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Original Article

Canine babesiosis in Austria in the 21st century – A review of cases

Anja Joachim [a,*], Maria Sophia Unterköfler [a], Anja Strobl [b], Karin Bakran-Lebl [c], Hans-Peter Fuehrer [a], Michael Leschnik [b]

[a] Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Austria
[b] Clinical Unit of Internal Medicine Small Animals, Department of Companion Animals, University of Veterinary Medicine Vienna, Austria
[c] Institute for Medical Microbiology and Hygiene, AGES - Austrian Agency for Health and Food Safety Ltd., Austria

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ABSTRACT

A retrospective study on 699 cases of canine babesiosis presented to veterinary clinics in eastern Austria were evaluated for the location where infection had presumably taken place. Of these, 542 (77.54%) had acquired the infection in Austria, while the majority of non-autochthonous cases came from neighboring countries, most notable Hungary. Both groups were recorded primarily in Vienna, eastern Lower Austria and Burgenland, but cases from the southern (Styria, Carinthia) and western (Upper Austria, Tyrol, Salzburg) provinces of the country were also recorded. Records were made all year round, with most cases in spring (46.6%) and fall (48.4%). The annual cases ranged from four to 58 (mean: 31.8) with large fluctuations and no visible trend for an in- or decrease. The tick vector of *Babesia canis*, *Dermacentor reticulatus,* is present in Austria but displays a very patchy distribution, and its occurrence and activity are not readily foretold, which might be a reason why its presumably increasing density in Europe is not reflected by increased incidences of canine babesiosis. Another factor that may influence the numbers of cases per year could be the application (or non-application) of acaricidal or repellent compounds. A limitation of this study is that bias is exerted by the location of the participating clinics, and by the unknown rate of infections that does not induce clinical symptoms and is likely not presented in veterinary practices and clinics. The data, however, clearly show that at least the lowlands of Austria are endemic for *B. canis*, and appropriate tick control must be advised all year round.

1. Introduction

Canine babesiosis can be caused by a variety of *Babesia* species. In Europe, *Babesia vogeli*, transmitted by the Brown Dog tick *Rhipicephalus sanguineus*, prevails in the Mediterranean and southeastern regions where the vector is abundant, while *Babesia canis*, transmitted by the ornate dog tick or meadow tick *Dermacentor recticulatus*, is prevalent in central and western Europe. In addition, rather focal occurrence of “small” canine *Babesia* species, i.e. *Babesia gibsoni* and *Babesia vulpes* (syn. *Theileria annae, Babesia microti*-like), has been reported (Solano Gallego and Baneth, 2011; Baneth et al., 2019; Bajer et al., 2022a, 2022b). Recently published works strongly indicate a spread of *D. reticulatus* in central and northern Europe (Zygner et al., 2009; Radzijevskaja et al., 2018; Drehmann et al., 2020; Grochowska et al., 2022; Daněk et al., 2022), and, with it, an increased reporting of *B. canis* infections in some countries (Dwużnik-Szarek et al., 2022; Helm et al., 2022). By contrast, in Switzerland temporal epidemic foci were previously described that did not seem to have persisted in more recent times (as reviewed by Bajer et al., 2022a, 2022b).

Babesia canis infects erythrocytes and can cause subclinical or mild, but also life-threatening infections. Thrombocytopenia, anemia, and other hematological changes can develop from low to baseline to severe levels and lead to lethargy, inappetence, coagulopathies, renal failure, and occasionally neurological signs with coma and even fatal outcomes (Strobl et al., 2020; Beletić et al., 2021). The presence of *B. canis* in Austria has previously been described (Leschnik et al., 2008, 2012; Duscher et al., 2013; Strobl et al., 2020; Sonnberger et al., 2021; Bajer et al., 2022a, 2022b), and we hypothesized that annual cases were increasing. Moreover, it was assumed that the majority of these cases were autochthonous and are reported not only from eastern Austria (where a high abundance of *D. reticulatus* ticks is recorded (Rubel et al., 2020; Dirks et al., 2021; Sonnberger et al., 2022) but throughout the

* Corresponding author at: Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Veterinaerplatz 1, A-1210 Vienna, Austria.

