

Vector-borne diseases imported to Poland between 2021 and 2023

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Abstract

Introduction: The aim of the study was to monitor the occurrence of selected vector-borne diseases in anaemic dogs arriving in or returning to Poland from areas endemic for these diseases. Material and Methods: The study involved 497 dogs, of which 184 came to Poland from Ukraine with their owners fleeing the war. Other animals returned to the country from holidays spent in Croatia (n = 96), Turkey (n = 79), Italy (n = 48), Bulgaria (n = 42), Albania (n = 36) and Romania (n = 12). Molecular biology methods were used for detection of pathogens transmitted by the vectors. Results: Molecular tests revealed the presence of vector-borne pathogens in 79 dogs. The most commonly diagnosed infection was caused by Babesia canis (27 dogs), followed by infections with Anaplasma phagocytophilum (in 20 dogs), Mycoplasma haemocanis (15 dogs), Bartonella henselae (7 dogs), Ehrlichia canis (4 dogs), Hepatozoon canis (3 dogs), Babesia gibsoni (2 dogs) and Leishmania infantum (1 dog). Most of the sick dogs (n = 39) came from Ukraine. In dogs spending holidays with their owners outside Poland, vector-borne diseases were most often detected after their return from Turkey (n = 16), and next in descending order from Croatia (n = 7), Italy (n = 6), Albania (n = 4), Bulgaria (n = 4) and Romania (n = 3). Conclusion: The wider migration crisis and increasingly frequent trips of owners with their dogs to areas of endemic infectious and parasitic diseases observed in recent years are the main risk factors for the occurrence of these diseases in Poland. Therefore, constant monitoring of vector-borne diseases, especially in dogs returning from holidays and arriving in Poland from abroad, seems to be crucial for their early detection and introduction of appropriate therapy.

Keywords: animal movements, dogs, vector-borne diseases.

Introduction

Vector-borne diseases are a significant, growing problem in human and veterinary medicine. These illnesses are transmitted by arthropod vectors, and companion animals may be their reservoir for other animals and people.

The emergence of vector-borne diseases may be driven by several factors, including climate change, deforestation, land-use change, urbanisation, human population growth and migration, habitat fragmentation, animal movements and biodiversity loss (11). These

factors may influence the emergence of biological vectors of infectious and parasitic diseases in areas previously free from them. This is confirmed by, for example, changes in the geographical distribution of important tick vectors, such as *Ixodes ricinus* (25, 36).

Poland is a country where vector-borne diseases such as babesiosis (caused by *Babesia canis*), granulocytic anaplasmosis and Lyme disease are relatively frequently diagnosed in dogs (55, 56). In recent years, isolated cases of infections with *Babesia gibsoni* (47), *Hepatozoon canis* (50), *Leishmania infantum* (18, 41) and *Dirofilaria immitis* (29) have also

been observed. This may be a consequence of the increased frequency of tourist trips with pet dogs to countries where these diseases occur. The influx of Ukrainian war refugees and their animals from areas where vector-borne diseases are endemic is another factor that may lead to introduction of the pathogens referred to into Poland.

The aim of the study was to monitor the occurrence of vector-borne diseases in dogs arriving in or returning to Poland from areas where these diseases are endemic.

Material and Methods

The study was conducted in the years 2021–2023. It included 497 anaemic dogs of different breeds aged from 6 months to 14 years. The sex distribution was 297 male and 200 female dogs. The dogs which came to Poland from Ukraine totalled 184: 16 in 2021, 141 in 2022, and 27 in 2023. Other animals returned to the country from spring or summer holidays spent in Croatia (96 dogs: 28 in 2021, 31 in 2022 and 37 in 2023), Turkey (79 dogs: 8 in 2021, 42 in 2022 and 29 in 2023), Italy (48 dogs: 26 in 2022 and 22 in 2023), Bulgaria (42 dogs:

19 in 2021, 16 in 2022 and 7 in 2023), Albania (36 dogs: 3 in 2021, 20 in 2022 and 13 in 2023) and Romania (12 dogs: 1 in 2021, 8 in 2022 and 3 in 2023) (Table 1).

In anamnesis, information was obtained about any anti-ectoparasite prophylaxis which had been sought for the animals. This had been administered to 36 of the dogs from Ukraine and 72 dogs spending holidays in Croatia, 65 having been in Turkey, 42 in Italy, 30 in Bulgaria, 31 in Albania and 8 in Romania.

The animals demonstrated different clinical symptoms (Table 2). Haematological examination revealed anaemia in all dogs and thrombocytopaenia in 413 of them (Table 3). Blood was collected from all animals for molecular testing to analyse for selected vector-borne diseases (babesiosis, anaplasmosis, ehrlichiosis, haemotropic mycoplasmosis, leishmaniasis, hepatozoonosis and bartonellosis). The study was conducted in accordance with the Directive of the European Parliament on the protection of animals used for scientific purposes (Directive 2010/63/EU), and all owners of the dogs agreed to their inclusion in the study. Blood sampling was a part of the clinical procedure and no local ethics committee approval was required.

