Research Article

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Ixodid Ticks on Domestic Ruminants: an Investigation in the Valley of Maritsa River in Plovdiv Region, Bulgaria

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Abstract: The species composition, distribution, seasonal dynamics and indices of infection of ixodid ticks parasitizing sheep *Ovis aries* (L., 1758), goats *Carpa aegagrus hircus* (L., 1758) and cattle *Bos taurus* (L., 1758) in four municipalities near Maritsa River in Plovdiv Region were studied. In 2011-2016, 584 sheep, 521 goats and 114 cattle were tested at several locations. The overall condition and the condition of the skin and visible mucosal surfaces of animals were observed. Eight ixodid tick species parasitizing domestic ruminants were recorded. *Rhipicephalus bursa* (Canestrini & Fanzago, 1877) was the most widespread tick in the region and the predominant species on sheep and goats, and *Hyaloma plumbeum* (Panzer, 1796) predominated on cattle. Some differences in the indices of infection and seasonal dynamics of the spread of ixodid ticks were observed on the different species of domestic ruminants. There were no serious changes in the overall condition and the condition of the skin and visible mucosal surfaces of the infected animals.

Key words: cattle, goats, Ixodidae, Plovdiv Region, sheep

Introduction

Ixodidae ticks are obligate blood-sucking arthropods. They have great epidemiological importance for the distribution of infectious and parasitic diseases in humans and domestic and wild animals, as they can be reservoirs, vectors and (or) transient host for tick-borne pathogens (Pavlovic *et al*. 2016). This is due to their biological characteristics, e.g. the ability of transovarial and three-phase transmission of a large number of pathogens (Balashov 1967) and falling into a state of hibernation at low temperatures or lack of food (Harlan & Foster 1990). This allows maintenance of natural foci of infectious and parasitic diseases, regardless of seasonal changes in climate in a given area or long-term lack of a susceptible host. The global economic losses of tick-borne diseases are supposed to be many billions of dollars (Jongejan & Uilenberg 2004). This determines the important health and economic significance of the ixodid ticks, which is why they are subject to a number of studies (Arnaudov *et al*. 2014).

In Bulgaria, approximately 30 species have been recorded. They belong to the following genera: *Ixodes, Dermacentor, Rhipicephalus, Boophilus, Amblyomma, Hyalomma* and *Haemaphysalis* (Beron 1973-1974). The majority of research in the country, however, has been conducted more than 4 decades ago. These studies were conducted mainly in connection to the detection of an outbreak of tick-borne encephalitis in Iskra Village, Parvomay Municipality in 1953 (Arnaudov & Antov 1976, Georgiev *et al*. 1971, 1975, Sarbova 1956). This issue, as well as the fact that the dynamics of climate change could lead to drastic changes in the species composition of ixodid ticks in a given geographical area (Estrada-Peña &

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Venzal 2007) necessitates the study of the species composition and distribution of ixodid ticks. A specific feature of the Plovdiv Region is that range farming is very well-developed, especially along the Maritsa River. It is densely populated and there are important national and international transport corridors that pass through it. This sets a higher risk of transmission of zoonotic diseases through ixodid ticks.

The combination of these factors shaped the focus the present study: determining the distribution, species composition, seasonal dynamics and performance of the infection of ixodid ticks on sheep, goats and cattle in four municipalities near Maritsa River in Plovdiv Region.

Materials and Methods

Study area

Work was carried out in four municipalities near Maritsa River in Plovdiv Region: Saedinenie, Stamboliyski, Sadovo and Parvomai, 42°15'–42°50' N and 24°33'–25°13' E (Fig. 1). The Plovdiv area has a humid climate that is mild, with no dry season (year-round rainfall). Its climate is slightly influenced by the Mediterranean Sea. Summers are hot and muggy, with thunderstorms. Seasonality is moderate (Köppen-Geiger classification - Cfa). The av. temperature is 12.3°C. The annual temperature deviation is 22.5°C. The total annual rainfall averages at 492 L/m (www.plovdiv.climatemps.com).

Almost the entire area where the study was conducted is utilized for agriculture. The natural vegetation occupies a very limited area. The forests are represented by small torn habitats mainly on lands, which are not suitable for farming. The plants that prevail there are of the genus *Quercus* and hornbeam. With a secondary origin, there are mesophilic grasslands that have occurred in place of elm, pedunculate oak, field ash and other forests. Willow and poplar riverside forests as well as longose vegetation predominate along the Maritsa River and its major tributaries (Gruev & Kuzmanov 1994).