E-mail addresses: Anja.Joachim@vetmeduni.ac.at (A. Joachim), Maria.Unterkoefler@vetmeduni.ac.at (M.S. Unterköfler), Anja.Strobl@vetmeduni.ac.at (A. Strobl), Karin.Bakran-Lebl@ages.at (K. Bakran-Lebl), Hans-Peter.Fuehrer@vetmeduni.ac.at (H.-P. Fuehrer), Mi-chael.Leschnik@vetmeduni.ac.at (M. Leschnik).

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country. For this we retrieved data on 699 cases referred to veterinary clinics in eastern Austria from 2001 to 2022 and evaluated their geographical distribution, whether they were autochthonous or imported, and changes in in their rates over seasons and years.

2. Materials and methods

Cases of canine babesiosis reported from two large veterinary clinics and two associated diagnostic laboratories in eastern Austria from September 2001 until March 2022 were included. The clinics and laboratories were located in northern Burgenland, and Vienna. The diagnosis had been made by detection of parasite stages in stained blood smears or by molecular detection (PCR) of Babesia DNA in blood conducted in the veterinary diagnostic laboratories. In addition, all dogs showed clinical signs of acute babesiosis such as pallor (confirmed in hematology as anemia), hemoglobinuria, jaundice, petechiae or ecchymosis in skin or mucous membranes, lethargy, fever and/or gastrointestinal disturbances.

Of all dogs, age, gender, breed, and the place of residence for the previous three weeks or longer were recorded.

Since no central register of dogs in Austria is available, we estimated a "virtual" dog population for the provinces and districts by relating the Austrian dog population with the human population making the following calculations:

$$\frac{\text{Percentage of households with one or more dogs (per province)}}{100} \times \text{number of households (per district)}$$

(n = 629.120 dogs; Statistik Austria Konsumerhebung 2019/20; www.statistik.at; accessed 20.08.2022).

For a more detailed evaluation of this proxy calculation, data only for Vienna (available at Statistiken zu Hunden in Wien - Offizielle Statistik der Stadt Wien) were calculated separately and returned a mean deviation of 38.6% (min 10.2%, max 63.0%) from our calculation based on the total populations of citizens and dogs (for details see Suppl. file 2).

Autochthonous infections were defined as infections of animals that had not spent time abroad within three weeks prior diagnosis of canine babesiosis (according to the owner's information). Animals with a stay in endemic countries outside Austria within three weeks (including dogs originating from abroad) prior to the occurrence of clinical signs were grouped as "imported infections".

Maps for the distribution of cases (grouped as "autochthonous" or "imported"), divided into two decades (2001–2011 and 2012–2022) and grouped according to the season were created using R version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria) based on the zip code by district. Open access shapefiles were retrieved from the Statistik Austria homepage (www.statistik.at; accessed 01.03.2022).

Data regarding age of the included dogs and cases per year were tested for normality (Shapiro-Wilk test) and logistic regression (glm) in R.

3. Results

A total of 699 cases of canine babesiosis reported from veterinary clinics in eastern Austria from September 2001 until March 2022 were included.

The age of the animals ranged from 1 month to 17 years (median: 5 years; interquartile range: 6 years) and was not normally distributed; around half of the positive dogs were between two and six years old (see Suppl. File 1). They were of mixed gender (144 intact females, 161 neutered females, 300 intact males, 93 neutered males, 1: no information) and of different (pure or mixed) breeds.

3.1. Cases by year and season

Over the evaluated years, the annual cases reported ranged from four to 58 (Fig. 1) with an annual mean of 31.8 ± 16.9 cases. Cases were normally distributed over time and logistic regression calculation did not reveal an increase or decrease over time.