Table 1. Non-indigenous and travelling dogs screened for vector-borne diseases, by country of origin or destination

Country	Number of examined dogs	8 1		Number of infected dogs protected against ectoparasites	
Ukraine	184	36	39	4	
Croatia	96	72	7	2	
Turkey	79	65	16	3	
Italy	48	42	6	1	
Bulgaria	42	30	4	1	
Albania	36	31	4	0	
Romania	12	8	3	0	
Total	497	284	79	11	

Table 2. Clinical signs observed in non-indigenous and travelling dogs with vector-borne diseases

	_	Number of dogs with the particular clinical sign									
Identified pathogen	Number of infected dogs	Apathy	Fever	Pale mucus membranes	Icterus	Lameness/ muscle pain	Gastroenteric problems	Neurological signs	Discolouration of the urine	Dermatological signs	Epistaxis
Brucella canis	27	27	19	23	4	0	10	0	24	0	0
Babesia gibsoni	2	2	2	2	0	0	0	0	2	0	0
Anaplasma phagocytophilum	20	20	11	6	0	4	3	1	0	0	4
Mycoplasma haemocanis	15	15	8	15	0	0	0	0	0	0	0
Bartonella henselae	7	7	5	6	0	0	1	0	0	0	0
Ehrlichia canis	4	4	3	3	0	2	1	0	0	1	2
Hepatozoon canis	3	3	1	3	0	2	0	0	0	0	0
Leishmania infantum	1	1	1	1	0	0	0	0	0	1	0
No infection	418	379	306	361	16	4	83	4	0	2	0
Total	497	458	356	420	20	12	98	5	26	4	6
P-value		0.002	0.078	0.016	0.540	8.517×10^{-5}	0.999	0.581	2.200×10^{-16}	0.121	1.369×10^{-5}

Table 3. Haematological disorders observed in non-indigenous and travelling dogs with vector-borne diseases

		Number of dogs with the particular haematological disorder						
Identified pathogen	Number of infected dogs	Anaemia	Thrombo- cytopaenia	Normal PLT	Leukocytosis	Leukopaenia	Normal WBC	
Brucella canis	27	27	27	0	2	21	4	
Babesia gibsoni	2	2	2	0	1	1	0	
Anaplasma phagocytophilum	20	20	20	0	3	1	16	
Mycoplasma haemocanis	15	15	9	6	10	0	5	
Bartonella henselae	7	7	7	0	2	0	5	
Ehrlichia canis	4	4	4	0	0	1	3	
Hepatozoon canis	3	3	1	2	3	0	0	
Leishmania infantum	1	1	0	1	1	0	0	
No infection	418	418	343	75	26	19	373	
Total	497	497	413	84	48	43	406	
P-value		-	0.191	0.191	1.807×10^{-7}	1.949×10^{-10}	2.200×10^{-16}	

PLT - platelet count; WBC - white blood cell count

Table 4. Primers and PCR conditions for detection and identification of Anaplasma/Ehrlichia spp., Babesia canis, Babesia gibsoni, Bartonella henselae, Hepatozoon canis, Mycoplasma haemocanis and Leishmania infantum in samples of non-indigenous and travelling dogs

Pathogen	Primers	Target gene	Amplicon siz (base pairs (bp))	e PCR conditions	Reference	
Anaplasma/ Ehrlichia spp.	EHR 521: (5'-TGT AGG CGG TTC GGT AAG TTA AAG-3') EHR 747: (5'-GCA CTC ATC GTT TAC	16S RNA	247 bp	35 cycles: denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 45 s	Pancholi <i>et al.</i> (37)	
	AGC GTG-3')			72 C 101 43 S		
	BAB GF2: (5'-GTC TTG TAA TTG GAA TGA TGG-3')			50 cycles: denaturation at 92°C for 60 s, annealing at	Adaszek and	
Babesia canis	BAB GR2: (5'-CCA AAG ACT TTG ATT TCT CTC-3')	18S RNA	559 bp	52°C for 60 s extension at 72°C for 90 s	Winiarczyk (3)	
	d3: (5'-TCC GTT CCC ACA ACA-CCA GC-3')	-	182 bp	50 cycles: denaturation at 92°C for 60 s, annealing at	Fukumoto et al.	
Babesia gibsoni	d4: (5'-TCC TCC TCA TCA TCC TCA TTC G-3')	P18/BgTRAP		52°C for 60 s extension at 72°C for 90 s	(16)	
Bartonella henselae	BART-LC-GEN-F: (5'-ATG GGT TTT GGT CAT CGA GT-3')	Citrate synthase	250 bp	40 cycles: denaturation at 96°C for 60 s, annealing at 60°C for 60 s extension at 72°C for 90 s	Staggemeier et al. (43)	
	BART-LC-HEN-R: (5'-AA ATCGACATTAGGGTAAAGTTTTT-3')	gene			ei ui. (43)	
Hepatozoon canis	HepF: (5'-ATA-CAT-GAG-CAA-AAT-CTC AAC-3')	18S RNA	666 bp	34 cycles: denaturation at 95°C for 30 s, annealing at 53°C for 30 s extension at	Inokuma <i>et al.</i> (24)	
	HepR (5'-CTT-ATT-ATT-CCA-TGC-TGC-AG-3')			72°C for 90 s		
	SYBR_Forward (5'-AGC AAT RCC ATG TGA ACG ATG AA-3')			4011		
Mycoplasma haemocanis	SYBR_Reverse 1: (5'-TGG CAC ATA GTT TGC TGT CAC TT – 3')	16S RNA	103 bp	40 cycles: denaturation at 95°C for 30 s, annealing at 60°C for 60 s extension at 72°C for 90 s	Willi et al. (52)	
	SYBR_Reverse 2: (5'-GCT GGC ACA TAG TTA GCT GTC ACT-3')			72 € 101 70 \$		
Leishmania infantum	N13A(5'-AAC TTT TCT GGT CCT CCG GG-3')	kinetoplast DNA minicircle	120 bp	40 cycles: denaturation at 94°C for 30 s, annealing at 58°C for 30 s extension at	Francino et al. (15)	
	N13B (5'-CCC CCA GTT TCC CGC CC-3')	D. A miniciple		72°C for 30 s	(13)	