Tick collection

The investigation was conducted in 2011-2016. During the study, 584 sheep, 521 goats and 114 cattle at 9 localities were examined (Fig. 1). The animals came from farms with different number of animals kept in them. Only herds that were grazing have been taken into consideration. The animals were examined monthly, during the period from the exit to the pasture to the retread to the barn (March – October). The examination of animals for infection was performed in the following order: head (between the horns and ears), neck, back, under the tail, groin and udder. After the collection, the tick samples were separately stored in 70% ethanol and labelled with the date and the field name until the species determination. Tick species were identified under a binocular magnifier (Karl Zeiss) according to the identification keys by Estrada-Pena *et al.* (2004) and Pomerantsev (1950).

The quantity determination of the infection of hosts by ixodid ticks was represented by the following parameters:

1. The prevalence of infection, P%. It is the percentage of occurred parasitic infections of the investigated animals in the area as a whole and of the settlements in it. It is determined by the formula: number of infected hosts / number of examined hosts x 100.

2. Intensity of infection, II. It represents the number of ticks of one species found on one infected animal.

The data obtained were analyzed using Chi-square test (χ^2 test) aiming to determine the relationship between infections with a certain type of tick and the host species (GraphPad InStat 3.1 Softwere). The confidence level was kept at 95%.

The overall condition (vitality, behaviour, appetite and water intake) and the condition of the skin and visible mucosal surfaces of invaded animals were observed.

Results and Discussion

Species composition of ixodid ticks invading domestic ruminants in the investigated area

For the purposes of the present study, 2277 ixodid ticks were collected and identified. It was found that they belonged to eight species: *Rhipicephalus bursa, Rhipicephalus sangiuineus* (Latreille, 1806), *Hyaloma plumbeum, Dermacentor marginatus* (Sulzer, 1776)*, Ixodes ricinus* (Linneaus, 1758)*, Boophylus calcaratus* (Birula, 1895)*, Haemaphysalis sulcata* (Canestrini & Fanzago, 1878) and *Haemaphysalis punctata* (Canestrini & Fanzago, 1878). Sheep were found to be hosts of six of tick species, goats had six species and three species were found on cattle (Table 1).

Parameters of infection, seasonal dynamics and epidemiological features of the identified ixodid ticks

Rhipicephalus bursa is the most widespread in the region (n=801). It is the predominant parasitic species in sheep and goats and is also found in cattle. Its infection rate was the highest in sheep and the lowest in cattle (Table 1). The Chi-square test showed there

Fig. 1. Localities in the Plovdiv Region where the field studies were carried out (1 - Saedinenie; 2 -Tsarimir village; 3 -Tseretelevo village; 4 - Stamboliyski; 5 - Sadovo; 6 - Parvomai; 7 - Gradina village; 8 - Byala Reka village; 9- Pravoslaven village)

Table 1. Number (n) and indicators of infection (P% - prevalence, II – intensity of infection) of ticks on domestic ruminants in Plovdiv Region

Ticks / Host	*Rhipicephalus bursa* (n=801) P% II	*Rhipicephalus sanguineus* (n=511) P% II	*Ixodes ricinus* (n=188) P% II	*Dermacentor marginatus* (n=590) P% II	*Hyaloma plumbeum* (n=44) P% II	*Boophylus calcaratus* (n=57) P% II	*Haem. punctata* (n=59) P% II	*Haem. sulcata* (n=27) P% II
Sheep	29.45 1–8	18.66 1–5	9.93 1–5	13.52 1–12	-	-	3.60 1–2	2.05 1–3
Goats	22.65 1–5	22.07 1–6	11.51 1–5	14.58 1–11	-	-	4.22 1–2	1.53 1–2
Cattle	5.26 1–2	-	-	-	28.07 1–3	15.79 1–9	-	-

was a statistical correlation between the infections with a specific tick and the host species. The biggest differences within the infestation rates have between cattle, on the one hand, and sheep and goats, on the other ($p < 0.01$). The differences in the infestation rates between sheep and goats are lower but also with statistical significance ($p < 0.05$).

Rh. bursa was found during the entire period of the study, and the highest infestation levels were recorded in June (Fig. 2). In our view, the spread of this tick species is due to the climate conditions in the research area (humid and hot weather) as *Rh. bursa* is a thermophilic species (Hoogstraal & Valdes 1980). This makes it widely distributed in the Mediterranean Region (Estrada-Pena & Santos-Silva 2005, Papadopoulos et al. 1996). Its epidemiological significance is associated with its ability to be a carrier of babesiosis, theileriosis and anaplasmosis in domestic animals and the Nairobi sheep disease (Friedhoff 1997, Taylor *et al*. 2013). The tick is one of the vectors of the agents of the Crimean-Congo hemorrhagic fever (CCHF) and of the Q-fever (Taylor *et al*. 2013). As a two-host parasite, *Rh. bursa* has its significant importance in causing epidemics and outbreaks of human CCHF because of their large numbers during certain periods and their aggressiveness in seeking human hosts (Hoogstraal 1979).

