PREVALENCE OF TICK INFESTATION IN DOGS FROM IASI AREA (ROMANIA)

Gabriela-Victoria MARTINESCU[1], Larisa IVĂNESCU [1], Raluca MÎNDRU [1], Lavinia ANDRONIC [1] and Liviu MIRON [1]

e-mail: martinescugabi11@yahoo.co.uk

Abstract

Dogs and cats play an important role in the spread of tick species and in the transmission of pathogens. *Dermacentor reticulatus* occurs in areas by varied types of climate, vegetation and a high availability of potential hosts, while *Ixodes ricinus* follows a similar seasonal pattern each year. This study was carried out between 2017 and 2021, where we examined 189 canine patients of different breeds, aged between 2 months and 18 years. We collected 435 ticks which were placed individually according to the animal in eppendorf tubes in ethyl alcohol and numbered, until morphological identification. The collected ticks were predominantly adults with different feeding status, belonging to the species: *Ixodes ricinus* (41.6%), *Dermacentor reticulatus* (57.7%) and *Rhipicephalus sanguineus* complex (0.69%). *Dermacentor reticulatus* was predominant, with two peaks of activity in spring and autumn. We also found 2 specimens of *D. reticulatus* males, in January, outside the normal period of activity. The second species identified, *Ixodes ricinus*, shows three peaks of activity, having the most intense period of activity in the spring months. The second peak is observed in summer, and the third in autumn. The prevalence of the species *Rhipicephalus sanguineus* complex is low, of only 0.69% of the total ticks collected from dogs.The aim of this study was to determine the prevalence of tick infestations collected from canine patients, in order to assess a possible change in the activity periods of the species.

Key words: Dermacentor; seasonality; Ixodes; epidemiology;

Pets play an important role in the spread of native tick species and the transmission of pathogens to other animals and humans (Buczek et al., 2021). Dogs are usually infested by adult ticks (Földvari et al., 2005; Dutto et al., 2009), in smaller numbers by nymphs (Földvari et al., 2005; Dutto et al., 2009; Estrada-Peña et al., 2017) and sporadically by larvae (Dutto et al., 2009; Hansford et al., 2016; Manilla, 1998; Kumsa et al., 2011; Saleh et al., 2019). The most common body sites infested with ticks are the head (ear area, eyes and muzzle), legs and neck (Dutto et al., 2009; Lorusso et al., 2010; Wright et al., 2018), and the frequency of tick attachment is associated with dog breed and temperament (Lorusso et al., 2010).

The ticks that attach to dogs differ depending on the seasonal activity of the species involved. These activity patterns are supported by previous studies examining the seasonal activity of ticks collected from hosts or natural habitats (Lengauer et al., 2006; Dautel et al., 2008; Kohn et al., 2011; Cull et al., 2017).

Infestation of dogs with ticks, especially the species *Dermacentor reticulatus*, has increased in recent years in some parts of Europe (Heile et al., 2006; Beck et al., 2014). Warmer winters, but also extended autumn and spring seasons, cause the expansion of tick distribution at higher altitudes but also in northern latitudes (Beugnet et al., 2013; Dantas-Torres, 2015). *D. reticulatus* may be active in January and February, shortly after the snow cover disappears (Karbowiak, 2014). Also, the fat bodies present in some *Dermacentor reticulatus* ticks are used to survive the winter (Zajac et al., 2020).

Dermacentor reticulatus occurs in areas characterized by varied types of climates, vegetation and a high availability of potential hosts, therefore presenting ecological plasticity and high adaptability to periodic unfavorable conditions (Zajac et al., 2020). This species has been identified in western Siberia as well as in the steppes of Ukraine and Russia of the European continent, where the temperate climate is characterized by extremely high annual temperature values and low annual precipitation (Rubel et al., 2016; Miller, 2019).

In endemic areas of central Europe, *Dermacentor reticulatus* shows a frequency comparable to *Ixodes ricinus* in dogs, and represents 45% of ticks in Germany (Beck et al., 2014) and 49% of ticks in Hungary (Földvari et al., 2005).