Each sample was labelled with a unique number without details of the owner of the dog. All blood samples were analysed in a BIONOTE Vcheck M10 analyser (VetExpert, Poland), which isolated whole blood DNA and amplified the DNA of *Leishmania* spp., Babesia spp., Mycoplasma haemocanis, Hepatozoon spp., Ehrlichia canis, Anaplasma spp. and Bartonella spp. in a real-time PCR (Canine Vector 8 Panel). All DNA samples positive in the BIONOTE Vcheck M10 analyser were also amplified by standard real-time PCR using a Rotor-Gene thermocycler (Corbett Research, Mortlake, Australia). The list of primers used for all studied pathogens and the reaction conditions are presented in Table 4. The real-time PCR with SYBR Green 1 dye was carried out in thin-walled test tubes with a capacity of 100 μL. A DyNAmo HS SYBR Green qPCR Kit (Finnzymes, Espoo, Finland) was used in the method allowing a high-specificity reaction to be conducted. The reaction mixture with a volume of 20 µL consisted of the following components: 2 µL of the DNA matrix, 0.4 µL of each primer, 10 µL of Master Mix containing a hot start version of the modified polymerase Tbr (Thermus brockianus), buffer for the polymerase Tbr, dNTP, MgCl₂ and the intercalating SYBR Green 1 dye and water to 20 µL.

The size of the groups recommended using Fisher's exact test to evaluate the effectiveness of ectoparasite prevention. The null hypothesis assumed that the two

categorical variables are independent, *i.e.* that the probability of developing an illness is the same for protected and unprotected animals (the odds ratio is 1). Calculations were performed using the RStudio package. Fisher's exact test was also applied in the statistical analysis in relation to clinical signs and haematological indicators. In this case, the null hypothesis assumed that the probabilities of symptoms occurring and normal or exceeded reference values in the haematological examination are the same for both infected and uninfected animals. Results were considered statistically significant at a P-value ≤ 0.05 . Calculations were performed using the RStudio package (39).

Results

Molecular tests revealed the presence of vector-borne pathogens in 79 dogs. The most commonly diagnosed infection was *Babesia canis* (27 dogs), followed by *Anaplasma phagocytophilum* (20 dogs), *Mycoplasma haemocanis* (15 dogs), *Bartonella henselae* (7 dogs), *Ehrlichia canis* (4 dogs), *Hepatozoon canis* (3 dogs), *Babesia gibsoni* (2 dogs) and *Leishmania infantum* (1 dog). All positive results obtained in the BIONOTE Vcheck M10 analyser were confirmed in a standard real-time PCR test (no false-positive results were found).

Table 5. Results of vector-borne disease pathogen isolation and amplification from samples of non-indigenous and travelling dogs by country of origin or destination

	Number of dogs infected with the particular pathogen											
Country	Babesia canis	Babesia gibsoni	Anaplasma phagocytophilum	Ehrlichia canis	Hepatozoon canis	Leishmania infantum	Mycoplasma haemocanis	Bartonella henselae	Total			
Ukraine	15	0	12	1	0	0	8	3	39			
Croatia	6	0	0	1	0	0	0	0	7			
Turkey	0	0	4	0	3	1	4	4	16			
Italy	1	0	2	2	0	0	1	0	6			
Bulgaria	2	0	2	0	0	0	0	0	4			
Albania	1	1	0	0	0	0	2	0	4			
Romania	2	1	0	0	0	0	0	0	3			
Total	27	2	20	4	3	1	15	7	79			

Table 6. The effectiveness of ectoparasite prevention and probability of infection in the group of protected non-indigenous and travelling dogs