Infection with *Rhipicephalus sanguineus* was established only in goats and sheep (Table 1). The Chi-square test showed there was no correlation between the infections with a specific tick and the host species. The differences in the infection levels between the sheep and goats were not significant at $p<0.05$. Maximum of infestation with this arachnid was in May and June (Fig. 2). *Rh. sanguineus* is the most common ixodid tick in the world (Dantas-Toress 2010). This study also found a widespread infestation with it in ruminants, although its main host is the dog. This high infestation in ruminants in May and June can be associated with its biological characteristics, i.e. abundant food intake and moulting of the larvae and nymphs in the summer (Dantas-Toress *et al*. 2011). *Rh. sanguineus* as a three-host parasite can be a vector or reservoir of certain pathogens (e.g., *Rickettcia conorii* and *Ehrlichia canis*). The tick has the ability of maintaining the pathogen in nature, through several generations, by transovarial and transstadial passages (Dantas-Torres 2008). Its role in the transmission of pathogens to humans is well documented, in spite of its relatively low anthropophily (Dantas-Torres 2008).

Ixodes ricinus is a parasite on sheep and goats but not in cattle (Table 1). The Chi-square test showed that the differences in the infestation levels between

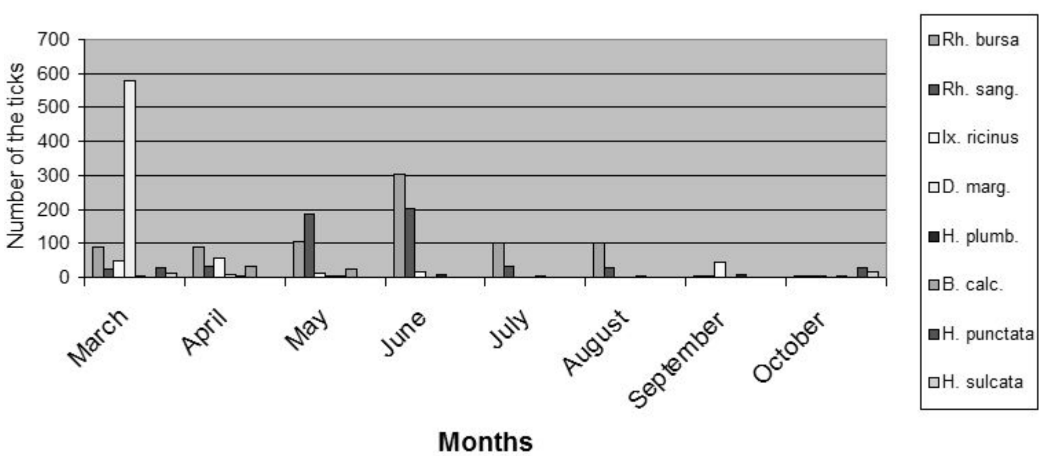

Fig. 2. Population dynamics of the recorded ixodid tick species by months in the studied area

the sheep and goats are not significant (p>0.05). A determining factor for its distribution is the high air humidity (Estrada-Pena & Santos-Silva 2005). This explains the discovery of two waves of infestations (spring and autumn) during the wet months (March-April and September) and his absence during the dry periods (Fig. 2).

Ixodes ricinus is a widespread species in different parts of Europe (Gray 1991, Daniel *et al.* 2003, Lindgren *et al.* 2000). In some areas of the former Yugoslavia and Northern Greece, it is the predominant tick species in small and large domestic ruminants (Omeragic 2011, Pavlidou *et al.* 2008, Pavlovic *et al.* 2013, 2014, 2016). This tick has a particularly important epidemiological significance because it is a vector of some of the most dangerous transmissible infections - Lyme disease, tularemia, babesiosis and tick-borne encephalitis (Goddard 2007, Gray 1991, Lindquist & Vapalahti 2008, Sarbova 1956). Its epidemiological significance will grow for two main reasons. On the one hand, its prevalence is currently increasing under the influence of environmental, climate and anthropogenic factors (Lindgren *et al.* 2000). Also, the rates of infection of *Ix. ricinus* with *Borelia burgdorferi*, various types of *Rickettsia, Babesia* and *Ehlichia* are very high in many European countries (Dumitrache *et al.* 2012).

