STUDY ON TICKS REMOVED FROM PATIENTS FOR INFECTION WITH *BORRELIA BURGDORFERI* AND THEIR NUMBER DEPENDING ON TEMPERATURE AND PRECIPITATION IN 2016-2021

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ABSTRACT

Background: Deacarization, applied epidemiological measures, and climatic factors affect the abundance of ticks. On the other hand, ticks themselves are a factor of great epidemiological and epizootic importance. Studies concerning the influence of climatic factors on ticks and their infection with *B. burgdorferi* are still limited in Bulgaria. The aim was to investigate the abundance of ticks in relation to temperature and precipitation, as well as the infection with *B. burgdorferi* of ticks removed from patients during the period 2016-2021.

Materials/methods: A total of 10,907 ticks were collected from patients and classified according to species and stage of development. Nested PCR was performed targeting two sites of the spacer region between 5S and 23S of *B. burgdorferi* sensu lato rRNA. Weather data were collected from free Internet meteorological sites.

Results: It was found that 92-96% of the ticks belonged to the species *Ixodes ricinus.* PCR data were obtained for infestation of ticks in 2016-2021. Only in 2018, the highest number of ticks was observed in May, while in the other five years - in June, which was analyzed in relation to the average temperature and precipitation.

Conclusion: An increase in tick abundance was observed at average temperatures around 20°C, with rainfall on the days before the peak. During the 6-year period, the highest number of ticks was collected in June 2021, which could be explained with the high average temperatures and abundant precipitations during the preceeding spring months (April – June).

Key words: *B. burgdorferi, ticks, temperature, precipitation*

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INTRODUCTION

Lyme borreliosis is the most common tick-borne infection in North America and Europe (1). The tick *Ixodes ricinus* is the main vector. As a result of climate change, associated with global warming (2), this species is constantly expanding its distribution area. Tick-borne diseases require an established vector population, a pathogen and suitable environmental and climatic conditions across the cycle of transmission in humans (3). Climate influences the life cycle of ticks, as well as the reproduction rate of bacteria inside the vector and human hosts (4). This means that uprising temperature can reduce the incubation period of pathogens and the life cycle of vectors, thus boosting the transmission risk through elevated vector populations. Long-term seasonal changes can affect the prevalence of vector-borne diseases in Europe (5). *I. ricinus* is present throughout Europe with an expansion to higher latitudes in Sweden (6) and, to higher altitudes elevations in the Czech Republic (7), Austria (8), Norway and Germany (4). On the average, more than 1500 ticks removed from patients per year were tested for *Borrelia burgdorferi* infection during the study period. It is known that ticks are susceptible to climatic determinants as humidity and temperature. The number of ticks removed from our patients varied significantly through the years, from over 2000 in 2018 and 2020 to less than 1500 in 2016 and 2021 . In 2018 , the highest number of ticks was reached one month earlier as compared to the other years of the study. What are the reasons for the observed annual differences ? The aim of the study

was to investigate *B. burgdorferi* infection in ticks removed from patients and their numbers in relation to the average temperature and precipitation over the 6-year period.

MATERIAL AND METHODS

A total of 10,907 ticks were collected from patients as follows: 1,158 in 2016, 1,895 in 2017, 2,347 in 2018, 1,985 in 2019, 2,052 in 2020 and 1,470 in 2021. They were identified morphologically with a microscope "Leika" and determined to the species and stage of development (9). Nested PCR were performed targeting two sites from the spacer region between 5S and 23S of *B. burgdorferi* sensu lato rRNA (10). Weather data were collected from freemeteo.bg (11) and meteoblue.com (12). Diagrams were made for the four months of interest: April, May, June and July, when most of the ticks were brought to the laboratory for examination. Data on Lyme disease morbidity in humans during 2016-2020 were obtained from NCIPD (13).