Country	Number of protected and infected dogs	Number of non- protected and infected dogs	Number of protected and non-infected dogs	Number of non- protected and non- infected dogs	P-value	95 % confidence interval	Odds ratio
Ukraine	4	35	32	113	0.115	0.097-1.260	0.405
Croatia	2	5	70	19	0.009	0.009-0.747	0.111
Turkey	3	13	62	1	2.845×10^{-11}	$9.562 \times 10^{-5} 4.478 \times 10^{-2}$	0.005
Italy	1	5	41	1	2.062×10^{-5}	$1.123 \times 10^{-4} 1.262 \times 10^{-1}$	0.007
Bulgaria, Romania and Albania	1	10	61	18	2.007×10^{-5}	$6.752 \times 10^{-4} - 2.415 \times 10^{-1}$	0.031
Total	11	68	266	152	2.200×10^{-16}	0.042 - 0.184	0.092

The majority of infected animals presented typical symptoms of specific vector-borne diseases (Table 2). The performed haematological examinations revealed anaemia (in all 79 dogs), thrombocytopaenia (in 70 dogs), leukopaenia (in 24 dogs) and leukocytosis (in 22 dogs). In 33 dogs from this group (41.8%), the white blood cell count was within the reference range. Relating this result to the group of animals in which no vector-borne infection was found by molecular testing and in 373 of which a leukocyte count in the reference range was found (89.2%), it can be concluded that this difference was statistically significant (P-value = 2.200×10^{-16}).

Most of the sick dogs (n = 39) came from Ukraine. Of these, 15 were infected with Babesia canis, 12 with Anaplasma phagocytophilum, 8 with Mycoplasma haemocanis, 3 with Bartonella henselae and 1 with Ehrlichia canis. In dogs travelling outside Poland, vector-borne diseases were most often detected after their return from Turkey (n = 16; 4 Anaplasma phagocytophilum infections, 4 Mycoplasma haemocanis, 4 Bartonella henselae, 3 Hepatozoon canis and 1 Leishmania infantum), and less often in descending order after travel to Croatia (n = 7; 6 Babesia canis infections and 1 Ehrlichia canis), Italy (n = 6; 2 Anaplasma phagocytophilum, 2 Ehrlichia canis, 1 Babesia canis and 1 Mycoplasma haemocanis), Albania (n = 4; 2 Mycoplasma haemocanis, 1 Babesia canis and 1 Babesia gibsoni), Bulgaria (n = 4; 2 Anaplasma phagocytophilum and 2 Babesia canis) and Romania (n = 3; 2 Babesia canis and 1 Babesia gibsoni) (Table 5).

Only four of the positive animals from Ukraine had received prophylaxis against ectoparasites in the previous three months. Of the animals that became infected while on holiday, only two dogs had received prophylaxis against ectoparasites before travelling to Croatia, three before travelling to Turkey, one to Italy and one to Bulgaria, and none had before travelling to Albania or Romania (Table 6).

The effectiveness of ectoparasite prevention was verified using the F test, in which for all countries except Ukraine, H0 was rejected (confidence intervals \neq 1), and the P-values indicate that there were statistically significant differences between the groups. For the data collected in this study, the odds ratios suggest that the probability of infection is between 0.11 and 0.005 lower in the group of protected dogs (Table 6).

Discussion

The results of our studies indicate that both the increased frequency of tourist trips with pet dogs to countries where vector-borne diseases are endemic and the influx of immigrants with their dogs from Ukraine to Poland may constitute risk factors for the occurrence of a wider range of diseases than covered in this report and diseases considered exotic in this country.

It is commonly known that the countries which the study dogs visited or from which they originated are

endemic areas for vector-borne diseases. It is confirmed by numerous literature data. In Ukraine, such pathogens as Babesia spp., Anaplasmataceae, Rickettsia spp., Bartonella spp., Mycoplasma haemocanis or Hepatozoon spp. have been found in both ticks and dogs (21, 31). The results of the study by Mrljak et al. (35) indicate that Croatia is still facing the problem of arthropod-borne diseases and that the seroprevalence of such pathogens as Babesia canis, Anaplasma phagocytophilum, Leishmania infantum or Ehrlichia canis even among dogs that do not present any clinical symptoms may be high. That research reported respective prevalences for those disease agents of 20.00%, 6.21%, 1.38% and 0.46%. In Turkey, studies comprising asymptomatic stray dogs showed the presence of DNA of Babesia canis, Hepatozoon spp., Hepatozoon canis, Dirofilaria immitis and Ehrlichia canis in 5.3% (7/133), 27.1% (36/133), 5.3% (7/133), 1.5% (2/133) and 9.8% (13/133) of the dogs, respectively. The observations of Guo et al. (19) of pet dogs, kennel dogs and shepherd dogs in the Turkish city of Konya contrast somewhat, showing the presence of genetic material of *Babesia* spp. (2.1%), Hepatozoon spp. (4.2%) and Mycoplasma spp. (24%), the last being detected more frequently in kennel dogs (31.9%) than in pet (21.4%) and shepherd dogs (13.8%). It should be emphasised that this country is also an endemic area for leishmaniasis (28, 51).

