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A short-term and long-term relationship between occurrence of acute canine babesiosis and meteorological parameters in Belgrade, Serbia

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ABSTRACT

Belgrade, the capital of the Republic of Serbia, is an endemic location for canine babesiosis caused by *Babesia canis*. This tick-borne disease occurs seasonally in regions with moderate continental climate. However, recent clinical data show that: 1) some cases of canine babesiosis have been recorded during the winter, and 2) canine babesiosis is spreading to the northern parts of Europe with a colder climate, which is a region previously free of this disease. Our study investigates the occurrence of canine babesiosis in different seasons over 2013–2016, and explores a short-term link between meteorological parameters and number of diagnosed cases of canine babesiosis in Belgrade. It also looks into possible long-term correlations that span one year before the onset of the disease. Based on 872 recorded cases over four years, our results show a bimodal seasonal distribution of canine babesiosis, with a pronounced peak in the spring, and a less conspicuous one in the autumn. Throughout the year, even over the coldest and warmest periods, there is a broad range of temperatures and relative humidities when the disease is recorded. Over one year prior to the spring and autumn onset of the disease, we found a noticeable impact of temperature and relative humidity, and to a lesser extent, of atmospheric pressure and cloud cover, on the number of diagnosed cases. These findings imply short-term and long-term relationships between occurrence of acute canine babesiosis and certain meteorological parameters, and they open further questions that need to be investigated in order to understand the epidemiology of this disease.

1. Introduction

Belgrade, the capital of the Republic of Serbia, is located in a region endemic for canine babesiosis caused by *Babesia canis* (Kovačević Filipović et al., 2018), a protozoan transmitted by a tick vector – *Dermacentor reticulatus* (Földvári et al., 2016). Free-roaming outdoor dogs are considered a major reservoir for *B. canis* and *B. canis* is maintained in the tick population by transstadial and transovarial transmission (Uilenberg, 2006). Despite an occasional detection of the parasite in wild canids (Cardoso et al., 2013; Duscher et al., 2013; Hodžić et al., 2015; Hornok et al., 2013a; Juwaid et al., 2019), their capability to maintain *B. canis* has not yet been proven (Földvári et al., 2016). The population of free-roaming dogs in the Belgrade urban and suburban area increased abruptly at the end of the last century, and this coincided with a recognition of an increased number of clinical cases of canine babesiosis (Krstic et al., 1994). A general relationship between the *D. reticulatus* activity and meteorological parameters has been described in different regions (Földvári et al., 2016) and a seasonal occurrence of the disease has been noted (Leschnik et al., 2008; Welc-Faleciak et al., 2009). Still, a relatively large number of clinical cases during the winter months in Belgrade (personal communication with veterinary practitioners in Belgrade), when the average temperatures are lower than predicted for the *D. reticulatus* activity, prompted us to investigate a link between the canine babesiosis occurrence and meteorological parameters. A large number of clinical cases recorded in a relatively small geographical area, could enable a precise description of a meteorological influence on canine babesiosis occurrence in different seasons. Moreover, site-specific combinations of temperature, relative humidity and precipitation, could have a critical impact on the seasonal character of tick activity and thus an incidence of the disease.

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Tick-borne diseases are affected by a number of ecological parameters. Among the most important are vector competency, tick population dynamics, seasonal and diel activity of the vector, host specificity and other host-related factors, and habitat requirements that include micrometeorological parameters (Sonenshine and Mather, 1994). *D. reticulatus* is a tick whose life cycle generally lasts one to two years, and the development from eggs to adults is usually completed within a few months, between spring and autumn (Kahl and Dautel, 2013; Nosek, 1972). Further, *D. reticulatus* is a three-host tick, with each stage feeding only once (Milutinović et al., 2012). Although *Dermacentor* spp. larvae/nymphs have been found on dogs during mid-summer in Hungary (Hornok et al., 2013b), these developmental stages predominantly infest small mammals for their blood meal, while adults infest larger mammals, including dogs. In the majority of cases, canine babesiosis seems to occur as a result of a blood meal by an adult *D. reticulatus* carrying *B. canis* since its conception. Another important characteristic of adult *D. reticulatus* is that in Central Europe, both winter and summer diapause are very short or do not exist at all, enabling a prompt activity of ticks when meteorological conditions are favourable (Földvári et al., 2016; Szymanski, 1987; Tharme, 1993).

Recently, *B. canis* has been detected in golden jackals and parasitising *D. reticulatus* ticks in parts of Serbia further south from Belgrade (Sukara et al., 2018), making the vector and the pathogen more prevalent in the Balkans than could be inferred from the European Centre for Disease Prevention and Control database (European Centre for Disease Prevention and Control (ECDC, 2017). South of Belgrade, the maximum of seasonal activity of *D. reticulatus* has been noted in the spring at a mean temperature of 10 °C and relative humidity of 73% (Milutinović and Radulovic, 2002), i. e., in conditions that exclude the ticks' activity during the cold months. Still, as already mentioned, clinical cases of canine babesiosis have been recorded in Belgrade in winter time. Hence, our analysis investigates the seasonality of the disease occurrence and within it, as a special case, winter months.

