

STUDY ON TICK INFESTATIONS OF SMALL RUMINANTS, IN SOUTHERN ROMANIA

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Abstract

Ticks (Acari: Ixodidae) are the most important vectors of zoonotic diseases, presenting a high risk of causing diseases in animals and humans. The purpose of this study was to investigate the prevalence of ticks and the identification of tick species infesting small ruminants in Southern Romania. The study was undertaken from March 2019 to October 2020, including nine sheep and goats flocks in four Prahova County premises. A total number of 2463 sheep and 1202 goats were examined and 76.53% of sheep and 81.2% of goats respectively were infested. Overall, from the infested animals 2233 ticks were collected and the following species were morphologically identified: *Haemaphysalis punctata*, with an overall prevalence of 66.5%, followed by *Dermacentor marginatus* (24.5%) and *Ixodes ricinus* (9.0%). The annual dynamics of tick infesting small ruminants in the investigated areas showed maximum tick abundance in April, for *H. punctata* and *Dermacentor marginatus*, and May for *I. ricinus*, respectively. To reduce the high prevalence of ticks and their impact on productivity in small ruminants, immediate attention is required as control interventions.

Key words: ticks, infestations, small ruminants, southern Romania.

INTRODUCTION

Ticks (Acari: Ixodidae) are the most important vectors worldwide, since they transmit numerous diseases to animals and humans such as tularemia, brucellosis, Q fever, rubella, tick-borne encephalitis (Sonenshine, 1991; Mitrea, 2011; Ioniță & Mitrea, 2017). It is reported that about 10% of the known tick species are vectors for a variety of pathogens (viruses, bacteria, protozoa) of animals and humans. Of them, a high number are causative agents of tick-borne diseases that nowadays are re-emerging in many European countries and worldwide (Jongegan and Uilenberg, 2004; Estrada-Pena et al., 2018).

Tick infestations in small ruminant represent one of the most important condition affecting livestock industry; ticks are a major constraint on ruminant production, directly, as hematophagous parasites, but also indirectly, due their vectorial role for pathogens, such as *Anaplasma* spp., *Babesia* spp., *Theileria* spp. etc, that impact sheep health and their production (Jongegan, 1999; Genchi & Manfredi, 1999).

Over several generations, the tick has the ability to maintain the pathogen in nature

through transstadial and transovarial passages. Biological factors contributing to their high vector potential are their lifestyle, salivary secretions, and blood digestion (Sonenshine, 1991; Márquez-Jimenez et al., 2005; Dantas-Torres et al., 2012).

In Romania, the tick fauna is very diverse, including about 25 species that have been reported infesting animals and/or humans (Feider, 1965; Bădescu, 1967; Mihalca et al., 2012a; Ioniță et al., 2003). In recent years, there has been a significant increase in the abundance of tick infestations on animals (Mitrea et al., 2004; Ioniță et al., 2010). Among the tick species reported in Romania, the most widespread is *Ixodes ricinus* and the most abundant is *Dermacentor marginatus* (Mihalca et al., 2012). However, other species belonging to the following genera, *Haemaphysalis*, *Hyalomma*, *Boophilus*, or *Rhipicephalus* have been also reported (Ioniță et al., 2003; Ioniță et al., 2010; Mihalca et al., 2012; Sandor et al., 2017; Ioniță & Mitrea, 2017).

Studies on the prevalence of ticks in Romania, as well as their geographical distribution and seasonal dynamics were carried out in several areas of our country, in North-Eastern, South-

Eastern, or Western (allowing a good assessment of the tick density in the studied areas as well as the seasonal dynamics and/or risks for tick-borne pathogens of relevance for the animal and public health (Ioniță et al., 2003; Mitrea et al., 2004; Ionita et al., 2010; Chițimia, 2007; Mihalca et al., 2012; Raileanu et al., 2015). In addition, the increased abundance of tick populations associated with climate change and socio-economic conditions is a high potential risk for tick-borne diseases in Romania, as recently reported including by molecular methods (Ioniță et al., 2012, 2013, 2016, 2018; Ioniță & Mitrea, 2017).

Sheep and goat breeding is an important activity in many areas of Romania, particularly for production of meat, milk, and cheese. However, epidemiological surveys on ticks infesting small ruminants in Romania are still limited. Therefore, the purpose of this study was to determine the tick species infesting sheep in goats in some areas in Southern Romania, and the associated potential risks for tick-borne diseases.