In Italy, the vector-borne pathogens most frequently affecting dogs include *Borrelia burgdorferi* s.l. (83.5%), *Rickettsia* spp. (64.9%), *Anaplasma* spp. (39.8%) and *Ehrlichia canis* (28.7%) (34). Leishmaniasis is also endemic in a region of Italy, specifically in at least three different areas of Piedmont (Turin, Ivrea and Casale), where seroprevalence in resident dogs is 3.9–5.8% (14).

In Bulgaria, antibodies to Babesia canis, Anaplasma phagocytophilum, Ehrlichia canis, and more recently also to Leishmania infantum, are commonly found in the dog population (22, 32, 38, 44). The epizootic situation of vector-borne diseases in Albania and Romania is similar. The results of the study by Hamel et al. (20) show that in respect of the arthropodborne pathogens, dogs in Albania are most frequently infected by Babesia spp., *Hepatozoon* Leishmania infantum, Dirofilaria immitis, Anaplasma phagocytophilum, Anaplasma platys, Ehrlichia canis and Mycoplasma haemocanis, with prevalence rates ranging from 1 to 9%. Seroprevalence for *Babesia* spp., Leishmania infantum, Anaplasma spp. and Ehrlichia canis is 6.6%, 5.1%, 24.1% and 20.8%, respectively. According to Andersson et al. (7), the pathogens most commonly diagnosed in Romanian dogs with suspected vector-borne diseases are Babesia canis, Hepatozoon canis and Mycoplasma spp. Similarly to in other previously described countries, also here dogs are prone to infections with Anaplasma spp., Leishmania infantum or Babesia gibsoni (8, 10, 23).

Since the beginning of the war in Ukraine, the Polish–Ukrainian border has been crossed by millions of refugees, mostly women and children, very often with pets (26). After the Russian aggression against Ukraine, to facilitate refugees' passing through the border with pets, the Chief Veterinary Officer temporarily simplified the procedure for dealing with animals translocated for non-commercial purposes and accompanying refugees entering the EU from Ukraine through the Polish border (17). This procedure was designed to control rabies and focused on ensuring effective vaccination against it in dogs, cats and ferrets and tracking the identity (*via* microchipping) and intended location of the animal. Because of massive traffic at the border, no other preventive measures have been implemented to control other infectious diseases, including parasitic diseases.

While infection with *Babesia canis* or *Anaplasma phagocytophilum* is very common in native dogs (2, 35, 49) and does not pose a diagnosis or treatment problem for Polish veterinarians, such diseases as ehrlichiosis, hepatozoonosis, leishmaniasis, haemotropic mycoplasmosis, bartonellosis and babesiosis caused by *Babesia gibsoni* infections can be a challenge for veterinary practitioners in the country.

So far, data on the occurrence of these diseases in dogs in Poland are fragmentary. The first cases of hepatozoonosis caused by *Hepatozoon canis* invasions in Poland were described in 2021–2022 (50). The infected dogs came to Poland from Ukraine (together with refugees) (9), but there were also dogs suffering from it which had never left Poland (autochthonous cases) (50). Recently three new cases of hepatozoonosis were diagnosed in dogs which had returned from Turkey, where they accompanied their owners on holiday (own observations).

Babesia gibsoni infections were first reported in Poland in 2020 in three dogs (47). The studies included monitoring of tick-borne diseases based on blood samples delivered to an analytical laboratory taken from animals with thrombocytopaenia. Another case from 2022 involved a dog that had never left Poland (1).

A single case of canine leishmaniosis in Poland has been described so far. A three-year-old stray female dog was admitted to a veterinary clinic for generalised skin lesions and lethargy. Canine leishmaniosis was confirmed by several diagnostic methods: cytology (impression smears from skin lesions and fine-needle aspiration from lymph nodes), histopathology (skin biopsies) and serology (ELISA and IFAT) (41). As no history of the dog was available, it was assumed that the dog was infected somewhere in southern Europe (18).

The presence of *Bartonella henselae* DNA was detected by Mazurek *et al.* (33) in the blood of four dogs in eastern Poland. The dogs infected with bacteria came from urban (two individuals) and from rural (two individuals) areas. They had never left Poland and lived together with cats in the same household. None of the dogs showed any disease symptoms, so in these cases, the infection was subclinical, and the cats probably served as the source of direct (by scratching or by biting) or indirect (by fleas) infection of the dogs.

Mycoplasma haemocanis infections in dogs in Europe are relatively rare. According to the available literature, they have so far been found in France, Switzerland, Italy, Romania, Greece, Spain, Turkey and Portugal (5, 12, 53). In Poland, two cases of haemotropic mycoplasmosis were described in dogs aged 8 and 11 years, which developed symptoms of apathy and anaemia. Both animals suffered from comorbidities (spleen angiosarcoma and cauda equina syndrome) (27, 30), which may have contributed to the development of the infection.