Specifically, we looked into a short-term relation between the number of diagnosed cases and meteorological parameters that could facilitate the ticks' activity and, in turn, the occurrence of canine babesiosis. We also hypothesised that correlating the number of clinical cases with the meteorological data spanning a 12-month period prior to the onset of the disease, could yield valuable information about a long-term impact of meteorological parameters on population density of *D. reticulatus* infected with *B. canis*.

The aims of this work were to investigate: 1) the seasonal occurrence of acute canine babesiosis caused by large *Babesia* in Belgrade over a four-year period, 2013–2016; 2) a possible relation between the disease occurrence and temperature, relative humidity, cloud cover and atmospheric pressure over a 2-week period, which is an average predicted incubation time of canine babesiosis; and 3) a possible correlation between the disease occurrence and the meteorological parameters during one year before the onset of the disease.

2. Material and methods

We performed the analyses on data collected in Belgrade (44°49′N; 20°28′E), one of the largest cities in south-eastern Europe, with a population of about 1.7 million. It is situated at a confluence of the Sava and Danube rivers. The Belgrade climate is humid subtropical, with four seasons. According to the 1981–2010 climate normals, the mean annual temperature is 12.5 °C, with January the coldest (1.4 °C) and July the warmest month (23 °C), the annual mean relative humidity of 68% and the annual average precipitation of 690.9 mm (Republic Hydrometeorological Service of Serbia (RHMZ, 2018a). On average, there are 58 frost days and 39 days with snow cover per annum. The annual mean number of tropical days is 36.

2.1. Clinical data acquisition

Data were collected over four years 2013–2016, in two veterinary practices in Belgrade. The data records contained 872 diagnosed clinical cases of babesiosis induced by large *Babesia*. We applied the following criteria for inclusion of cases: 1) acute onset of clinical signs consistent with *Babesia* infection (24–48 hours of anorexia, fever, lethargy, pale or icteric mucous membranes); 2) large *Babesia* positive thin blood smear stained with BioDiff® (BioGnost, Croatia); 3) haematology changes consistent with acute babesiosis (thrombocytopenia, leukopenia and/or anaemia); and 4) positive response to treatment with imidocarb-dipropionate (6.6 mg/kg/body weight). All the dogs were naturally infected.

The original data records were kept on a daily basis. However, we combined the records from both practices into a weekly time series that were then correlated with meteorological data.

2.2. Meteorological parameters

We obtained the following daily mean meteorological parameters for Belgrade: temperature (TG), relative humidity (HU), atmospheric pressure (PP), precipitation (RR), and cloud cover (CC) from the Republic Hydrometeorological Service of Serbia (Republic Hydrometeorological Service of Serbia (RHMZ, 2018b). We calculated the mean weekly values of the meteorological parameters from their daily records. Please note that hereinafter, "temperature" refers to "weekly mean temperature", and the same stands for the other meteorological variables.

2.3. Correlation analysis

As the first step in our analysis, we used Shapiro-Wilk test to assess whether the time series followed the normal distribution. Since the test was not conclusive, we calculated Spearman's correlation coefficients which quantify a monotonous relation between two variables. Only statistically significant Spearman's coefficients (significance level 0.05) are shown.

In the correlation calculations, we also introduced a time lag, given in number of weeks, which shifts correlation pairs in the arrays. When time lag equals zero, correlation pairs comprise of values recorded in the same week. For example, number of canine babesiosis cases and temperature for the same week are correlated. When time lag equals -1, number of diagnosed cases is correlated with temperature recorded one week earlier. In our analysis, we let time lag vary between 0 and -52, thus spanning a whole year.

3. Results and discussion

3.1. Seasonal occurrence of canine babesiosis

The occurrence of canine babesiosis in Belgrade shows a seasonal cycle (Fig. 1). Two maxima in the occurrence can be discerned: a relatively broad and prominent winter/spring maximum between weeks 1 and 18, i.e. from January to the beginning of May, and a much narrower and less pronounced autumn peak between weeks 41 and 47, i.e. from early October to late November. In-between the maxima, there are two local minima: a broad one that spans the summer season (from mid-June to the beginning of October) and a narrow one in late autumn.

Fig. 1.

The frequency distribution (not shown) of 872 clinical cases recorded over the four investigated years, gives the most frequent value (mode) of 1 case/week, and the midpoint (median) of 3 cases/week. There were 39 weeks with only one case of canine babesiosis diagnosed, which made ˜5% of the total number of cases. The weeks with the number of recorded cases around the median, between 2 cases/week and 4 cases/week, made up further ˜20% of the total number of cases.