Dermacentor marginatus was found to parasitize sheep and goats in the studied area but not on cattle (Table 1). Infection with this arachnid was found only in March, and in April and May only single individuals were found on sheep. During June - October, infections of *D. marginatus* were not detected (Fig. 2). The ticks were found on the head between the horns and in the tail area. The high intensity of infection is notable – 1-12 in sheep and 1-11 in goats and, in this regard, it has the second highest total number (n= 590). The Chi-square test showed that the differences in the infestation levels between the sheep and goats are not significant (p>0.05).

According to Rubel *et al.* (2016), the geographic distributions of the species in Europe spreads from Portugal to Ukraine and also in some eastern regions; e.g. in Kazakhstan in the areas between 33°N and 51°N. In Romania, it is the predominant tick species on domestic and wild animals (Dumitrache *et al.* 2012). The second highest levels are in Bosnia and Herzegovina (Omeragic 2011) and in Belgrade area (Pavlovic 2013). Its development is related to the presence of forest vegetation (especially of species *Quercus*) and the relatively high altitude, 800-1000 m a. s. l. (Estrada-Pena & Santos-Silva 2005). In the present study, this parasite was found in places with lower altitude (100-200 m a. s. l.) but mainly in March. In our previous study (Arnaudov *et al.* 2014), a prevalence of *D. marginatus* was found in sheep and goats in areas with altitudes above 600 m and natural oak vegetation. *D. marginatus* is a vector of a number of rickettsial pox diseases, including those caused by *Rickettsiae* with the newly discovered genotype RpA4 (Selmi *et al.* 2009, Špitalská *et al.* 2012, Vitorino *et al.* 2007). In recent studies, it was found that this arachnid is infected with various rickettsiae to a high level (Marquez *et al.* 2006, Špitalská *et al.* 2012).

Hyalomma plumbeum infestation was found only in cattle where it is the predominant parasitic species. The prevalence was 28.07%, and intensity of infection was 1-3 (Table 1). There were no seasonal changes in the infection (Fig. 2). In another study, we have found an infection in sheep and goats with it but in areas with high altitude (Arnaudov *et al.* 2014). This tick has important epidemiological significance as it was the main vector of tick-borne encephalitis during the epidemic in Iskra Village, Plovdiv Region, in 1953 (Sarbova 1956), and also it is the vector of the Crimean-Congo hemorrhagic fever (Papa *et al*. 2004, Whitehouse 2007) and West Nile virus fever (Koopmans *et al*. 2007).

Boophylus calcaratus infestation was found only in cattle. Infection of the tick was registered only during April - May (Fig. 2). The infestation of animals is related to their pasture in open areas. A specific site of infection was found – ticks were found on the head, neck and around the tail. The value of the intensity is high – 1-9 (Table 1). This tick is adapted to tropical and subtropical climate; therefore, it occurs mainly in Asia and in North Africa (Taylor *et al*. 2013). As a single-host tick, not parasitizing on humans, *B. calcaratus* has no epidemiological significance but in cattle, it is a carrier of significant diseases, such as babesiosis and anaplasmosis (Taylor *et al*. 2013). From an epidemiological point of view, it is particularly important that it can transmit the infectious agent transovarially and the pathogen's ability to be preserved in the tick for three generations, not necessarily invading a host in that period.

Haemaphysalis punctata and *Haemaphysalis sulcata* infections were the most limited (Table 1). Individual ticks of both species were found only in the cold months, March and October, on sheep and goats (Fig. 2). The Chi-square test showed that the differences in the infestation levels between sheep and goats were not significant ($p>0.05$). In addition to Bulgaria, *H. punctata* and *H. sulcata* are found mainly in the countries of the Mediterranean region (Estrada-Pena *et al*. 2004). The results of this study are close to the results of similar investigations on *H. punctata* in other Balkan countries (Omeragic 2011, Papadopoulos *et al*. 1996, Pavlovic *et al*. 2016).

Results from examination of animals invaded by ticks

There were no serious changes in the overall condition and the condition of the skin and visible mucosal surfaces of the invaded animals.

Conclusions

Eight species of ixodid ticks infecting domestic ruminants were identified in the investigated area. *Rhipicephalus bursa* was the most widespread and prevalent parasitic species on sheep and goats. On cattle, the predominant species was *Hyalomma plumbeum*. There are species and seasonal variations of infections associated with the biology of ticks and the climatic conditions of the area.

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