The majority of cases were reported in fall, September to November (48.40%) and spring, March to May (46.60%), while in summer (4.00%) and winter (7.00%) only few cases were noticed (Fig. 2).

In the two decades that were evaluated, 367 cases (52.5%) were reported 2001–2011, and 332 cases (47.5%) were reported in 2012–2021 with similar annual caseloads (33.4 ± 14.4 respectively 30.2 ± 19.7 cases/year).

3.2. Autochthonous vs. imported cases

Based on the distinction between autochthonous and imported cases, the majority of reported dogs (77.5%) originated from Austria at the time of submission and had not been outside the country three weeks before submission to veterinary hospital according to the owner's information, while the rest originated mostly from (or had spent time in) Hungary (15.9%), Serbia (1.6%) Slovenia (1.1%), Slovenia, Croatia, Poland, Romania, Ukraine (0.6% each), Bosnia (0.3%) and in single cases from other countries (Table 1).

Both the cases considered as autochthonous and those that were considered as imported were mostly recorded from the eastern part of Austria, the provinces of Burgenland (most notably in Neusiedl/See with 23 non-imported and three imported cases over the whole period), Lower Austria and Vienna and, to a lesser extent, Styria where single cases occurred in a number of districts from 2002 to 2012.

The most westerly autochthonous infections were noted in in Tyrol with single infections in the districts of Imst, Innsbruck-Land, and Kufstein 2007–2016 (Fig. 3).

3.3. Origin of dogs in autochthonous cases

When all non-imported cases were considered by district of residence, primarily dogs from Vienna and neighboring districts were recorded (Fig. 4**, left panel**).

Calculating the proportion of cases by population density of the districts, however, more districts from Lower Austria and Burgenland stood out (Fig. 4**, right panel**).

3.4. Geography and climate of districts with reported cases

Considering the autochthonous cases by mean elevation of the district they were recorded in 167 districts (excluding Vienna) with average 272 (±146.9) m above sea level, with the majority below 200 m (61; 36.5%; including most of the districts around lake Neusiedl) or between 200 and 400 m (145, 50.3%, including districts in the Vienna Basin in Lower Austria). The highest districts with positive cases are listed in Table 2. For comparison, the city (and province) of Vienna (with a total number of 117 cases) is elevated from 151 m (Lobau, 22nd district, Donaustadt) to 542 m (Hermannskogel, 19th district Döbling) and is on

Fig. 1. Annual cases of canine babesiosis in Austria recorded from Sept 2001 to March 2022.

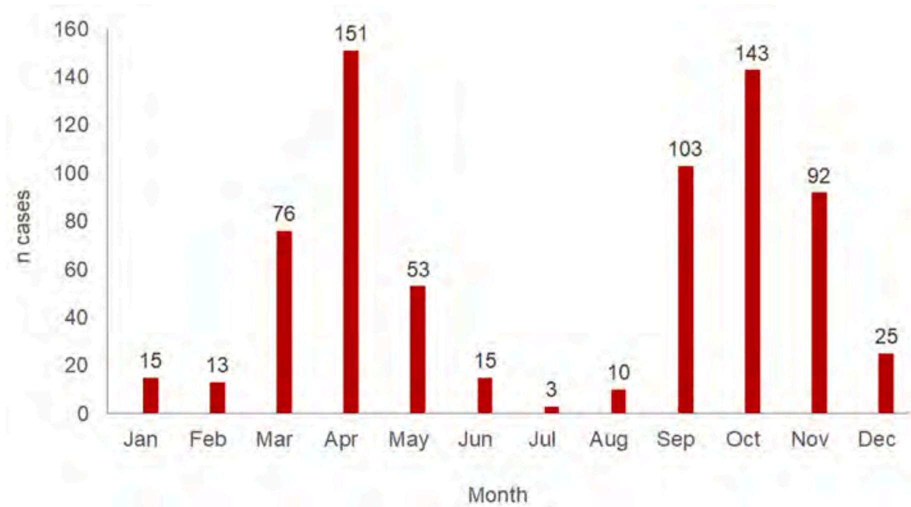

Fig. 2. Monthly cases of canine babesiosis recorded in Austria from Sept 2001 to March 2022.