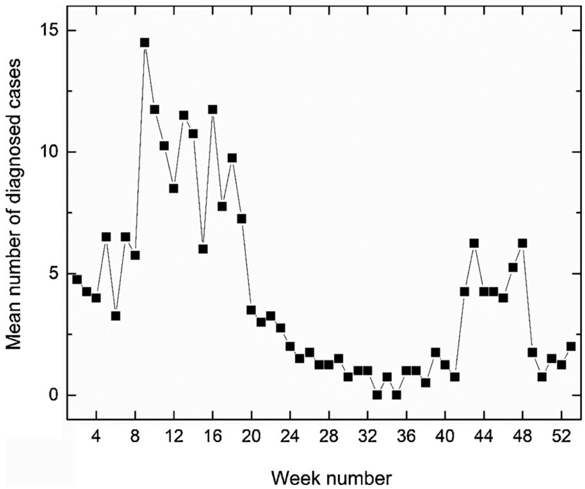

Fig. 1. The mean number of diagnosed cases of canine babesiosis per week, over 2013–2016.

Almost half of the total number of cases were diagnosed within the weeks with 5–11 cases/week. Finally, almost 30% of the total number of cases were diagnosed within the weeks over which there were 12–28 recorded cases. This number of cases per week represents only 8% of the data points in the frequency distribution of number of diagnosed cases, and thus can be considered as outliers of the distribution.

We used the ranges of number of diagnosed cases per week to investigate the seasonality of canine babesiosis. Specifically, we looked into four periods of the year: "Jan-Feb" from the beginning of the year to mid-February; "Feb-May" from the second half of February to the end of May; "Jun-Sep" spanning June, July, August and September; and "Oct-Dec", i.e. October, November and December. We based the length of these periods on the number of diagnosed cases per week. For example, we used extremely high number of cases per week, between 12 and 28, to separate the Feb-May period from its surrounding seasons: in mid-February from the Jan-Feb period, and at the end of May from the Jun-Sep period. On the other hand, we discriminated the Jun-Sep from the Oct-Dec period by the occurrence of 5–11 cases/week, and thus defined Jun-Sep as a period over which only 0–4 cases/week were diagnosed. Fig. 2 shows the seasonal distribution of weeks with a given number of diagnosed cases.

Some interesting features in the seasonality of babesiosis occurrence can now be discerned. For example, the Jun-Sep period, spanning the four warmest months in Belgrade (June–September), was "a quiet period" when babesiosis was only occasionally diagnosed. When diagnosed, the number of cases was never greater than 4 per week. On the

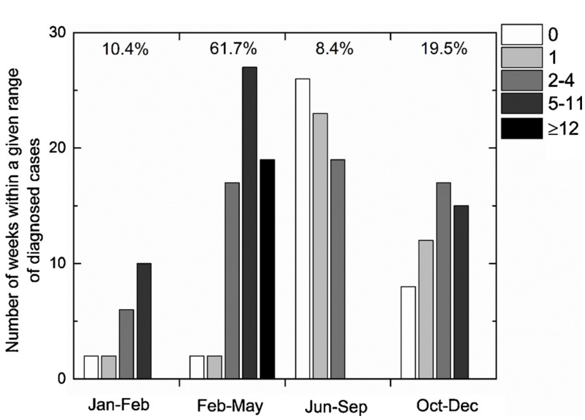

Fig. 2. The seasonal distribution of weeks with a given number of diagnosed cases. The numbers at the top represent the total number of cases in each season, as a percentage of the total number of cases.

other hand, by far the largest number of cases (more than 60% of the total) was diagnosed in the Feb-May period. Only during this period, a very high number of cases, more than 12 cases/week, was recorded. Over 2013–2016, there were 16 such instances: 3 occurred in February, 8 in March, 4 in April, and another in May. Our results are in agreement with the highest activity of *D. reticulatus* in the spring as already described in Földvári et al. (2016).

In other parts of Europe, e.g. northern France, eastern parts of Austria, in Hungary near the Austrian border, Croatia, and eastern and central Poland, that have four distinguished seasons like in Serbia, the number of acute canine babesiosis cases was similarly distributed as in our study (bimodal pattern), but in Austria and eastern Poland, unlike the rest of the regions, the maximum season was in the autumn rather than in the spring (Leschnik et al., 2008; Matijatko et al., 2012; Rene-Martellet et al., 2013; Welc-Faleciak et al., 2009). This difference in the disease peak occurrence needs further investigation. In regions with a warmer climate, such as southern France, cases are recorded throughout the year, with a decrease during the warm summer season (Rene-Martellet et al., 2013). Further away from Serbia, a spreading of the infected *D. reticulatus* to northern Europe resulted in autochthonous clinical cases in Norway (Oines et al., 2010) and the United Kingdom (Cook et al., 2016). Although canine babesiosis is focally distributed in Europe (Rubel et al., 2016), the border line of the pathogen and the vector's habitat is shifting northwards (Chitimia-Dobler, 2015; Dautel et al., 2006; Kloch et al., 2017; Paulauskas et al., 2015).