RESULTS AND DISCUSSION

It was found that between 92-96% of ticks belonged to the species *I. ricinus.* There was an increase in the number of nymphs studied over the 6-year period.

PCR data for infestation of ticks from Sofia city were found in 21.03% (184/875) of the ticks investigated in 2016, 20.7% (249/1,203) of the ticks in 2017, 9.47% (155/1,636) of the ticks in 2018, 22.47% (387/1,722) of the ticks in 2019, 15.02% (273/1,817) of the ticks in 2020 and 20.41% (258/1,264) of the ticks in 2021.

A comparative study for *B. burgdorferi* infestation of ticks from 2018 to 2021 was conducted in the five locations in Sofia with the highest number of ticks removed from patients.

Table 1. *Infection of ticks with* **B. burgdorferi** *in five places in Sofia in 2018-2021*

Region in Sofia	Infection in 2018	Infection in 2019	Infection in 2020	Infection in 2021
Cemetery parks	5/50 (10%)	17/76 (22%)	13/62 (21%)	19/84 (23%)
South park	9/72 (12.5%)	12/33 (36%)	8/42 (19%)	14/56 (25%)
Borisova garden park	4/50 (8%)	9/52 (17%)	14/ 66 (21%)	9/49 (18%)
Neighbour-hood "Iztok"	4/13 (31%)	7/22 (32%)	5/33 (15%)	11/43 (26%)
Neighbour-hood Bankya	1/35 (3%)	6/23 (26%)	5/38 (13%)	7/31 (23%)

In addition, a tick infestation was compared to morbidity in humans during the same period 2016-2020.

Table 2*. Comparative study on tick infestation and morbidity in humans in 2016-2020*

Year	Infestation of ticks with *B. burgdorferi*	Number of cases / morbidity in humans in Bulgaria
2016	184/875 (21.03%)	290/ 4.05%000
2017	249/1,203 (20.70%)	402/ 5.66%000
2018	155/1,636 (9.47%)	599 / 8.50%000
2019	387/1,722 (22.47%)	375 / 5.36%000
2020	273/1,817 (15.02%)	160 / 2.3%000

The highest number of ticks in 2018 was observed during the month of May, and the peak in tick abundance in 2016, 2017, 2019, 2020 and 2021was found in June. Data on the monthly number of ticks (for April, May, June and July) were analyzed against the average temperature and precipitation during the 6-year study period (Fig. 1, 2, 3, 4, 5, 6, 7, 8).

According to our data, the species *I. ricinus* is associated with over 92% of tick bites of humans in the country. An increase in the number of nymphs studied over the years was also associated with an increase in the number of bites, due to their pronounced anthropophilia and difficult detection. Nymphs were less infected with the causative agents of Lyme disease as compared to imagos (15.93% vs. 20.29%) only in 2016. Tick infestations varied between 10.68% in 2018 and 27.58% in 2019 during the 6-year study period, showing the important role of ticks in *B. burgdorferi* transmission. A meta-analysis of the distribution and prevalence of bacteria from the group *B. burgdorferi* s.l. in European ticks (14) showed that the most commonly reported *B. afzelii, B. garinii, B. valaisiana*, and *B. burgdorferi* sensu stricto largely overlap across Europe.

The highest prevalence occurred in areas with small amplitude of the mean annual temperatures (7°C–17°C), together with a moderate spring rise of the vegetation. The study established an average of 2% infection with *B. burgdorferi* s.l. of 82,000 questing nymphs with prevalence from <1% to >46%.

The highest rate of tick infection with *B. burgdorferi* in Sofia was detected in 2019 (22.47%), and the lowest - in 2018 (9.47%). A significant decrease in tick infection was found in 2018 (9.47%), compared to 2017 (20.70%). The data were analogous to those established by us in 2003/2004, when there was a decrease from 25.42% to 7.21%, (P<0.05) (15). These data showed that statistically significant fluctuations in tick infection are observed periodically over the years.