MATERIALS AND METHODS

In order to characterize the tick species infesting small ruminants in Southern Romania, a study was carried out, from March 2019 to October 2020, including nine sheep and goats flocks in four districts of Prahova County.

Ticks were collected from animals during of the clinical examination. The entire animal was examined for ticks, with special attention at the head, udder, perineum, lower abdominal area, and the internal parts of the legs. When present, ticks were removed and stored into vials containing 70% ethanol. The vials were labelled with the date of collection, location, and host species and were transported to the Parasitology laboratory of the Veterinary Faculty of Bucharest, for morphological identification.

The tick identification was performed under a stereomicroscope, by using morphological keys (Estrada et al., 2004).

RESULTS AND DISCUSSIONS

The infestation with ticks on small ruminants was investigated in nine localities belonging to four districts of the Prahova County, Romania.

For this, there were examined nine flocks of sheep and goats, from which three flocks in Valea Călugărească (44°57'40"North, 26°9'20" East), four in Urlați (44°59'28"North, 26°13'50"East), one in Iordăcheanu (45°02'45"North 26°14'20" East) and one in Drăgănești (44°49'41"North, 6°17'14"East). A total number of 2463 sheep and 1202 goats were examined for tick infestation and 76.53% of sheep and 81.2% of goats, respectively were infested.

From the infested animals, altogether, 2233 ticks were collected and three tick species were identified, namely: *Haemaphysalis punctata*, with an overall prevalence of 66.5% (731 females and 101 males at goats; 562 females and 90 males at sheep), followed by *Dermacentor marginatus* (24.5%) (144 males, 102 females, collected from goats and 185 males, 115 female collected from sheep), and *Ixodes ricinus* (9.0%) 93 females, 24 males at goats; 72 females and 14 males at sheep) (Tables 1, 2, 3).

According to the host species, the frequency of the tick species identified was as following: *D. marginatus*, 546 (24.5%), of which 300 in sheep and 246 in goats; *H. punctata*, 1484 (66.5%) of which in sheep 652 and goats 832, and *I. ricinus*, 203 (9.0%), of which 86 in sheep and 117 in goats. The prevalence of the tick species identified is shown in Figures 1, 2.

Table 1. Prevalence of tick species infesting sheep and goats in Southern Romania (Prahova county)

Tick species/ Host	<i>Haemaphysalis punctata</i>	<i>Dermacentor marginatus</i>	<i>Ixodes ricinus</i>
Total (n = 2233)	1484 (66.5%)	546 (24.5%)	203 (9.0%)
Sheep (n = 1038)	652 (62.8%)	300 (28.9%)	86 (8.3%)
Goats (n = 1195)	832 (69.6%)	246 (20.6%)	117 (9.8%)

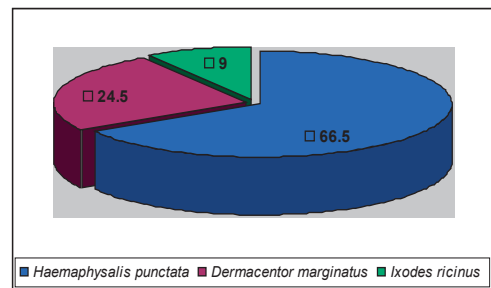


Figure 1. Proportion of Ixodidae tick species populations in small ruminants in Prahova County

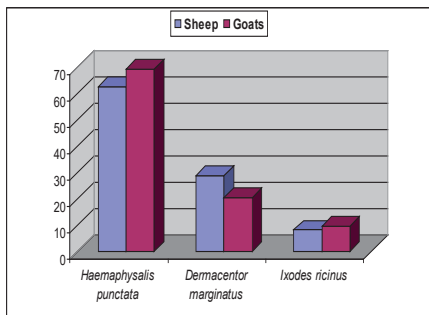


Figure 2. Prevalence and specific structure of Ixodidae tick populations in sheep and goats, in Prahova County

Of the examined sheep 76.5% (n=1885) were infested with ticks; from them 1038 ticks were collected, of which 62.8% (n=652) were identified as *Haemaphysalis punctata*, 28.9% (n=300) as *Dermacentor marginatus*, and 8.3% (n=86) were *Ixodes ricinus* (table 2).

Out of the 1202 examined goats, 81.2% (n=976) were infested with ticks; of them a number of 1195 ticks were collected; of them, 69.6% (n=832) were *Haemaphysalis punctata*,

20.6% (n=246) *Dermacentor marginatus*, and 9.8% (n=117) were *Ixodes ricinus* (table 3).