Table 1
Country where *B. canis* transmission/infection had occurred; $n = 699$.

Country	Year(s)	N dogs [Percent]
Austria	2001–2022	542 [77.54]
Bosnia	2012,2015	2 [0.29]
Croatia	2009,2010,2013	4 [0.57]
Germany	2015	1 [0.14]
Greece	2015	1 [0.14]
Hungary	2001–2020	111 [15.88]
Italy	2005	1 [0.14]
Poland	2004	4 [0.57]
Portugal	2004	1 [0.14]
Romania	2016,2017	4 [0.57]
Serbia	2009–2011, 2013, 2015,2017,2019,2020	11 [1.57]
Slovakia	2005,2007,2009,2010,2012,2015,2016	8 [1.14]
Slovenia	2005,2008,2013,2018	4 [0.57]
Thailand	2014	1 [0.14]
Ukraine	2011,2014,2018,2022	4 [0.57]

average about 200 m above sea level (www.wikipedia.org; accessed 06.07.2022; www.topographic-map.com/maps/64z2/%C3%96sterreich/; accessed 06.07.2022.

4. Discussion

In this study we evaluated 699 cases of canine babesiosis presented at veterinary clinics in Eastern Austria in 2001 to 2022. The majority of cases was located in Vienna, Lower Austria and Burgenland, which was expected as these areas represented the most probable origin of clients of the participating clinics in eastern Austria. In addition, Vienna and lower Austria are the provinces with the largest populations; www.statistik.at; accessed 20.08.2022). Consequently, the "top ten" of the districts with cases of canine babesiosis also located nearby two large clinics that provided most case records. When we related the numbers of cases to the presumed size of the dog population in the districts, differences were noted, and one district stood out, Rust, a small community

