.l.) was dominated by oaks, whereas site C (1185 m a.s.l.) was characterized by mixed wood with a predominance of black alders (*Alnus glutinosa*), and including sycamore maples (*Acer pseudoplatanus*), silver firs (*Abies alba*) and European ashes (*Fraxinus excelsior*). Part of the material collected in 2009-2011 was used in laboratory analysis for the detection of *Rickettsia slovaca* in *D. marginatus* and rodent tissues, as described in Martello et al. (2013). In 2012, trapping was carried out in high stand beech (*Fagus sylvatica*) wood habitat, with poor undergrowth and thick leaf litter, between 1200 and 1650 m a.s.l., near to the altitudinal limit of the tree vegetation (approximately, 1700 m a.s.l.); traps were set 20 m apart in two parallel lines in three transects, for a total of 764 trap nights. Sherman live traps (230x80x90 mm, Sherman Live Traps Co., Tallahassee, FL) and Ugglan live traps (240×60x90 mm, Grahnab, Sweden) were baited with cereals and apples and provided with cotton to protect against the cold temperature during the night.

Captured rodents were anesthetized with a mixture of medetomidine and ketamine, as described in Amore et al. (2007). They were individually identified, in 2009, by ear punch. Starting in 2010, a microchip (transponder AEG ID162 ISO, AEG, Germany) was subcutaneously injected in the interscapular region. Rodent species or genus, sex, and weight were recorded. Each processed animal was carefully screened for the presence of ticks on the entire body. Any attached tick was removed and stored in 70% ethanol. After examination, anesthetized mice were injected with atipamezole HCl (Antisedan®, Pfizer Animal Health, Rome, Italy) to reverse the effects of medetomidine, and were released at the capture site after they had completely recovered from the anaesthesia. Animal capture sampling procedures were approved by the Bioethics Commission of the University of Turin.

**Statistical analysis of tick infestation.**

Ticks were identified by species and stage by using keys from Manilla (1998). The prevalence of infestation and 95% confidence interval (CI), were calculated by tick species and stage, year, month, and host species or genus, by using the FREQ procedure in the SAS System 9.3 (SAS, 2011). Data from recapture of the same rodents during the same trapping session were excluded from the analysis. Mean numbers of ticks per host, standard deviations (SD), and negative binomial dispersion parameters (*k*) were obtained using intercept-only, generalised linear models (GLM) using PROC GENMOD. Negative binomial error (log link) was used to take into account the aggregated distribution of ticks among hosts (Littell et al., 2006).

## Results

### Small rodent trapping.

Trapping yielded 215 *Apodemus* spp. (76 females, 139 males; 80 individuals in 2009, 52 in 2010, 30 in 2011, 53 in 2012), and 57 *Myodes glareolus* (18 females, 39 males; 35 individuals in 2010, 18 in 2011, 4 in 2012). Mean (SD) weight was 24.7 grams (g) (5.9) for *Apodemus* spp., and 27.3g (5.5) for *M. glareolus*. It was not possible to distinguish between the wood mouse *Apodemus sylvaticus* and the yellow-necked mouse *Apodemus flavicollis*. In fact, the distinction between these two species, on the basis of morphological characters, is considered as unreliable, especially in the Southern parts of their geographic range, including central Italy (Barciova and Macholán, 2009; Bugarski-Stanojević et al., 2013; Jojić et al., 2014) .

### Infestation of small rodents by *I. ricinus.*

A total of 485 larvae and 9 *I. ricinus* nymphs were collected from *Apodemus* spp., whereas 17 larvae and one nymph were collected from *M. glareolus.* Prevalence (95% CI) of infestation by *I. ricinus* larvae was 54.4% (47.5, 61.2) on *Apodemus* spp. and 17.5% (8.7, 29.9) on *M. glareolus*. Prevalence of infestation by nymphs was 3.7% (1.6, 7.2) on *Apodemus* spp. and 1.7% (0.04, 9.4) on *M. glareolus*. The mean (SD) number of *I. ricinus* larvae per individual host was 2.3 (4.1) for *Apodemus* spp. and 0.30 (0.73) for *M. glareolus*; the mean (SD) of nymphs was 0.04 (0.21) for *Apodemus* spp. and 0.02 (0.13) for *M. glareolus*. Subsequent statistical analysis was carried out limited to *Apodemus* spp., due to the small sample size for *M. glareolus*.

Prevalence of infestation by *I. ricinus* larvae on *Apodemus* spp. was 48.8% in 2009, 61.5% in 2010, 56.7% in 2011, 54.7% in 2012. *I. ricinus* nymphs were collected from *Apodemus* spp. in 2009, when prevalence was 7.5%, and in 2012, when one individual out of 53 was infested (prevalence = 1.9%). No *I. ricinus* nymphs were found on *Apodemus* spp. in 2010 and 2011.

Infestation levels (prevalence and mean number of ticks per host) by *I. ricinus* larvae on *Apodemus* spp. peaked in June, followed by a decline in July-August, and by a second, lower peak in September (Table 1). Aggregation of *I. ricinus* larvae on *Apodemus* spp. was maximum in August, as shown by the smallest negative binomial parameter, *k*, during this month (Table 1). *I. ricinus* nymphs were most frequently found in July (Table 1).