With regards to sex ratio characteristics, among the collected specimens, the proportion of females was higher for two of the identified species, namely *Haemaphysalis* and *Ixodes*. Therefore, the highest abundance was registered for females of *H. punctata* (87.8%) in goats and 86.2% in sheep, higher than in males. For *D. marginatus* the abundance of males was slightly higher than of females, representing 61.7% in sheep and 58.5% in goats. The overall lowest abundance was found in *I. ricinus*, in the both, sheep and goats, but also females predominated, comparative with males (tables 2, 3).

There is known that sex ratio is influenced also by mating strategies, as reported in questing tick (Hornok, 2009). For instance, *Ixodes* ticks are known to mate also on the vegetation (Kiszewski et al., 2001). Also, this may indicate a transition in the relevant period towards higher number of males questing (to fertilize females on animals) (Randolph et al., 2002).

Table 2. Number and prevalence (%) of ticks by category (species, male, female) collected from domestic goats, in nine study areas in Prahova County

Tick species	Number of collected female ticks (%)	Number of collected male ticks (%)	Number of collected total ticks (%)
<i>Haemaphysalis punctata</i>	731 (87.8%)	101 (12.2%)	832 (69.6%)
<i>Dermacentor marginatus</i>	102 (41.5 %)	144 (58.5%)	246 (20.6%)
<i>Ixodes ricinus</i>	93 (79.5 %)	24 (20.5%)	117 (9.8%)
Total No ticks (%)	926 (77.5%)	269 (22.5%)	1195

Table 3. Number and prevalence (%) of ticks by category (species, male, female) collected from domestic sheep in nine study areas in Prahova County

Ticks species	Number female ticks (%)	Number male ticks (%)	Number of collected total ticks (%)
<i>Haemaphysalis punctata</i>	562 (86.2%)	90 (13.8%)	652 (62.8%)
<i>Dermacentor marginatus</i>	115 (38.3 %)	185 (61.7%)	300 (28.9%)
<i>Ixodes ricinus</i>	72 (83.7%)	14 (16.3 %)	86 (8.3 %)
Total No. ticks (%)	749 (72.2%)	289 (27.8%)	1038

Elective places for attaching of ticks were the inner face of the thigh, the sternal region, the lower abdominal region, and the cervical region. The tick infestation was higher in animals with poor body conditions.

The frequency of tick species and their distribution by host species and originating locality are detailed in Tables 4 and 5.

Table 4. The distribution and the specific structure of the Ixodidae tick population in sheep, in nine study areas of the Prahova County (Southern Romania)

Zones /No. hosts infested by tick (n=1885)	Pantazi/ 272	Racheri/ 249	Varfurile/ 100	Valea Mielor/ 268	Cherba/ 170	Valea Nucetului/ 305	Valea Pietrei/ 200	Iordacheanu/ 199	Draganesti/ 122
Ticks species (n=1038)	Number ticks; % (n=152)	Number ticks; % (n=133)	Number ticks; % (n=104)	Number ticks; % (n=123)	Number ticks; % (n=97)	Number ticks; % (n=182)	Number ticks; % (n=99)	Number ticks; % (n=96)	Number ticks; % (n=52)
<i>Haemaphysalis punctata</i> (n=652)	81 [53.3%]	61 (45.9%)	58 (55.7%)	87 (70.7%)	73 (75.2%)	129 (70.9%)	65 (65.7%)	70 (72.9%)	28 (53.8%)
<i>Dermacentor marginatus</i> (n=300)	61 (40.1%)	56 (42.1%)	40 (38.5%)	25 (20.4%)	16 (16.5%)	42 (23.1%)	26 (26.3%)	16 (16.7%)	18 (34.6%)
<i>Ixodes ricinus</i> (n=86)	10 (6.6%)	16 (12.0%)	6 (5.9%)	11 (8.9%)	8 (8.3%)	11 (6.0%)	8 (8.0%)	10 (10.4%)	6 (11.6%)

Table 5. The distribution and the specific structure of the Ixodidae tick population in goats in nine study areas of the Prahova County (Southern Romania)