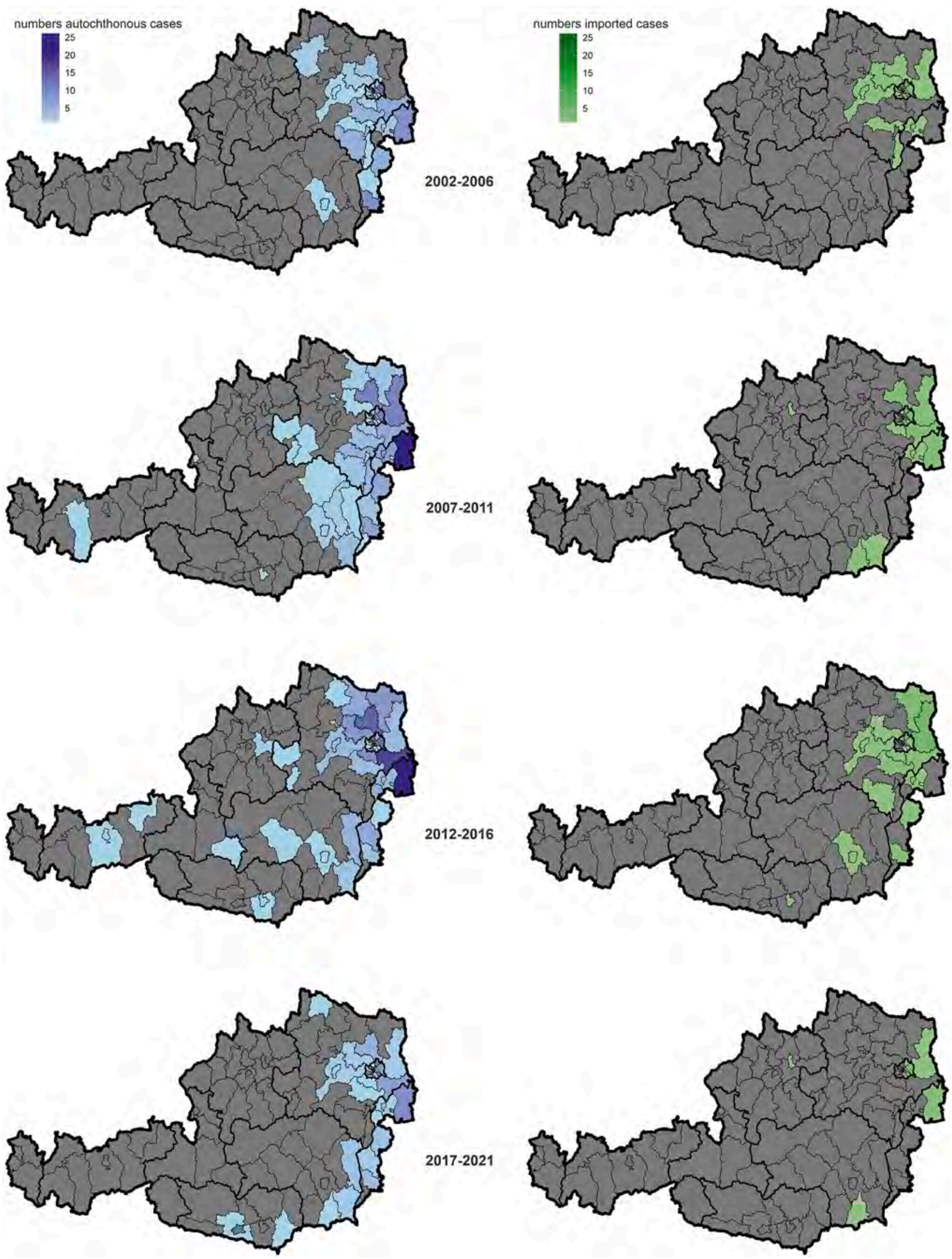

Fig. 3. Autochthonous (left, in blue) and imported (right, in green) cases 2002–2021 by geographic area (based on districts) in five year-intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