Ticks belonging to the genera *Ixodes* and *Rhipicephalus* are the most widespread in Italy,

[1] Iasi University of Life Sciences (IULS), Faculty of Veterinary Medicine, Romania

according to Maurelli et al. (2018). The authors of the study identified a prevalence of the species *Rhipicephalus sanguineus* of 27.5% in dogs from Northern Italy and 36.1% in those from the central and southern regions, while the prevalence of the species *Ixodes ricinus* was 25.6% in the regions of Northern Italy and only 10.8% in dogs from the center and south of the country. Only a few dogs were infested with *Dermacentor* spp. (0.6%).

In Romania, Raileanu et al. (2018) collected ticks from 13 sites distributed in the urban area of Iasi, identifying three species of ticks: *Ixodes ricinus* with a prevalence of 95.3%, *Haemaphysalis punctata* – 3.9% and *Dermacentor reticulatus* – 0.8%. Similar results were found by Pavel in his doctoral thesis research (2014) where the prevalence of the species *Ixodes ricinus* was the highest (92.39%), followed by *Haemaphysalis punctata* (6.94%) and *Dermacentor reticulatus* (0.59%).

Cull et al. (2018) consider that *Ixodes ricinus* follows a similar seasonal pattern each year, although there may be a variation in peak activity in May, June and July depending on the year. Also, they observed an increase of activity in the autumn months.

Predictive spatiotemporal patterns (eg. type of vegetation, climatic conditions or wildlife population density) of tick abundance may be important to reduce the incidence of tick-borne diseases (Hartemink et al., 2015; Sgroi et al., 2022). Also, the relationship between temperature and tick developmental stages is not linear for ixodid ticks (Hollingsworth et al., 2015).

The aim of this study was to determine the prevalence of ticks collected from canine patients, in order to assess a possible change in the activity periods of the species.

MATERIAL AND METHOD

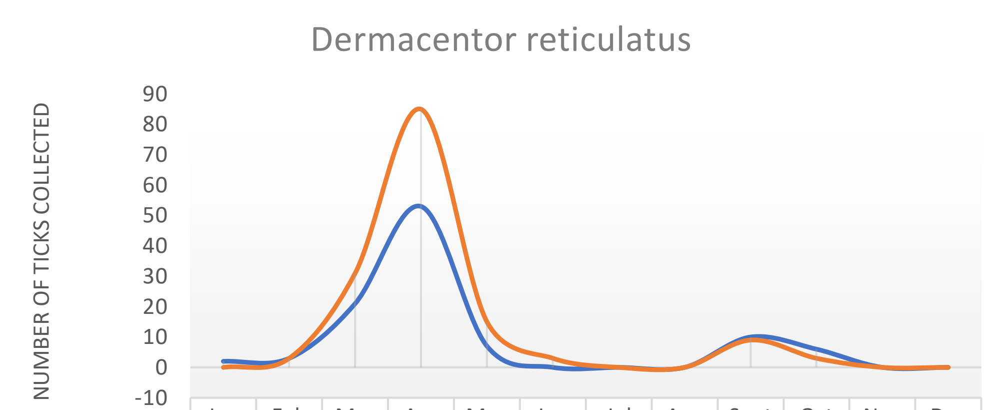
The study was carried out between 2017 and 2021 within the Parasitic Diseases Clinic and the Emergency Department of the Faculty of Veterinary Medicine Iași. During this period, 411 dogs of different breeds, aged between 2 months and 18 years, which were presented for various paraclinical investigations, were examined. All the ticks were removed with a special devices, which were placed individually according to the animal in eppendorf tubes (2ml–5ml), in ethyl alcohol with a concentration of 70% and numbered, until morphological identification.

After the general clinical examination, we found the presence of ticks attached to a number of 189 canine patients (45.99%).

The morphological identification was carried out within the Department of Parasitic Diseases of the Faculty of Veterinary Medicine Iasi, using standard taxonomic keys (Estrada-Pena et al., 2004; Dantas-Torres et al., 2013), as well as the Zeiss Stemi 305 stereomicroscope.