Zones / No. hosts infested by tick (n=976)	Pantazi/ 155	Racheri/ 150	Varfurile/ 80	Valea Mielor/ 95	Cherba/ 60	Valea Nucetului/ 198	Valea Pietrei/ 99	Iordacheanu/ 89	Draganesti/ 50
Ticks Species (n=1195)	Number ticks; % (n=192)	Number ticks; % (n=165)	Number ticks; % (n=127)	Number ticks; % (n=113)	Number ticks; % (n=98)	Number ticks; % (n=199)	Number ticks; % (n=126)	Number ticks; % (n=109)	Number ticks; % (n=66)
<i>Haemaphysalis punctata</i> (n=832)	121 (63.0%)	105 (63.7%)	93 (73.2%)	85 (75.2%)	72 (73.5%)	135 (67.8%)	89 (70.6%)	88 (80.7%)	44 (66.7%)
<i>Dermacentor marginatus</i> (n=246)	56 (29.2%)	47 (28.5%)	25 (19.7%)	15 (13.3%)	11 (11.2%)	38 (19.1%)	19 (15.1%)	18 (16.5%)	17 (25.8%)
<i>Ixodes ricinus</i> (n=117)	15 (7.8%)	13 (7.8%)	9 (7.1%)	13 (11.5%)	15 (15.3%)	26 (13.1%)	18 (14.3%)	3 (2.8%)	5 (7.5%)

The annual dynamics of the tick population recorded in the study area registered their peak in spring, due to the environmental and vegetation conditions. The maximum population abundance for *D. marginatus* and *H. punctata* was in April, while for *I. ricinus*, the maximum was in May followed by a sudden decrease in June due to low humidity. Interspecific variations of the annual dynamics were observed. The appearance of ticks was observed for *D. marginatus* in March, unlike *I. ricinus* where the beginning of the invasion was in May. The maximum value of the invasion indices was for in April, for *H. punctata* and *D. marginatus* and in May for *I. ricinus*. The dynamic curve decreased in the

summer months for all three species and started to re-increase in the autumn, this being about 25% of the spring maximum. These differences were found both in sheep and goats.

The abundance of Ixodidae ticks is influenced by the ecological conditions (Ioniță, 2004). In the plain area, the temperature is higher and the humidity is low, compared to the hill area, where the temperature is lower and the humidity is higher.

Based on the registered data during the study period (2019-2020), diagrams of the annual dynamics of the tick populations identified for each species are presented in fig. 3 and 4.

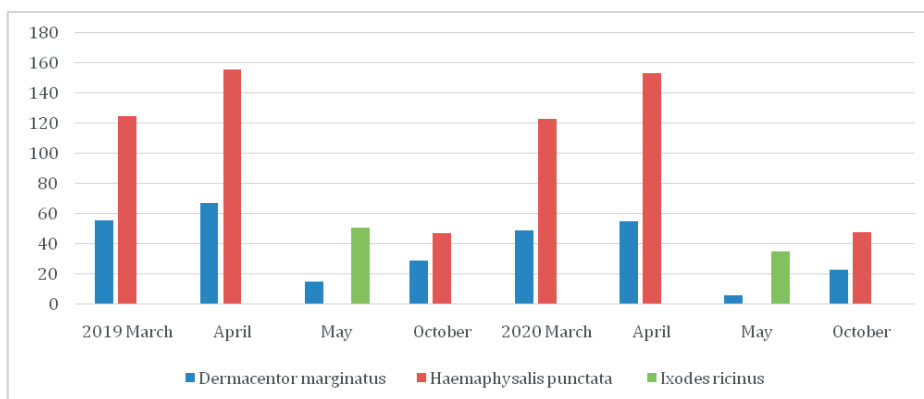


Figure 3. Annual dynamics of the recorded ixodid tick species in sheep, in the studied

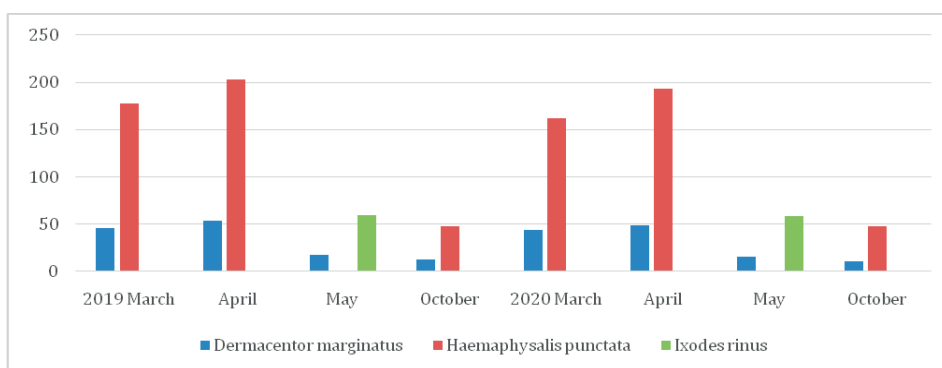


Figure 4. Annual dynamics of the recorded ixodid tick species by months in goats, in the studied area