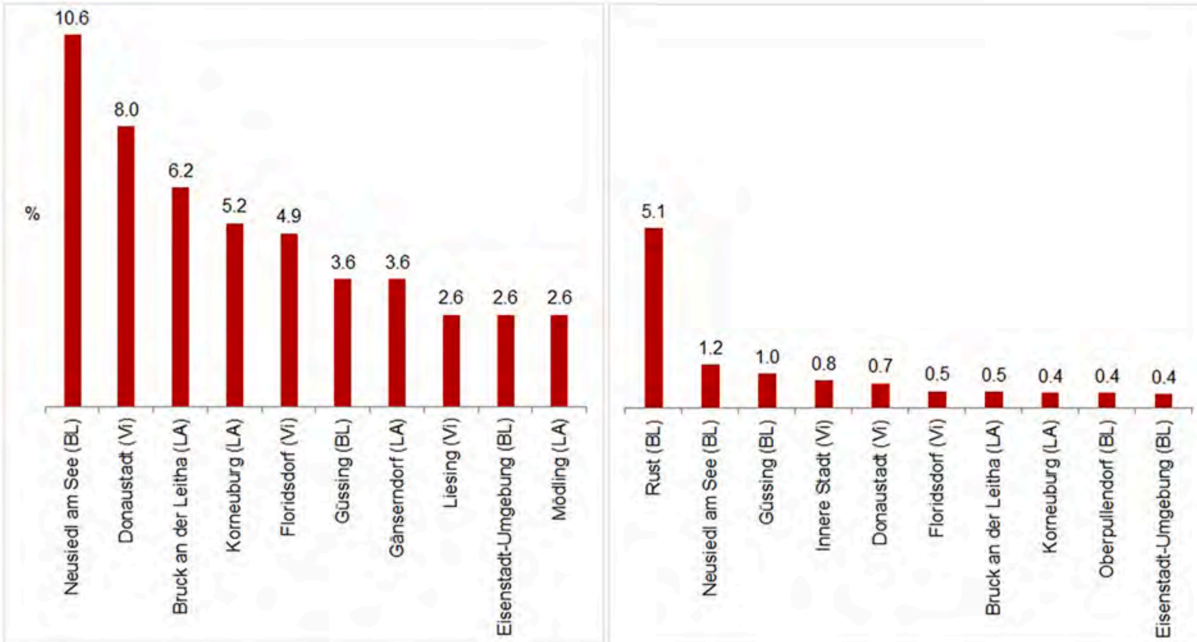

Fig. 4. Cases by district (in brackets: province - BL: Burgenland, VI: Vienna, LA: Lower Austria) (a) Top ten districts as proportions of all cases; (b) Top ten districts as proportions of all cases in relation to the population in the districts (Statistik Austria: Statistik des Bevölkerungsstandes, Statistik der natürlichen Bevölkerungsbewegung, Wanderungsstatistik. Provided: 29.06.2021. https://www.statistik.at/blickgem/pr1/g30604.pdf; Statista Research Department, https://de.statista.com/statistik/daten/studie/1098254/umfrage/hunde-in-oesterreich/; accessed: 21.01.2022) over the period of 2001 to 2022. For details on the locations see Suppl. File 3.

Table 2
"Top ten" districts from which positive cases were reported by mean elevation above sea level (www.wikipedia.org; accessed 06.07.2022; www.topographic-map.com/maps/64z2/%C3%96sterreich/; accessed 06.07.2022). For details on the locations see Suppl. File 2.

District	Mean elevation above sea level [m]	N positive dogs
Ferndorf (Villach-Land, Carinthia)	560	5
Kindberg (Bruck-Mürzzuschlag, Styria)	565	11
Hall in Tirol (Innsbruck Land, Tyrol)	574	9
Friedberg (Hartberg-Feistritz, Styria)	601	1
Schweiggers (Zwettl, Lower Austria)	633	4
Sankt Marein-Feistritz (Murtal, Styria)	698	4
Lichtenegg (Wiener Neustadt/Bucklige Welt, Lower Austria)	770	34
Grafenschlag (Zwettl, Lower Austria)	780	4
Arzl im Pitztal (Imst, Tyrol)	880	7
Sankt Margarethen im Lungau (Tamsweg, Salzburg)	1065	6

on lake Neusiedl, which is known for its large community of visitors especially during summer. However, it was not clear whether all the positive dogs located there were originally from Rust or had just visited from other areas of Austria, as the lake is a prominent holiday location for the population of Vienna and surroundings. As dogs travel frequently with their owners, we could not fully exclude "travel infections" in the sense of locally acquired infections, although the dogs' travel history was part of the anamnestic routine in the enrolled veterinary clinics. As regards the most frequently listed district for cases in total as well as cases in relation to the calculated dog population, 7/10 of the districts with the highest rates in each of the two categories overlapped, indicating the possibility of increased infection risks in these areas.

In the large majority of cases the infection appeared to be acquired in Austria, however, the case numbers or rates most likely do not reflect Austria as a whole due to the bias of the location of the clinics. A questionnaire survey conducted in 2010 among small animal practitioners in Western Europe revealed frequent cases of *B. canis* infections (163 cases reported by 151 veterinarians (ca. 15% of all registered practices in Austria), mostly from Burgenland (annual incidence 2.0–5.5) but also from Lower Austria, Styria, and Tyrol with low annual incidences of <0.2% in 2010 (Halos et al., 2014). A previous study that evaluated 240 cases of canine babesiosis in the Clinic of the University of Veterinary Medicine, Vienna, Austria, determined 59.6% of the cases to be autochthonous (Strobl et al., 2020). In the present study the rate of infections acquired in Austria was higher (77.5%), and the difference can be attributed to the larger data set that also included cases recorded in a second clinic and a veterinary laboratory.