Suspicion of vector-borne diseases is based on information obtained from the owners during the anamnesis, clinical examination and additional tests. Haematological examination may show abnormalities characteristic of some of the nosological entities in question. They include anaemia, thrombocytopaenia and leukopaenia in the course of babesiosis (4, 42, 48, 54), thrombocytopaenia in the course of anaplasmosis, ehrlichiosis and bartonellosis (6, 40, 45), and anaemia in the course of haemotropic mycoplasmosis (46). These data overlap with our observations; however, it should be noted that the dogs in which no vector-borne diseases were found by molecular testing also presented symptoms of anaemia (it was a criterion for inclusion in the study) and, in the majority of animals, symptoms of thrombocytopaenia. The only statistically significant difference observed in haematological examinations between the infected and uninfected dogs was the leukocyte count. In the group with negative results of PCR tests for vector-borne diseases, only 10.8% of the dogs had abnormal leukocyte counts, compared to 58.2% of the infected dogs. A definitive diagnosis of vector-borne diseases must therefore be supported in each case by more sensitive diagnostic methods.

Undoubtedly, a factor contributing to the development of vector-borne diseases is omission of proper protective measures against ectoparasites. Only 11 (13.9%) of the 79 studied infected dogs had benefited from ectoparasite prevention measures, and 68 (86.1%) of those 79 had not been protected. These data were statistically significant. Currently, there are numerous medications on the veterinary products market that reliably prevent ectoparasite infestations. They are available in different versions: spot-on, tablets, collars, *etc*. The role of veterinarians is to continually educate pet owners about the need for taking such measures, preferably throughout the year and especially when owners will be travelling abroad with their pets. Such behaviour significantly minimises the risk of vector-borne diseases in dogs (13).

Conclusion

Our observations and the above review of the literature indicate that although vector-borne diseases occur in dogs in Poland, that the number of their cases is increasing in the country undoubtedly has its main factor in the migration crisis and increasingly frequent trips of

owners with their dogs to areas of endemic infectious and parasitic diseases. Among dogs returning from holidays and arriving in Poland from abroad, constant screening for these diseases using analytic techniques that can detect several pathogens simultaneously and quickly seems to be crucial for their early recognition and introduction of appropriate therapy.

Since many vector-bone diseases are zoonoses, their proper monitoring and adequate prevention against ectoparasites in dogs are also important elements of human health protection.

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References

- Adaszek Ł., Łukaszewska J., Augustynowicz M.: Inwazja Babesia gibsoni u psa – opis przypadku (Babesia gibsoni infection in a dog – a case report – in Polish). Magazyn Wet 2022, 09, 31.
- Adaszek Ł., Winiarczyk S.: Molecular characterization of *Babesia canis canis* isolates from naturally infected dogs in Poland. Vet Parasitol 2008, 152, 235–241, doi: 10.1016/j.vetpar.2007.12.024.
- Adaszek Ł., Winiarczyk S.: Application of the SYBR Green realtime HRM PCR technique in the differentiation of the *Babesia* canis canis protozoa isolated in the areas of eastern Poland. Parasitol Res 2010, 106, 1253–1256, doi: 10.1007/s00436-010-1784-3
- Adaszek Ł., Winiarczyk S., Skrzypczak M.: The clinical course of babesiosis in 76 dogs infected with protozoan parasites *Babesia* canis canis. Pol J Vet Sci 2009, 12, 81–87.
- Aktas M., Ozubek S.: Molecular survey of haemoplasmas in shelter dogs and associations with *Rhipicephalus sanguineus* sensu lato. Med Vet Entomol 2017, 31, 457–461, doi: 10.1111/mve.12244.
- Álvarez-Fernández A., Breitschwerdt E.B., Solano-Gallego L.: Bartonella infections in cats and dogs including zoonotic aspects. Parasit Vectors 2018, 11, 624, doi: 10.1186/s13071-018-3152-6.
- Andersson M.O., Tolf C., Tamba P., Stefanache M., Waldenström J., Dobler G., Chiţimia-Dobler L.: Canine tick-borne diseases in pet dogs from Romania. Parasit Vectors 2017, 10, 155, doi: 10.1186/s13071-017-2092-x.
- Andersson M., Turcitu M.A., Stefanache M., Tamba P., Barbuceanu F., Chitimia L.: First evidence of *Anaplasma platys* and *Hepatozoon canis* co-infection in a dog from Romania - a case report. Ticks Tick Borne Dis 2013, 4, 317–319, doi: 10.1016/j.ttbdis.2012.12.006.
- Bajer A., Alsarraf M., Topolnytska M., Tołkacz K., Dwużnik-Szarek D., Rodo A.: Vector-borne parasites in dogs from Ukraine translocated to Poland following Russian invasion in 2022. Parasit Vectors 2023, 16, 430, doi: 10.1186/s13071-023-06042-2.