In a summary of this part, we note that canine babesiosis occurrence in Belgrade showed a bimodal pattern, with two maxima–a pronounced one in the spring and a less marked one in the autumn–that jointly gave more than 80% of the total number of diagnosed cases. Further 10% of the cases were diagnosed over January and the first half of February. It is also worth noting here that our database was limited by the fact that no further information on the patients' behaviour were given. For example, knowing whether the dogs were outdoor or indoor dogs, or whether they had travelled abroad prior to the diagnosis, would help us in a more detailed interpretation of the results.

3.2. Short-term relationship–two weeks before the onset of the disease

There are no precise data on incubation period for canine babesiosis after natural infection, but literature data point to the period of 4–21 days with the average period of two weeks (Bilić et al., 2018). Under that assumption, we chose a time lag of two weeks to look into the possible influence of the meteorological parameters on the activity of infected *D. reticulatus*. We separated the results according to the number of cases diagnosed per week and period (Table 1), which enabled us to compare the change in the meteorological parameters with the increasing number of diagnosed cases within each season.

For example, over the Feb-May period, an increase in number of cases per week from 0 to 1 coincided with a temperature increase from 9.2 °C to 16.2 °C. However, further increase in the number of cases was concurrent with a decrease in temperature, such that for the weeks with 12–28 diagnosed cases, the temperature was 9.2 °C. Similarly, the other meteorological parameters showed no apparent regularity in their values corresponding to different number of diagnosed cases over this period of the maximum disease occurrence. On the other hand, during the Jun-Sep period, an increase in the number of cases coincided with a decrease in temperatures, an increase in relative humidity, and a decrease in atmospheric pressure (Table 1). This finding implies a need to further investigate whether *D. reticulatus* ticks are more active during cooler and more humid summer days (or morning and evening hours) with a somewhat lower atmospheric pressure, and whether their activity increases in warmer autumn days, since the number of diagnosed cases of babesiosis and temperature show a simultaneous rise.

We make a note here that the above reasoning should be taken with some caution for two reasons. First, when the conclusions are drawn on the results obtained for very few data points, such as two weeks for the

Table 1
Values of meteorological parameters recorded two weeks prior to diagnosed cases. The data are separated into sets depending on the number of cases per week and period.

Cases/week	Period	Number of weeks	TG [°C]	HU [%]	PP [mbar]	RR [mm]	CC [okta]
0	Jan-Feb	2	−2.3	78	1016.3	0.9	4.7
	Feb-May	2	9.2	72	1003.3	2.2	5.8
	Jun-Sep	26	23.6	62	1005.4	3.0	3.4
	Oct-Dec	8	7.0	77	1015.6	1.0	4.7
1	Jan-Feb	2	6.4	75	1000.0	1.5	5.5
	Feb-May	2	16.2	67	1005.0	6.8	4.2
	Jun-Sep	23	22.9	62	1004.7	1.7	3.2
	Oct-Dec	12	8.6	80	1007.7	2.6	5.2
2–4	Jan-Feb	6	1.7	79	1008.3	1.6	5.2
	Feb-May	17	14.1	68	1000.0	3.9	4.8
	Jun-Sep	19	21.3	64	999.3	2.3	3.6
	Oct-Dec	17	9.5	77	1010.1	1.7	4.4
5–11	Jan-Feb	10	6.4	77	1002.7	1.2	4.9
	Feb-May	31	10.2	69	1000.6	2.4	5.0
	Oct-Dec	15	13.6	74	1005.0	1.4	3.7
12–28	Feb-May	16	9.2	67	1008.8	0.8	4.3

Jan-Feb and Jun-Sep periods, for 0 and 1 cases/week (Table 1), a large uncertainty is expected, and a greater number of data points could bring a notable change to the mean value. Second, our discussion of the tick activity implicitly omitted the possibility that the meteorological variables also exert influence on the availability of hosts (e.g., pet dogs spending more time outside during cooler summer days and warmer autumn days, which are probably preferred weather conditions of their care-takers).

Our results suggest that within each period, there are combinations of meteorological parameters that could enhance the activity of infected ticks. For that reason, we looked into the number of diagnosed cases as a function of two variables, temperature and relative humidity, within each period (Fig. 3). We chose these two meteorological parameters as they have been shown to be the major factors influencing the host-seeking activity (Hubalek et al., 2003). If weather conditions leading to a high tick activity, and consequently, a high number of diagnosed cases of canine babesiosis (5–11 cases/week), were termed “favourable”, then each period, apart from Jun-Sep, showed its specific combination of favourable conditions.