On the basis of the obtained data, a number of cases was suspected to be acquired outside Austria, especially in Hungary, which borders

Table 3
Records for canine *B. canis* infections in European countries that were documented for suspected non-autochthonous *Babesia* infections (ref. Table 1).

Country	Reference(s)
Bosnia	Ćoralić et al. (2018)
Croatia	Mrljak et al. (2017)
Germany	Zahler and Gothe (1997); Barutzki et al. (2007); Silaghi et al. (2020); Helm et al. (2022)
Greece	Diakou et al. (2019)
Hungary	Földvári et al. (2005); Máthé et al. (2006); Hamel et al. (2012)
Italy*	Morganti et al. (2022)
Poland	Welc-Faleciak et al. (2009), Dwużnik-Szarek et al. (2021)
Portugal	Dordio et al., (2021)
Romania	Hamel et al. (2012)
Serbia*	Davitkov et al. (2015); Kovačević Filipović et al. (2018); Strobl et al. (2021)
Slovakia*	Majláthová et al. (2011); Víchová et al. (2016)
Slovenia**	Duh et al. (2004)
Ukraine	Hamel et al. (2013); Bajer et al. (2022a, 2022b)

* Also endemic for *B. gibsoni* (Davitkov et al., 2015; Kovačević Filipović et al., 2018; Strobl et al., 2021; Víchová et al., 2014; Carli et al., 2021; Víchová et al., 2014.

** Also endemic for *B. vogeli* (Duh et al., 2004).

Austria to the East and is a popular holiday destination for Austrians and their dogs. Hungary also offers specific hunting trips (e.g. https://www.jagdreisen.at/laender/jagen-in-ungarn; accessed 15.08.2022) – a travel opportunity also for hunting dogs and possibly a number of parasite as well, since neither Hungary nor Austria request antiparasitic treatment prior to entering the country. For the majority of the countries in question, the presence of *B. canis* has been recorded in the literature (Table 3). In the single case of a dog imported from Thailand the infectious agent was diagnosed as *B. vogeli* which is endemic in South-East Asia (Piratae et al., 2015; Buddhachat et al., 2020; Colella et al., 2020).

The reported cases consisted of slightly more male than female dogs (56.3 vs. 43.7%). As gender data are not available for the Austrian dog population as a whole, a correlation between sex and parasite infection could not be established. Previous works suggested such testosterone as an influential factor (Hughes and Randolph, 2001), and an influence of sex was noted in infections with *Babesia rossi* (Mellanby et al., 2011); however, Strobl et al. (2020) could not confirm this for *B. canis* infections. This issue clearly warrants further investigations.