- 10. Cazan C.D., Ionică A.M., Matei I.A., D'Amico G., Muñoz C., Berriatua E., Dumitrache M.O.: Detection of *Leishmania infantum* DNA and antibodies against *Anaplasma* spp., *Borrelia burgdorferi* s.l. and *Ehrlichia canis* in a dog kennel in South-Central Romania. Acta Vet Scand 2020, 62, 42, doi: 10.1186/s13028-020-00540-4.
- Colwell D.D., Dantas-Torres F., Otranto D.: Vector-borne parasitic zoonoses: emerging scenarios and new perspectives. Vet Parasitol 2011, 182, 14–21, doi: 10.1016/j.vetpar.2011.07.012.
- Cortese L., Beall M., Buono F., Buch J., Pacifico L., Neola B., Palatucci A.T., Tyrrell P., Fioretti A., Breitschwerdt E.B., Veneziano V., Chandrashekar R., Piantedosi D.: Distribution and risk factors of canine haemotropic mycoplasmas in hunting dogs from southern Italy. Vet Microbiol 2020, 251, 108910, doi: 10.1016/j.vetmic.2020.108910.
- 13. Dantas-Torres F., Otranto D.: Best practices for preventing vector-borne diseases in dogs and humans. Trends Parasitol 2016, 32, 43–55, doi: 10.1016/j.pt.2015.09.004.
- Ferroglio E., Maroli M., Gastaldo S., Mignone W., Rossi L.: Canine leishmaniasis, Italy. Emerg Infect Dis 2005, 11, 1618– 1620, doi: 10.3201/eid1110.040966.
- Francino O., Altet L., Sánchez-Robert E., Rodriguez A., Solano-Gallego L., Alberola J., Ferrer L., Sánchez A., Roura X.: Advantages of real-time PCR assay for diagnosis and monitoring of canine leishmaniosis. Vet Parasitol 2006, 137, 214–221, doi: 10.1016/j.vetpar.2006.01.011.
- Fukumoto S., Xuan X., Shigeno S., Kimbita E., Igarashi I., Nagasawa H., Fujisaki K., Mikami T.: Development of a polymerase chain reaction method for diagnosing *Babesia* gibsoni infection in dogs. J Vet Med Sci 2001, 63, 977–981, doi: 10.1292/jvms.63.977.
- 17. General Veterinary Inspectorate: Temporary procedure for dealing with animals moved for non-commercial purposes of up to 5 animals, accompanying refugees entering the EU from Ukraine through the Polish external border during a military conflict. GIW, Warsaw, 2022, www.wetgiw.gov.pl.
- 18. Gójska-Zygner O., Zygner W.: Disease and sudden death case study: a 16-year-old retriever. Vet Times 2010, 40, 20–24.
- Guo H., Sevinc F., Ceylan O., Sevinc M., Ince E., Gao Y., Moumouni P.F.A., Liu M., Efstratiou A., Wang G., Cao S., Zhou M., Jirapattharasate C., Ringo A.E., Zheng W., Xuan X.: A PCR survey of vector-borne pathogens in different dog populations from Turkey. Acta Parasitol 2017, 62, 533–540, doi: 10.1515/ap-2017-0064.
- Hamel D., Shukullari E., Rapti D., Silaghi C., Pfister K., Rehbein S.: Parasites and vector-borne pathogens in client-owned dogs in Albania. Blood pathogens and seroprevalences of parasitic and other infectious agents. Parasitol Res 2016, 115, 489–499, doi: 10.1007/s00436-015-4765-8.
- Hamel D., Silaghi C., Zapadynska S., Kudrin A., Pfister K.: Vector-borne pathogens in ticks and EDTA-blood samples collected from client-owned dogs, Kiev, Ukraine. Ticks Tick Borne Dis 2013, 4, 152–155, doi: 10.1016/j.ttbdis.2012.08.005.
- Iliev P.T., Kirkova Z.T., Tonev A.S.: Preliminary study on the prevalence of endoparasite infections and vector-borne diseases in outdoor dogs in Bulgaria. Helminthologia 2020, 57, 171–178, doi: 10.2478/helm-2020-0016.
- Imre M., Farkas R., Ilie M.S., Imre K., Dărăbuş G.: Survey of babesiosis in symptomatic dogs from Romania: occurrence of *Babesia gibsoni* associated with breed. Ticks Tick Borne Dis 2013, 4, 500–502, doi: 10.1016/j.ttbdis.2013.06.006.
- 24. Inokuma H., Okuda M., Ohno K., Shimoda K., Onishi T.: Analysis of the 18S rRNA gene sequence of a Hepatozoon detected in two Japanese dogs. Vet Parasitol 2002, 26, 265–271, doi: 10.1016/s0304-4017(02)00065-1.
- Jaenson T.G.T., Jaenson D.G.E., Eisen L., Petersson E., Lindgren E.: Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30 years in Sweden. Parasit Vectors 2012, 5, doi: 10.1186/1756-3305-5-8.
- Jankowski M., Lazarus J.V., Kuchyn I., Zemskov S., Gałązkowski R., Gujski M.: One year on: Poland's public health initiatives and