Even in the Jan-Feb period, the favourable temperatures were between -4 °C and 7 °C, and relative humidity around 70—85% (Fig. 3). Our database showed more cases of canine babesiosis in January than in December (Fig. 1), even though January is, according to the 1981–2010 climate normals, the coldest month in Belgrade. However, over 2013–2016, December, with its four-year mean temperature of 3.2 °C, was somewhat colder than January with the mean of 3.8 °C. This finding might suggest that in the Belgrade region, *D. reticulatus* is the least active over the coldest month. Further, in the four investigated years, the total of six cases of babesiosis were recorded when the temperatures were between -4 °C and 0 °C. These data point to the possibility that infected *D. reticulatus* could be active and find a host even during few hours when maximal daily temperatures rise enough to enable their movement. Indeed, it has been shown that adult *D. reticulatus* could be active when soil temperatures are as low as 2.5 °C (Buczek et al., 2014), 1 °C (Nosek, 1972) and even -0.1 °C (Hubalek et al., 2003).

Another possibility that could theoretically contribute to the number of diagnosed cases is exacerbation and appearance of clinical signs in cases of asymptomatic *B. canis* infection. Asymptomatic *B. canis* infection has been documented in dogs (Bajer et al., 2013; Beck et al., 2009; Kovačević Filipović et al., 2018; Welc-Faleciak et al., 2009), grey wolfs (Beck et al., 2017), and golden jackals (Sukara et al., 2018). All authors agree that these canids are asymptomatic carriers of *B. canis*, but an uncertainty concerning the outcome of the infection exists. Several authors assume that a compromised immune system could lead to exacerbation or relaps of the disease (Bajer et al., 2013; Bourdoiseau, 2006), but this remains to be investigated, especially in cases that appear during very cold weather which can be seen as a stress factor for some individual dogs.

Over the Feb-May and Oct-Dec periods, the two peak seasons, the ranges of favourable temperatures and relative humidities were similar, with the Oct-Dec values somewhat shifted towards lower temperatures and higher relative humidities (Fig. 3). In the surveys of favourable meteorological parameters for *D. reticulatus* host-seeking activity, other authors reported temperature of 4—21 °C and relative humidity of 61—100% in eastern Poland (Bartosik et al., 2011), and 13–18 °C and 45–60% in southern Italy (Olivieri et al., 2017). Differences in the eastern Poland and southern Italy ranges of temperature and relative humidity for *D. reticulatus* reflect different climate conditions, and in turn, the tick’s ability to adapt to those differences.

On the other hand, if “optimum” conditions were linked to the extremely high number of diagnosed cases (larger than 12 cases/week), then the optimum temperatures were 3–16 °C and humidity 53–87%, however, only over the Feb-May period (Fig. 3). The 16 weeks that yielded the highest number of cases in our study recorded the mean temperature of 9.2 °C, relative humidity of 67% and mean precipitation of 0.8 mm (Table 1). This finding is in agreement with the optimum average temperature of 10 °C and a somewhat higher relative humidity of 73% for *Dermacentor* spp. activity in Serbia published for 1998–2000 (Milutinović and Radulovic, 2002). In that period, April was indicated as the peak season of the *Dermacentor* spp. activity (Milutinović and Radulovic, 2002). These two sets of data for Serbia demonstrate that the optimum temperature for the tick activity remained the same over the period of 15 years. However, the climate change has led to higher temperatures earlier in the year, and hence shifted the tick activity to February, March and April over 2013–2016 (Fig. 1).

Furthermore, during the Feb-May peak season, relative humidity lower than 60% did not seem to fully hinder the ticks’ activity (Fig. 3). A study in Italy showed that relative humidity greater than 70% for a period of 30 days during March, positively influenced ticks’ hydric balance and could give their peak activity in April, when humidity was lower, between 45% and 60% (Olivieri et al., 2017). This scenario is also probable for the maximum season in Belgrade, offering an explanation for cases of babesiosis seen at lower humidity levels.

In the Jun-Sep minimum season, the majority of cases were recorded when temperatures were 15–25 °C and relative humidity 55–77% (Fig. 3). However, there were six cases (0.69% of the total) with the temperature above 26 °C and relative humidity as low as 41%.