Canine babesiosis can take a variety of clinical courses in affected individuals (Solano Gallego and Baneth, 2011), so the exact time point of infection could not be determined in the evaluated cases. However, evaluation of the monthly cases showed year-round reports but two distinct peaks in spring and fall which is in line with the activity of *D. reticulatus*, the vector of *B. canis* (Drehmann et al., 2020; Duscher et al., 2013). Along with this, the majority of cases was reported from eastern Austria where this tick species is considered most prevalent (Rubel et al., 2020). While the clustering of the imported and probably the majority of the autochthonous infections in Vienna, eastern Lower Austria and Burgenland are attributable to the location of the clinics providing the data, cases from Styria, Upper Austria, Carinthia Salzburg and Tyrol and the most northerly tip of Lower Austria indicate that *B. canis* is probably spread throughout Austria. Here it was reported most frequently from the dry and warm lowlands of eastern Austria, but also from more humid areas of central and western Austria with elevations of up to 1000 m above sea level (for data on climate zones of Austria ref. Hiebl and Frei, 2016, 2018). We previously also documented *B. canis* in a red fox in Western Austria (Hodžić et al., 2018). The assumption that these are indeed endemic areas is also supported by the finding that through the five-year periods, cases were repeatedly reported from some of these regions. As *D. reticulatus* prefers, but is not limited to, areas with high humidity, it most likely prefers the vicinity of rivers and natural lakes, as well as the preference of deer as a suitable host (Silaghi et al., 2020), and it is widespread tick species that is considered to be expanding (Földvári et al., 2016); however, it has a very patchy distribution and its populations are difficult to assess (Enigk, 1958), and the infection rates of *D. reticulatus* specimen with *B. canis* are highly variable and not always correlated with infections in dogs reported from an area (as reviewed in Silaghi et al., 2020). It can also be speculated that the rate and yearly frequency of antiparasitic treatment for tick control may have influenced the annual rate of presented cases over time. Over the observation period, a number of acaricidal and/or tick repellent compounds have reached the veterinary pharmaceutical market. However, whether (and to what extent) this has influenced treatment applications and in turn transmission rates for tick-borne pathogens (separately or in relation to any changes of tick densities that may have occurred seasonally or over time) could not be uncovered here. Previous investigations on tick control and pathogen transmission in dogs in eastern Austria showed a poor owner compliance with the recommendations for tick control and consequently poor control of transmission of pathogens (Leschnik et al., 2013); this is somewhat mirrored in the present study where cases of *B. canis* infections did not seem to decrease during the observation period.

Since the data shown here were based on veterinary records, it can also be assumed that the numbers of *B. canis*-infections in the different areas were probably higher, firstly because affected animals were referred to other clinics, secondly because subclinical infections (Sonnberger et al., 2021), including reinfections of immune animals, were likely not presented. Therefore, molecular diagnostic tools are recommended to diagnose patients with submicroscopic parasitemia. Various PCR protocols can be used for the diagnosis of canine babesiosis causing parasites ranging from pan-apicomplexan protocols and RFLP to species-specific protocols (Zahler et al., 1998; Jefferies et al., 2007; Zintl et al., 2011).

Over the evaluated period, the number of annual cases fluctuated distinctly, but an increase of cases over time was not visible. In addition, an increase in records in eastern areas of the country or a spread of *B. canis* within Austria over the last 20 years was not detected, indicating that fluctuations in annual incidences are most likely not driven by spread of the parasite (or the vector) or at least not alone, but possibly by weather, focal presence of infected ticks or individual risk behavior, such as waiving the use of an acaricide or repellent on dogs or regular dog walks in preferred vector habitats (Leschnik et al., 2013), and have to be monitored over a longer time period, not compared on an annual basis.

Regarding the risk of increased transmission in the presence of infected canine hosts, the partial overlap of autochthonous with imported cases does not unequivocally explain this, due to the aforementioned bias of the locations. This phenomenon needs more in-depth analysis and larger data collection.

5. Conclusion

Retrospective evaluation of 699 confirmed cases of canine babesiosis diagnosed in dogs from Austria over the past 20 years shows that the majority of cases were autochthonous and occurred throughout the year, primarily in spring and autumn. Fluctuations but no steady in- or decrease in annual cases was recorded over the observed period. Most cases were reported from eastern parts of the country, which is presumably at least in part due to the location of the participating practices, however cases were also repeatedly reported from central and western districts, and also from areas with an average elevation of 750 m or more. Thus, it must be assumed that, except for the high-altitude alpine areas, Austria is generally endemic for *B. canis*. Prospective surveillance studies, including the vector tick *D. reticulatus*, should be conducted to monitor occurrence and possible spread of *B. canis* and its vector, and genetic typing of *Babesia* isolates should be carried out to determine possible connections between foci of infection. In addition, measures related to tick control on dogs should be monitored, evaluated and further promoted to keep tick-borne pathogens including *B. canis* at check.

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