RESULTS AND DISCUSSIONS

Following the centralization of the data, between 2017 and 2021, 411 dogs of different breeds and ages were examined, and a number of 435 ticks were identified on 189 dogs (45.99%),



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Males	2	3	21	53	7	0	0	0	10	6	0	0
Females	0	3	31	85	15	3	0	0	9	3	0	0

Figure 1 - Prevalence of *Dermacentor reticulatus* species

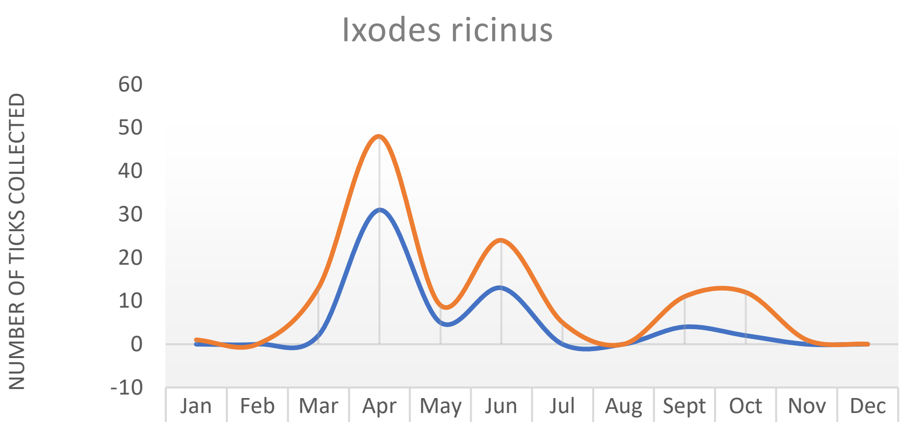
following the general clinical examination. The collected ticks were predominantly adults (99.54%), with different feeding status. This aspect was also observed by other authors (Földvari et al., 2005; Eichenberger et al., 2015; Dumitrache et al., 2014), where more than 90% of ticks collected from dogs were adults. The explanation of these results is given by the impossibility of macroscopic observation of nymphs and larvae during clinical examination.

Morphologically, we identified three species of ticks also exposed in: *Ixodes ricinus* (41.6%; 181/435), *Dermacentor reticulatus* (57.7%; 251/435) and *Rhipicephalus sanguineus* complex (0.69%; 3/435). Dogs can be infested with extremely many species of ticks. One such example was reported in Russia, where Livanova et al. (2018) detected 11 species of ticks, from four genera: *Ixodes*, *Dermacentor*, *Rhipicephalus* and *Haemaphysalis*. In southeastern Romania, Dumitrache et al. (2014) identified six species of ticks attached to dogs: the most common species was *R. rossicus* (95.6%), followed by *D. reticulatus* (3.2%), *I. ricinus* (0.5%), *H. marginatum* (0.3%), *R. sanguineus* s.l. (0.2%) and *I. crenulatus* (0.1%). Abdullah et al. (2016) conducted a study on the species of ticks infesting dogs in the United Kingdom, identifying in dogs that had not left the country in the last two weeks the following species: *Ixodes ricinus* (89.2%), *Ixodes hexagonus* (9.8%), *Ixodes canisuga* (0.78%), *Haemaphisalys punctata* (0.05%) and *Dermacentor reticulatus* (0.17%). Dogs that have left the country in the last two weeks were infested with one of the following species: *Ixodes ricinus* (67.4%), *Dermacentor variabilis* (2.3%) and *Rhipicephalus sanguineus* (30.2%), thus amplifying concerns about the importation of ticks through livestock company traveling to endemic areas.

According to the results obtained, reported in *Figure 1*, *Dermacentor reticulatus* was the dominant species, with two peaks of activity in the spring, in the months of March (52 specimens: 21 males; 31 females), April (138 specimens: 53 males; 85 females) and autumn – September (19 specimens: 10 males; 9 females), October (9 specimens: 6 males; 3 females). Duscher et al. (2013) observed the same frequency of activity of *Dermacentor reticulatus*, in spring and late autumn. Also, in this study we found 2 specimens of *D. reticulatus* males, in January, outside the normal period of activity. Similar results were also published in the study by Mierzejewska et al. (2015): males of *D. reticulatus* constituted the majority of ticks collected from dogs during winter, and Dautel et al. (2008) collected some unfed adult ticks from vegetation in January and February. The most common tick species identified in dogs in Russia was *Dermacentor reticulatus*, with a prevalence of 40.7%, followed by the species *Ixodes ricinus* (12.1%) and *R. sanguineus* (11.3%) (Livanova et al., 2018).