**Infestation of small rodents by *Dermacentor marginatus.***

A total of 442 *D. marginatus* larvae and 79 nymphs were collected from *Apodemus* spp., 34 larvae and 19 nymphs from *M. glareolus*. Prevalence (95% CI) of infestation by *D. marginatus* larvae was 23.3% (17.8, 29.5) on *Apodemus* spp. and 12.3% (5.1, 23.7) on *M. glareolus*. Prevalence of infestation by nymphs was 21.1% (11.4, 33.9) on *Apodemus* spp. and 13.9% (9.6, 19.3) on *M. glareolus*. The mean (SD) number of *D. marginatus* larvae per individual host was 2.1 (9.8) for *Apodemus* spp. and 0.60 (3.0) for *M. glareolus*, whereas the mean (SD) of nymphs was 0.37 (1.2) for *Apodemus* spp. and 0.33 (0.83) for *M. glareolus*.

Prevalence of infestation by *D. marginatus* larvae on *Apodemus* spp. was greater in 2009 (37.2%) and in 2012 (26.4%) than in 2010 (11.5%) and in 2011 (16.7%). The prevalence of infestation by nymphs *D. marginatus* on *Apodemus* spp. was 17.5% in 2009, 11.5% in 2010, 0.0% in 2011, 18.9% in 2012.

Greatest prevalences of infestation by *D. marginatus* larvae on *Apodemus* spp. were observed in July and August. The mean number of larvae per host peaked in July (Table 2). In June, the negative binomial parameter *k* was small, indicating a high degree of aggregation (Table 2).

Lowest infestation levels by *D. marginatus* larvae were observed in September, when the distribution was also aggregated. *D. marginatus* nymphs were not found on *Apodemus* spp. in June, and infestation levels by this stage were greatest in late summer, with a peak in August. Aggregation of nymphs among *Apodemus* spp. was maximum in July, when infestation was generally low (Table 2).

**Infestation of small rodents by other tick species.**

Two *Ixodes trianguliceps* larvae were found on one *Apodemus* spp. and one *M. glareolus*, whereas two *I. trianguliceps* nymphs were found on one *M. glareolus.*

## Discussion

The analysis of the infestation of small rodents in the period 2009-2012 demonstrated that *I. ricinus* invaded an area in the Northern Apennines of 1100-1650 m a.s.l., where investigations, which were carried out in 1994 showed that this tick species was rare or absent (Mannelli et al., 1997). Increasing population densities of wild ungulates, particularly roe deer (Carnevali et al., 2009) might have played a central role in the observed altitudinal range expansion of *I. ricinus* in our study area. This is in agreement with Medlock et al. (2013), who underlined the importance of changes in distribution and abundance of tick hosts as key determinants of vector range expansion. In the Apennines, increasing ungulate populations and reforestation were associated with the abandonment of traditional land use practices, such as sheep and dairy cow farming (Ciancio et al., 2006).

Climatic factors might be relevant for the expansion of *I. ricinus* at the limits of its altitudinal and latitudinal ranges (Medlock et al., 2013; Materna et al., 2008). Increasing summer temperatures occurred in the Northern Apennines in the last decades, as part of a general warming in the Mediterranean area (Giorgi, 2006; Baldi et al., 2006; Giorgi and Lionello, 2008), and this might have favored development and activity of *I. ricinus* at greater altitudes. Furthermore, milder winters, with reduced snow cover, might have favored roe deer populations (Gray et al., 2009). Interactions between land use and climate changes might, therefore, have contributed to the observed altitudinal range expansion of *I. ricinus* at our study area.

The time pattern of infestation of small rodents by immature ticks might be the combined result of the effects of factors associated with both the parasites, and the hosts (Rosà et al., 2007; Paulauskas et al., 2009; Paziewska et al., 2010; Pérez et al., 2012; Estrada-Peña et al., 2013). As a consequence, conclusions on seasonal activity of ticks, based upon rodent infestation, must be drawn with caution. In our study, the monthly activities of larvae of *I. ricinus* and *D. marginatus* on the same *Apodemus* spp. hosts, were different, probably reflecting marked differences in the life cycles of these two tick species. The observed peak of *I. ricinus* larvae on *Apodemus* spp. in June were probably derived from egg batches laid by females that fed in the spring and summer of the previous year. Gray (1982) working in Ireland showed that larvae from such egg batches became active in mid-late May and that the majority remained questing at a low level in the vegetation, possibly an adaptation to parasitizing rodents. In our study, *I. ricinus* larval activity declined in July-August, and it increased again in September. These larvae are presumably derived from autumn-feeding females that hatch from winter-diapausing eggs in late summer of the following year (Gray 1982).

The low levels of nymphal *I. ricinus* on small rodents at our study site, might be due to factors leading to a low attachment rate of this stage to small rodents (Gray et al., 1999; Amore et al., 2007). The collection of *I. ricinus* nymphs from the environment, and from host species other than rodents, would provide useful, and complementary information.