One of the limitations in acquisition of our data was that the diagnostic procedure included only blood smear examination and confirmation of the presence of large *Babesia* in erythrocytes. Except *B. canis* that has been shown to be the most prevalent (Davitkov et al.,

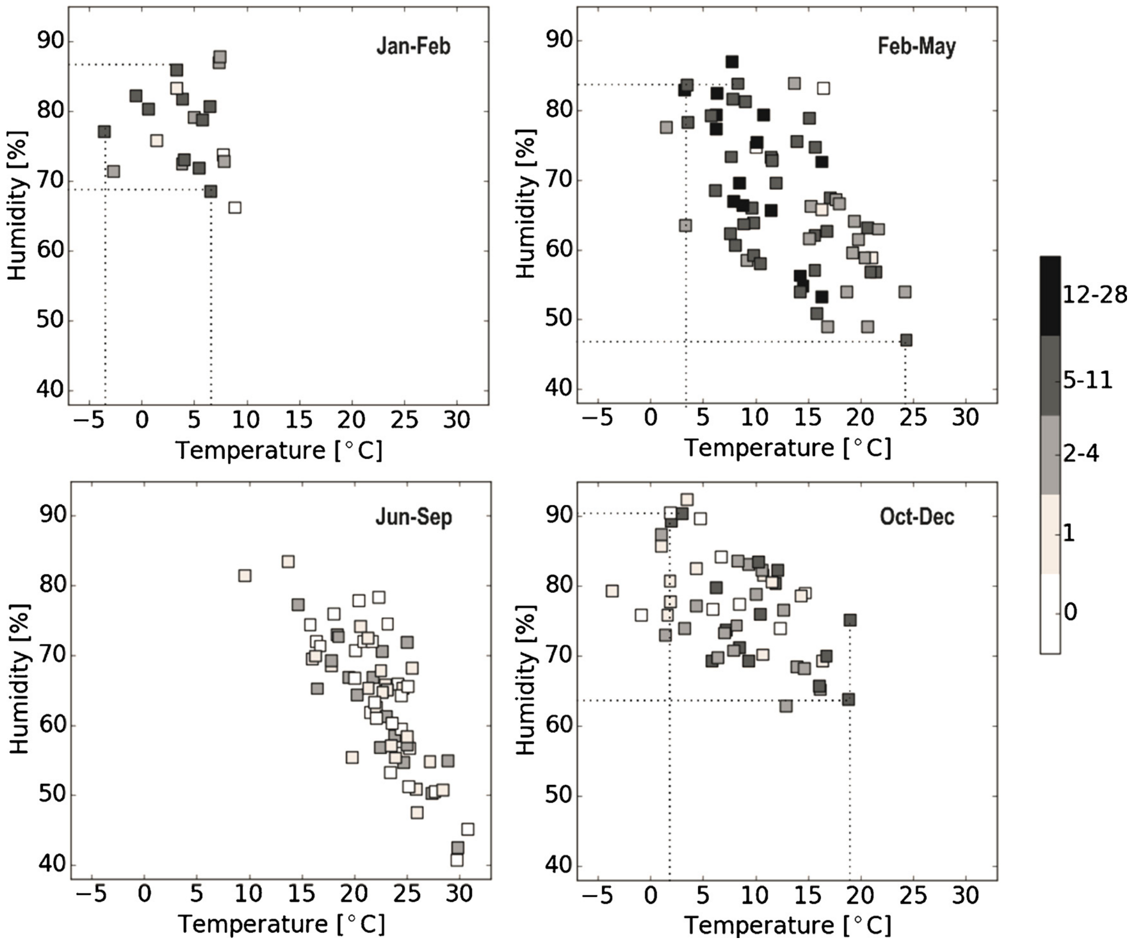

Fig. 3. Number of diagnosed cases as a function of temperature and relative humidity recorded two weeks prior to the diagnosis, for each season. The symbols are colour-coded according to the number of cases per week. The dotted lines mark the ranges of favourable temperatures and relative humidities in each season.

2015), *B. vogeli* has also been isolated from asymptomatic dogs (Gabrielli et al., 2015), and *B. vogeli* seroreactive dogs detected in our region (Kovačević Filipović et al., 2018). Thus, some of the cases, especially those diagnosed during the summer months, could be ascribed to *B. vogeli*, whose vector is *Ripicephalus sanguineus*, a tick more active during the warm season (Dantas-Torres, 2010). Few cases of clinical babesiosis due to infection with *B. vogeli* were reported in Italy (Solano-Gallego et al., 2008), Croatia (Beck et al., 2009) and across Europe (Carcy et al., 2015). Also, a simultaneous presence of *Dermacentor* larvae and nymphs on shepherd dogs in Hungary in mid-summer (Hornok et al., 2013b), raise the question of their possible role in *B. canis* transmission.

As a summary we can point out that canine babesiosis occurred in a broad range of temperatures and relative humidities that are in agreement with conditions previously published for *D. reticulatus* host-seeking activity. Low autumn and winter temperatures in a combination with high relative humidities, seem to provide favourable conditions for *D. reticulatus* host-seeking activity.