Unfortunately, some of our most representative parks (South park, Borisova garden) were among the localities with the highest number of ticks removed from people and brought for testing (Table 1). The risk of human infection was also high (between 10 and 23%) in the spring and autumn stew, when many people visit the Cemetery parks (Table 1).

Lyme disease comprises the most important vector-borne disease burden in the European Union with an estimated of 65,000 cases per year (2). As stated in the Annual Analyses of Acute Infectious Diseases in Bulgaria (13) during the 2016-2020 period the number of Lyme borreliosis cases ranged from 160 (2020) to 599 (2018). While morbidity varied between 4.05%000 and 5.66%000 (in 2016, 2017, 2019), tick infection rates ranged between 20.70% and 22.47% (Table 2). The comparison between the rates of tick infestation and the number of Lyme disease cases in humans showed that, the lowest tick infestation rate for the 6-year period (in 2018) corresponded to the highest morbidity rate in patients. Interestingly, these facts coincided with the highest number of ticks brought to the laboratory for analysis (2,347) and with a premature rise in the number of ticks (Fig 4) in that same year (2018). It should be noted that the high incidence of human cases in 2018 was based on data for several cities for which data on tick infestation was not available.

An increase in the number of ticks was observed at an average temperature around 20°C, with rainfall of 5-10 mm on the days in May and June in 2016 and 2017 (Fig. 1, 2, 4). The highest number of ticks was detected in the month of May only in 2018, in the presence of high temperatures, without sharp fluctuations, preceded by 30-40 days of precipitation (Fig. 3, 4). The increased number of ticks in June was in associated with an increase in temperatures and precipitation from mid-May to mid-June in 2019 (Fig. 5, 8). In 2020, most of the precipitations were observed in May accompanied by a smooth rise in temperatures, and leading to a peak number of ticks in June (Fig. 6, 8). In 2021, the average spring temperature was around 20°C, with a lot of rain in April, May and June, leading to the highest number of ticks during the 6-year period in June (Fig. 7, 8). According to a climate projection by 2040–2060, a 3.8% overall habitat enlargement for *I. ricinus* is anticipated in Europe. This correlates with the increased number of ticks and their extension into higher altitudes and latitudes in many areas like the Scandinavian and Baltic countries (2). Therefore, the increased number of ticks removed from people in Bulgaria during the years of the study was not surprising.

CONCLUSIONS

Data related to number and infestation of ticks can be applied in practice in connection with the