Small rodents were found infested by immature *D. marginatus*, confirming previous observations at the same location (Mannelli et al., 1997). Several ungulate species may serve as hosts for adult *D. marginatus*, wild boars being particularly important in the Northern Apennines (Selmi et al., 2008). The seasonal pattern of *D. marginatus* larvae on *Apodemus* spp. was characterized by greatest prevalence of infestation by larvae in July-August, and by peaks of the numbers of larvae per host in June-July, whereas nymphs were active in August-September (Table 2). These observations were in agreement with the relatively short life cycle of the tick species, whose immature stages are usually active and develop during the warmer month of the same year (Nosek et al., 1967; Cringoli et al., 2005). Winter survival of *D. marginatus* in the Northern Apennines is probably associated with the capability of adults to tolerate low temperatures (Dörr and Gothe, 2001).

Aggregation of immature ticks on rodents has been observed previously (Perkins et al., 2003; Kiffner et al., 2011) . Factors associated with aggregation of ticks on a small fraction of hosts include sex, movements, home range and grooming activity, as well as the patchy distribution of host-seeking ticks in the environment (Hughes and Randolph, 2001; Mannelli et al., 2012b). The role of individual factors, affecting infestation levels by ticks of different species on small rodents, deserves further investigations given their potential consequences for the transmission of agents of TBZ (Martello et al., 2013).

Levels of infestation by immature ticks (especially *I. ricinus*) were generally low on *M. glareolus*. This host species' relatively small home range, as well as it's apparent ability to develop immunity against *I. ricinus,* have been previously suggested as an explanation of lesser tick infestation on *M. glareolus* compared with *Apodemus* spp. (Dizij and Kurtenbach, 1995; Boyard et al., 2008).

The present study was carried out in a limited study area. Therefore, the altitudinal range expansion of *I. ricinus*, and the monthly pattern of activity of immature stages should be investigated at other locations in the Northern Apennines. Furthermore, trends and patterns of infestation of small rodents by *I. ricinus* and *D. marginatus*, and potential consequences on the transmission dynamics of agents of TBZ, should be followed in time.

**Conflict of interest**

No competing financial interests exist.

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Table 1. Infestation of *Apodemus* spp. by immature *Ixodes ricinus* in the Northern Apennines, Italy, from 2009 through 2012.

| Month (n) | June (48) | | | July (46) | | | August (72) | | | September (49) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tick stage | Prevalence (95% CI) | Mean (SD) | k | Prevalence (95% CI) | Mean (SD) | k | Prevalence (95% CI) | Mean (SD) | k | Prevalence (95% CI) | Mean (SD) | k |
| Larvae | 68.8 (53.7, 81.3) | 3.1 (5.4 ) | 0.60 | 50.0 (34.9, 65.1 ) | 1.4 (1.9 ) | 0.66 | 40.3 (28.9, 52.5) | 1.6 (3.7 ) | 0.25 | 65.3 (50.4, 78.3) | 3.2 (4.3) | 0.61 |
| Nymphs | 2.1 (0.05, 11.1 ) | 0.02 (0.14) | ND | 6.5 (1.4, 17.9) | 0.09 (0.35 ) | 0.14 | 2.8 (0.34, 9.7) | 0.03 (0.17) | ND | 2.0 (0.05, 10.9 ) | 0.02 (0.14) | ND |

n: number of examined hosts. Prevalence: percent of examined hosts with a least one tick. 95% CI: exact binomial 95% confidence limits of prevalence. Mean: mean number of ticks per examined host. SD: standard deviation. k: negative binomial distribution parameter, small values indicate aggregation of ticks among hosts. ND: not determined, due to only one or two individual infested by ticks.

Table 2. Infestation of *Apodemus* spp. by immature *Dermacentor marginatus* in the Northern Apennines, Italy, from 2009 through 2012.

| Month (n) | June (48) | | | July (46) | | | August (72) | | | September (49) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tick stage | Prevalence (95% CI) | Mean (SD) | k | Prevalence (95% CI) | Mean (SD) | k | Prevalence (95% CI) | Mean (SD) | k | Prevalence (95% CI) | Mean (SD) | k |
| Larvae | 8.3 (2.3, 20.0) | 2.4 (11.6) | 0.02 | 34.8 (21.4, 50.3) | 5.0 (17.0) | 0.1 | 37.5 (26.4, 49.7) | 1.1 (2.1) | 0.3 | 6.1 (1.3, 16.9) | 0.37 (2.3) | 0.02 |
| Nymphs | 0.00 (0.00, 7.4) | 0.00 (0.00) | ND | 4.4 (0.53, 14.8) | 0.20 (0.93) | 0.02 | 27.8 (17.9, 39.6) | 0.74 (1.6) | 0.23 | 16.3 (7.3, 29.7) | 0.35 (1.1) | 0.14 |

n: number of examined hosts. Prevalence: percent of examined hosts with a least one tick. 95% CI: exact binomial 95% confidence limits of prevalence. Mean: mean number of ticks per examined host. SD: standard deviation. k: negative binomial distribution parameter, small values indicate aggregation of ticks among hosts. ND: not determined, due to only one or two individual infested by ticks.