3.3. Long-term relationship–one year before the onset of the disease

Occurrence of canine babesiosis is a result of abundance of infected vectors and conditions in their habitat during development, availability of dogs and other susceptible canids, and climate (meteorological) conditions. This section gives the results of the correlation analysis (Fig. 4) that could help us to understand an influence of the meteorological parameters on the population of infected ticks. We introduced a lag to the correlation calculations that enables us to “look back into the past” and investigate an impact of weather conditions that is not immediately evident.

The correlation coefficients in Fig. 4 imply temperature as an important factor in the development of infected tick adults. First, the correlation coefficients given for Jan-Feb describe an influence of weather conditions on population of infected ticks that become active over this period. Within the week the cases were diagnosed (lag equals 0), there was a drop in temperature, i.e., the weather turned even colder over these cold months, which is a somewhat surprising result as we assumed that a rise in temperature could be a signal for tick activity. On the other hand, around three months prior to Jan-Feb, in late autumn, cloud cover seems to exert a major impact–its increase could be an environmental stimulus that poses as a signal for low activity. The effect of cloud cover could, however, be coupled with the length of photoperiod, as this is an environmental trigger for diapause commencement (Belozerov et al., 2002).

Following the Jan-Feb period, in the Feb-May maximum season, the negative correlation coefficients show that immediately (within four weeks) prior to feeding, ticks seem to prefer weather conditions characterised by cooler temperatures (Fig. 4). Over this one month prior to the disease diagnosis, temperature was the only influencing meteorological parameter. Temperature also showed a pronounced impact on the tick population 4–8 months before the maximum season, the period encompassing the preceding autumn and winter. The positive correlation coefficients over this period most likely indicate that warmer temperatures enable a better survival rate of the over-wintering ticks. Further, over this period, relative humidity seems to be another important meteorological parameter–the negative correlation coefficients

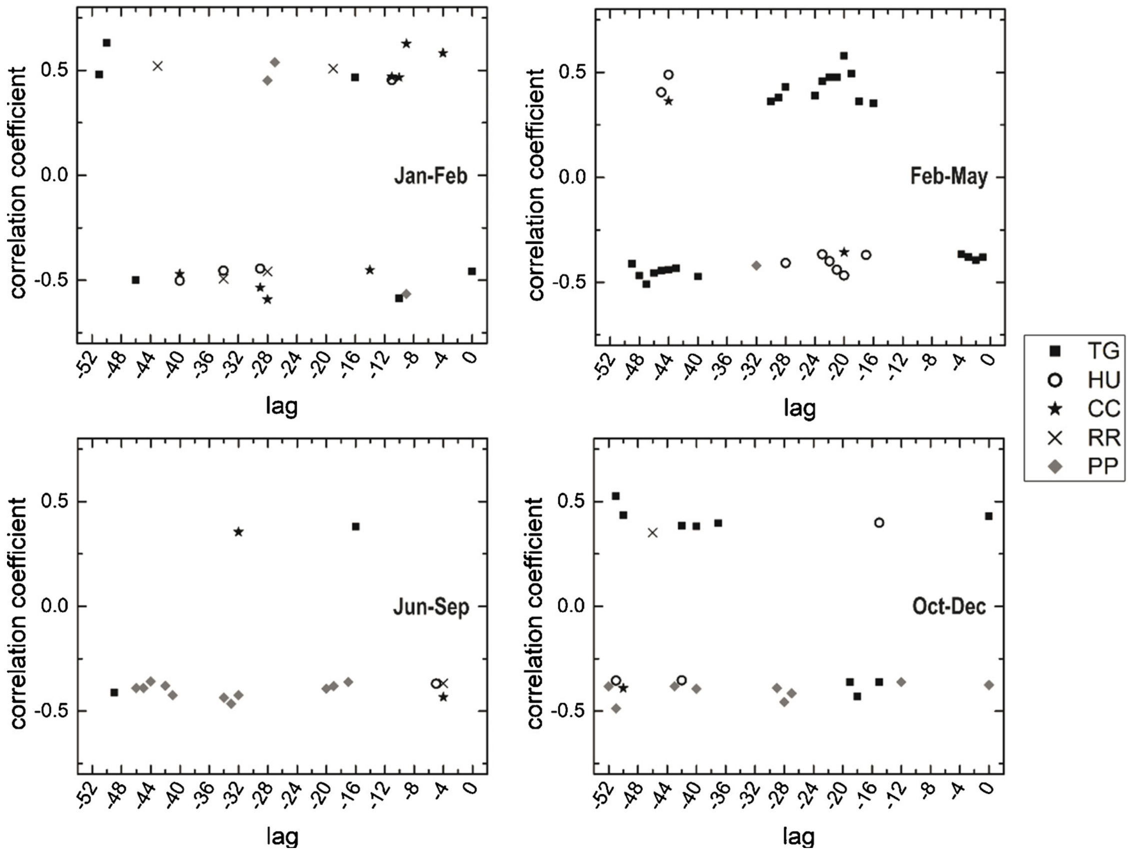

Fig. 4. Spearman's rank correlation coefficients between the number of diagnosed cases per week and meteorological parameters, as a function of lag given in weeks. Only the coefficient with the absolute value greater than 0.35, and statistically significant are given.