Ixodes ricinus is more active than *D. reticulatus* from April to October, and *Dermacentor reticulatus* was more frequently identified than *I. ricinus* from January to March, and from September to November (Beck et al., 2014), these data also being consistent with the results of our study. According to the study by Pavel et al. (2014) in Iasi, the seasonal activity of unfed ticks presented a bimodal pattern, with peaks of activity in spring and autumn, as follows: *Dermacentor reticulatus* recorded the first peak in March, decreasing in the following months and ceasing activity during the summer and the species *Ixodes ricinus* showed maximum activity in April and May.

The activity of the *Ixodes ricinus* species identified in our study shows three peaks of activity, with a downward trend in the colder months (*Figure 2*). Thus, the most intense period is identified in the spring months (March, April), followed by a decrease in May. The second peak is observed in June and the third, in September and October. In general, *Ixodes ricinus* is absent in the winter months (Mierzejewska et al., 2015), but incidentally, the presence of these species can also be observed in the months with low temperatures, as we also identified in this study, a single tick in month January. In the urban area of the city of Cluj, the activity of *Ixodes ricinus* larvae is unimodal, starting in May, while the nymphs and adults show a bimodal activity, with an increased activity observed from April to June (Borşan et al., 2020). The results obtained by Földvari et al. (2005), *Ixodes ricinus* and *Dermacentor reticulatus* were active throughout the year except July and December. In the study conducted in Northeast Germany, Beck et al. (2014) found that *Ixodes ricinus* can be found throughout the year, except for January, and the species *D. reticulatus* shows no activity in July.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Males	0	0	2	31	5	13	0	0	4	2	0	0
Females	1	0	13	48	9	24	5	0	11	12	1	0

Figure 2 - Prevalence of *Ixodes ricinus* species

R. sanguineus is a common species in the Mediterranean region, it is frequently imported via dogs to countries in Northern or Central Europe: Great Britain (Jameson et al., 2010; Tulloch et al., 2017; Gillingham et al., 2020), Germany (Menn et al., 2010; Röhrig et al., 2011; Schäfer et al., 2019) Netherlands, Denmark, Switzerland (Buczek et al., 2021), Belgium (Claerebout et al., 2013). Less frequently, this species can also be transferred from other endemic countries, for example, the Dominican Republic (Dongus et al., 1996) or Sudan (Buczek et al., 2021).

The present study identified a low prevalence of the species *Rhipicephalus sanguineus* complex, of only 0.69% of the total ticks collected from dogs, as follows: one larva collected in April, one nymph and one female in May. *Rhipicephalus sanguineus* had a prevalence of 0.1% in the study made by Beck et al. (2014), a value close to that reported in the present study. Maurelli et al. (2018) observed a peak infestation of *Rhipicephalus sanguineus* ticks during spring and summer, being collected from dogs in all months of the year. In Ukraine, *R. sanguineus* has been identified in dogs on the coast of the Black Sea and the Sea of Azov, and occasionally it has also been reported in cats and foxes (Nebogatkin, 2013; Rogovskyy et al., 2017). In Albania, the prevalence of *R. sanguineus* species identified in dogs was 8.1%, and that of *Ixodes ricinus* species was only 0.8% (Shukullari et al., 2017). *Rhipicephalus sanguineus* s.l. was identified in dogs from Spain with the highest prevalence (53%), followed by the species *Dermacentor reticulatus* (9%), *Ixodes ricinus* (9%) and *I. hexagonus* (4%). Adults of *R. sanguineus* were observed throughout the year, but the maximum peak of activity was in March-July (Estrada-Peña et al., 2017).

Following the clinical examination of the patients, we found that most canine patients - 41.2% (78/189) were infested with one tick, 23.3% (44/189) were infested with two ticks and 20.6% (39/189) with three ticks. Also, in 2/189 (1.1%) of the patients we identified increased infestations, with more than ten ticks, as shown in the data presented in *Table 1*. The intensity of tick infestations varies between 1 and 78 ticks/dog (Földvari et al., 2005). Dumitrache et al. (2014) identified a higher prevalence of single-species infestations, which was 92.3%. The largest tick infestation observed by Eichenberger et al. (2015) was 49 ticks per dog, belonging to the species *Ixodes ricinus*, but 55.6% of the dogs included in the study were infested with a single tick. In Europe, the role of dogs in the transmission of native and exotic tick species has been investigated in Germany (Abdullah et al., 2016; Hansford et al., 2016), Sweden (Jaenson et al., 2012), Switzerland (Eichenberger et al., 2015), Hungary (Földvari et al., 2005; Földvári et al., 2007), Cyprus (Tsatsaris et al., 2016), Spain (Estrada-Peña et al., 2017) and Portugal (Dantas-Torres et al., 2017).