The findings of this study add new data to update epidemiological knowledge regarding tick populations composition in goats and sheep from southern areas of Romania, particularly Prahova county. Therefore, in our study, the most prevalent tick species were *H. punctata* and *D. marginatus*, and in lower frequency and abundance *I. ricinus*. The bio-ecological features of these tick species are congruent with the characteristics of the environment and biotypes were from tick's host originated. All the three species identified in the present survey have been previously reported in different areas of Romania, including in areas from Southern Romania. For instance, some study reported higher prevalence values than the current study on *I. ricinus*, of 34.24%, in the South-East and North-East areas of Romania (Ioniță et al., (2003, 2010). It is recognized as the most widespread tick species in Romania, with the highest range of host species, including small

ruminants; its prevalence varies from 41.6% to 86.9%, according to the geographical area (Ionita et al., 2003, 2010; Dumitrache et al., 2012; Mihalca et al., 2012b).

D. marginatus is also widespread in Romania, with prevalence values from 9.5% to 34.2% (Ionita et al., 2010; Chitimia, 2007; Mihalca et al., 2012a). *H. punctata* in the current study is higher showed the highest prevalence in both sheep and goats, followed by *D. marginatus*. These findings are in line with similar studies in small ruminants from Italy (Rinaldi et al., 2004).

Tick infestation was compared with the global average prevalence in Europe. Therefore, a similar study in sheep in the southern Italian Apennines, reported the most frequent species, at the farm level, *D. marginatus* (37.6%), followed by *H. punctata* (29.4%), and with lower % *I. ricinus* (0.5%).

Our findings are different than those reported in neighboring countries, such as Serbia. For

instance, in a study investigating tick infestation in sheep and goats from Belgrade area revealed that 60.14% of examined sheep and 34.42% of goats were infested. The relative abundance analysis showed that in both sheep and goats *I. ricinus* was dominant (41.91%, in sheep, 64.42%, in goats). Other tick species recorded were as follows: in sheep - *D. marginatus* (32.91%), *Rhipicephalus bursa* (17.22%), *R. sanguineus* (6.72%), *H. punctata* (2.21%) and *D. reticulatus* (1.17%); while, in goats, were registered *Rhipicephalus bursa* (17.22%), *Rhipicephalus sanguineus* (6.72%), *H. punctata* (4.22%) and *D. marginatus* (2.91%) (Pavlović et al., (2013).

In a similar study in sheep and goats, in Bulgaria, the predominant species was found *Rhipicephalus bursa*, parasitizing on both goats and sheep in Plovdiv region (Arnaudov et al., 2014; Arnaudov & Arnaudov, 2017).

All these findings emphasize on the differences on the tick species composition of sheep and goats accordingly to the local geo-climatic characteristics that significantly impact the biology of ticks and their ecological niche, and consequently the ecology of tick-borne diseases (Márquez-Jiménez et al., 2005; Walter et al., 2016; Krčmar, 2019).

The knowledge on the tick species occurring in a particular area is of high relevance for the both, animal health and public health, as numerous tick-borne pathogens have also zoonotic potential, as recent molecular studies reported also on Romanian ticks (Ionita et al., 2013, 2016). Therefore, it is requiring additional in-depth studies on ticks to focus develop tick control strategies based on their spatiotemporal distribution, host preferences, and climatic niches, which could provide opportunities to assess the risk of infection at the local level through prevention and surveillance of critical points (Mitrea, 2002; Dantes-Torres et al., 2012).

CONCLUSIONS

This study provides new data on the species composition and epidemiology of ticks infesting sheep and goats in Southern Romania. The results confirmed the presence of *Dermacentor marginatus*, *Haemaphysalis punctata*, and *Ixodes ricinus* in the investigated

area and emphasize on the high potential risks for tick-borne diseases in animals and humans. Therefore, to reduce the high prevalence of ticks and their impact on productivity in small ruminants and the associated risks for tick-borne pathogens, immediate attention is required as control interventions.

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