suggest that less humid conditions are better for survival. On the other hand, earlier in the ticks' development, during the late spring and early summer, when eggs are laid and larvae feed and change to nymphs (Kahl and Dautel, 2013), temperature again seems to play a major role, and a decrease in temperature could be beneficial over this period of the *D. reticulatus* life cycle. Relative humidity in this period showed a significant influence only over a couple of succeeding weeks, when we found positive correlation coefficients. We note that this increase in relative humidity might reflect its importance over a short period, right after the eggs are laid.

In the correlation analysis for the Jun-Sep period (Fig. 4), the majority (more than 70%) of the number of cases per week were equal to 0 or 1. For that reason, the results should be taken with caution, and hence, we omitted their discussion.

The correlation coefficients that we obtained for the Oct-Dec period (Fig. 4) portray an influence of the weather conditions of the life cycle of pre-wintering ticks. First, a rise in temperature could be an environmental signal that prompts them to feed within the same week (the correlation coefficient for lag equal 0), while earlier, in the late summer, when the nymphs feed, a decrease in temperature seems to facilitate their development. Over the preceding autumn and winter, an increase in temperature and a decrease in relative humidity most likely provide better conditions for over-wintering. The larger the number of infected ticks that survived the winter, the larger the number of infected eggs laid in the spring, and thus, the larger the number of ticks that infect dogs in the autumn.

We based our discussion of the correlation coefficients only on the groups of coefficients that implied a consistent connection with the number of diagnosed cases, and thus excluded an analysis of sporadic instances. Furthermore, it should be emphasised that the assumption behind this correlation analysis is that the meteorological parameters influence the population density of infected ticks, and in turn, the occurrence of canine babesiosis. However, the weather conditions are only one of the influencing factors, and future studies could shed some light on possible differences in the behaviour of the non-infected and infected or even co-infected ticks. It might also be worth noting that larvae and nymphs of *D. reticulatus* are seemingly endophilic, inhabiting microhabitats of small rodents (Kahl and Dautel, 2013) and therefore, exposed to microclimate of these niche environments. However, the overall picture is supported by already known optimum weather conditions for *D. reticulatus* developmental biology. This fact justifies the use of databases of recorded cases of canine babesiosis for monitoring the spread of *D. reticulatus* and, consequently, climate change.

Finally, let us note that the urban green spaces in Belgrade are categorised as "under serious risk" resulting from climate change (Đokić and Grujić, 2015). The microclimate of the city parks is therefore expected to change in the near future, and our results imply a possible two-way influence of an increasing temperature on the population of *D. reticulatus*. On one hand, higher temperatures during summer could lead to a reduced number of ticks active in the ensuing autumn and spring. In contrast, milder autumns and winters might assist a higher rate of the ticks' cold-season survival, and so facilitate an increase in the canine babesiosis incidence. Still, the problem of the disease occurrence in the city parks could be relatively easily remedied by regular grass mowing (Bajer et al., 2017).

In a summary of this part, our results suggest that one of the major signals for lowering activity of the infected ticks is the increased cloud cover in late autumn (that coincides with shortening of the daylight). During this period, warmer and less humid weather seems to increase the infected ticks' survival chance. During the Feb-May maximum in the activity of ticks that leads to the maximum in the diagnosed cases of canine babesiosis, cooler conditions seem to facilitate the disease occurrence.

4. Conclusions

We analysed data records of diagnosed canine babesiosis cases in Belgrade over 2013–2016, and identified a bimodal seasonal distribution, with a pronounced peak in the spring, and a less conspicuous one in the autumn. This asymmetry in the peak seasons needs further investigation. Our results also suggest that the period of low activity is short, encompassing only the coldest month of the year, in our case, December. Still, throughout the year, even over the coldest and warmest periods, there was a broad range of temperatures and relative humidities when the disease was recorded. The obtained ranges are in a general agreement with the previously published conditions for *D. reticulatus* host-seeking activity, but the disease occurrence over the periods with more extreme weather conditions, raise several questions about its vectors, pathogens and disease pathogenesis.

Over one year prior to the onset of the disease, we found a noticeable impact of temperature and relative humidity, and to a lesser extent, of atmospheric pressure and cloud cover, on the number of spring and autumn diagnosed cases. These finding imply long-term relationship between occurrence of acute canine babesiosis and meteorological parameters, opening a question on its true nature.

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Declaration of Competing Interest

None.

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