Humans can also host tick species following travel to exotic countries, thereby bringing in new species (Gillingham et al., 2020). The future of the planet's climate is uncertain in the long term, so the impact of climate change on biodiversity and tick-borne diseases is unpredictable. Some causes and consequences of climate can vary in time and space, some being reversible (Dantas-Torres, 2015). The geographical range of many tick species depends mainly on the biology and ecology of the specific species, as well as on biotic and abiotic factors, since each species has environmental conditions and preferred biotopes that determine their geographical distribution and, consequently, the delimitation of risk areas for tick-borne diseases (Dutto et al., 2009).

Table 1

Prevalence of collected ticks from dogs after clinical examination

No. ticks present on the dog / Month	1	2	3	4	5	6	7	8	9	>10
Jan.	3	0	0	0	0	0	0	0	0	0
Feb.	1	1	1	0	0	0	0	0	0	0
Mar.	14	9	5	2	1	0	1	0	0	0
Apr.	**34**	**17**	**16**	**8**	**5**	**1**	**0**	**1**	**0**	**2**
May	4	2	3	0	1	1	0	0	0	0
Jun.	6	5	3	2	0	0	1	0	0	0
Jul.	4	1	0	0	0	0	0	0	0	0
Aug.	0	0	0	0	0	0	0	0	0	0
Sept.	5	6	4	0	1	0	0	0	0	0
Oct.	6	3	2	0	1	0	0	0	0	0
Nov.	1	0	0	0	0	0	0	0	0	0
Dec.	0	0	0	0	0	0	0	0	0	0
	78	**44**	**39**	**12**	**9**	**2**	**2**	**1**	**0**	**2**

Legend: nr. – number; Jan. – January; Feb. – February; Mar. – March; Apr. – April; Jun. – June; Jul. – July; Aug. – August; Sept. – September; Oct. – Octomber; Nov. – November; Dec. – December;

Temperature and relative humidity affect ticks' rate of host search as well as survival (Lindgren et al., 2006; Ruiz-Fons et al., 2012). In Northern European urban forests, the population dynamics of larvae and nymphs belonging to the species *Ixodes ricinus* are closely related, with data in the specialist literature on synchronous activity, thus suggesting a higher abundance of ticks and consequently a higher risk of pathogens that can infect humans and domestic animals (Cayol et al., 2017).

Human behavior is also a determinant of environmental protection, human and animal health, with an example being the avoidance of tick-infested habitats by humans that could change the risk pattern of disease transmission (Dantas-Torres, 2015). Urban and sub-urban areas are increasingly affected by vector expansion (Grochowska et al., 2020; Oechslin et al., 2017) due to environmental interventions. However, green areas inside or around cities exert a positive effect on the quality of life and human health (Cetin, 2015). The COVID-19 pandemic has shown the need to make cities more livable, but also the danger of close interaction with wildlife species, thus increasing the risk of exposure to various pathogens (Bellato et al., 2021).

The effects of tick parasitism on pets and their owners can be limited by the periodic application of various protection methods recommended by veterinarians.

CONCLUSIONS

The predominant tick species collected from dogs in Iasi county, in the period 2017-2021 was *Dermacentor reticulatus* (57.7%), followed by *Ixodes ricinus* (41.6%) and *Rhipicephalus sanguineus* complex (0.69%).

All ticks in this study were feeding at the time of collection, therefore the possibility of transmitting pathogens to the host was an increased one, being an indication of pathogens present in the local community.

Also, the increased prevalence of the *Dermacentor reticulatus* species in dogs explains the numerous cases of canine babesiosis reported in the area of Iași and the identification of the *Rhipicephalus sanguineus* complex species in this area reinforces the idea of continuing investigations to identify the pathogenic role on humans and animals.

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