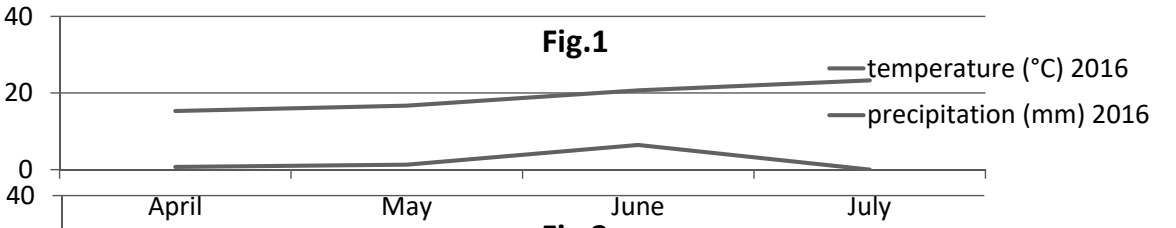

Fig.1

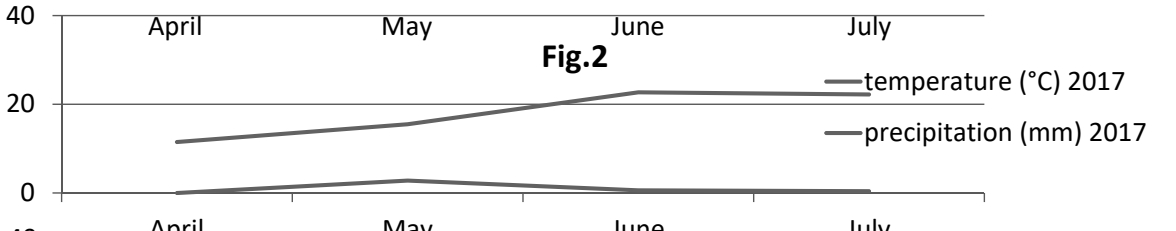

Fig.2

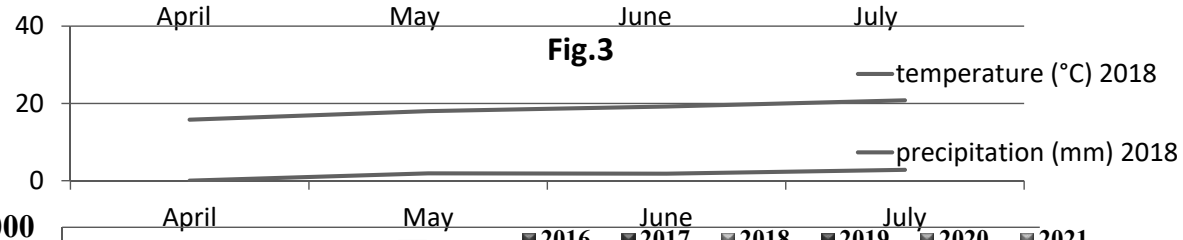

Fig.3

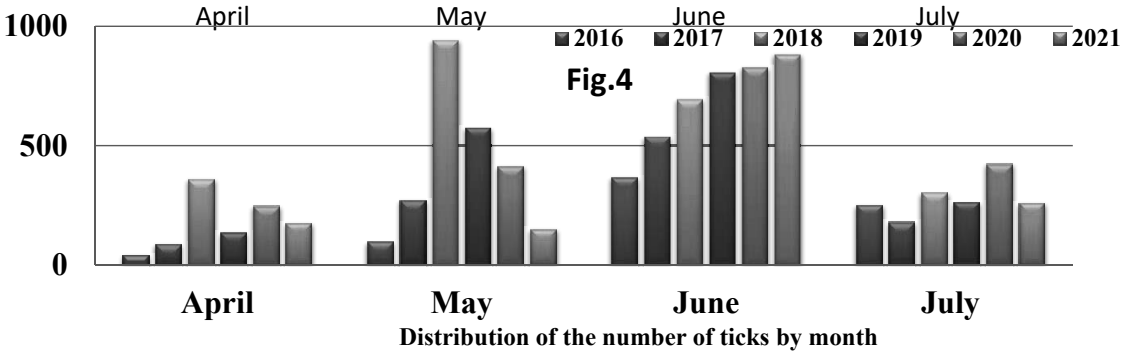

Fig.4

Distribution of the number of ticks by month

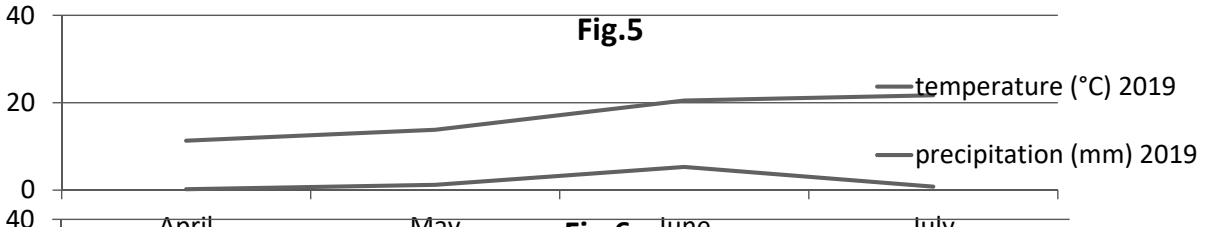

Fig.5

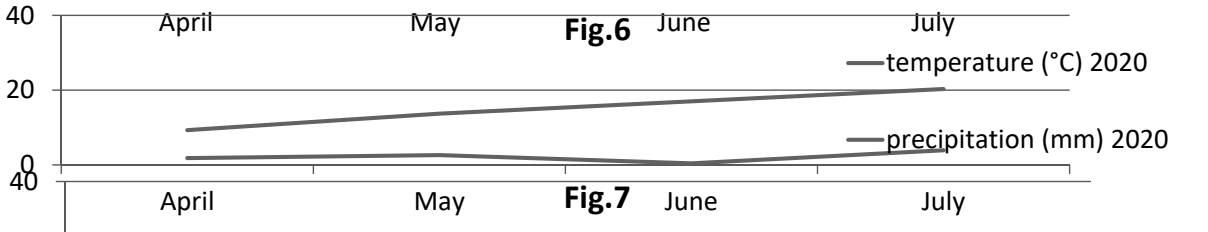

Fig.6

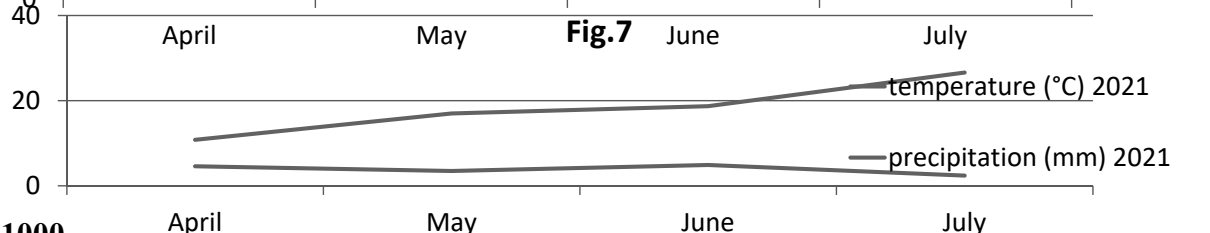

Fig.7

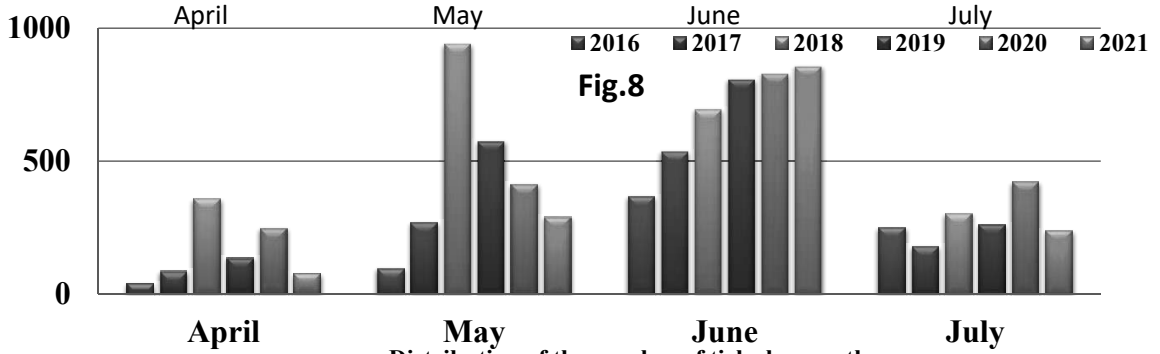

Fig.8

Distribution of the number of ticks by month

deacarization of lawns and the use of personal protective equipment. Temperature and precipitation data should also be taken into account when lawns are processed against ticks. Timely examination of the body after staying in lawns and rapid removal of ticks, especially the highly anthropophilic nymphes, reduces the risk of infection with Lyme borreliosis. This study indicates the importance of monitoring temperature and precipitation, for predicting the number and infestation levels of *I. ricinus* ticks associated with the incidence of Lyme disease

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