- national response to millions of refugees from Ukraine. Med Sci Monit 2023, 29, e940223, doi: 10.12659/MSM.940223.
- Kandefer-Gola M., Gola M., Pyrek P., Kinda W.: Mykoplazmoza hemotropowa u psa – opis przypadku (The haemotropic mycoplasma in dog – case report – in Polish). VetTrends 2019, 4, 1–3.
- Karakuş M., Arserim S.K., Erişöz Kasap Ö., Pekağırbaş M., Aküzüm D., Alten B., Töz S., Özbel Y.: Vector and reservoir surveillance study in a canine and human leishmaniasis endemic area in most western part of Turkey, Karaburun. Acta Trop 2019, 190, 177–182, doi: 10.1016/j.actatropica.2018.11.020.
- Krämer F., Schaper R., Schunack B., Połozowski A., Piekarska J., Szwedko A., Jodies R., Kowalska D., Schüpbach D., Pantchev N.: Serological detection of *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato and *Ehrlichia canis* antibodies and *Dirofilaria immitis* antigen in a countrywide survey in dogs in Poland. Parasitol Res 2014, 113, 3229–3239, doi: 10.1007/s00436-014-3985-7.
- Kuczorski M., Podleśna G., Gołańczyk J.: Naczyniakomięsak śledziony i mykoplazma hemotropowa psów – opis przypadku (Splenic angiosarcoma and canine haemotropic mycoplasma – a case report – in Polish). Magazyn Wet 2015, 06, 03.
- Levytska V.A., Mushinsky A.B., Zubrikova D., Blanarova L., Długosz E., Vichova B., Slivinska K.A., Gajewski Z., Gizinski S., Liu S., Zhou L., Rogovskyy A.S.: Detection of pathogens in ixodid ticks collected from animals and vegetation in five regions of Ukraine. Ticks Tick Borne Dis 2021, 12, 101586, doi: 10.1016/j.ttbdis.2020.101586.
- 32. Manev I.: Serological survey of vector-borne pathogens in stray dogs from Sofia area, Bulgaria. Vet Parasitol Reg Stud Reports 2020, 21, 100441, doi: 10.1016/j.vprsr.2020.100441.
- Mazurek L., Winiarczyk S., Skrzypczak M., Adaszek L.: Cats as a reservoir of *Bartonella henselae* for dogs. Ann Agric Environ Med 2019, 26, 669–671, doi: 10.26444/aaem/105396.
- Mendoza-Roldan J.A., Benelli G., Bezerra-Santos M.A., Nguyen V.L., Conte G., Iatta R., Furlanello T., Otranto D.: Seropositivity to canine tick-borne pathogens in a population of sick dogs in Italy. Parasit Vectors 2021, 14, 292, doi: 10.1186/s13071-021-04772-9.
- Mrljak V., Kuleš J., Mihaljević Ž., Torti M., Gotić J., Crnogaj M., Živičnjak T., Mayer I., Šmit I., Bhide M., Barić Rafaj R.: Prevalence and geographic distribution of vector-borne pathogens in apparently healthy dogs in Croatia. Vector Borne Zoonotic Dis 2017, 17, 398–408, doi: 10.1089/vbz.2016.1990.
- 36. Noll M., Wall R., Makepeace B.L., Newbury H., Adaszek Ł., Bødker R., Estrada-Peña A., Guillot J., da Fonseca I.P., Probst J., Overgaauw P., Strube C., Zakham F., Zanet S., Rose Vineer H.: Predicting the distribution of *Ixodes ricinus* and *Dermacentor reticulatus* in Europe: a comparison of climate niche modelling approaches. Parasit Vectors 2023, 16, 384, doi: 10.1186/s13071-023-05959-y.
- Pancholi P., Kolbert C.P., Mitchell P.D., Reed Jr K.D., Dumler J.S., Bakken J.S., Telford 3rd S.R., Persing D.H.: *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. J Infect Dis 1995, 172, 1007–1012, doi: 10.1093/infdis/172.4.1007.
- Pantchev N., Schnyder M., Vrhovec M.G., Schaper R., Tsachev I.: Current surveys of the seroprevalence of *Borrelia burgdorferi*, Ehrlichia canis, Anaplasma phagocytophilum, Leishmania infantum, Babesia canis, Angiostrongylus vasorum and Dirofilaria immitis in dogs in Bulgaria. Parasitol Res 2015, 114, 117–130, doi: 10.1007/s00436-015-4518-8.
- RStudio Team: RStudio: Integrated Development for R. RStudio, Boston, MA, USA, 2020.
- Sainz Á., Roura X., Miró G., Estrada-Peña A., Kohn B., Harrus S., Solano-Gallego L.: Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. Parasit Vectors 2015, 8, 75, doi: 10.1186/s13071-015-0649-0.

- 41. Sapierzyński R.: Canine leishmaniosis. Pol J Vet Sci 2008, 11, 151–158.
- Solano-Gallego L., Sainz Á., Roura X., Estrada-Peña A., Miró G.: A review of canine babesiosis: the European perspective. Parasit Vectors 2016, 9, 336, doi: 10.1186/s13071-016-1596-0.
- 43. Staggemeier R., Pilger D.A., Spilki F.R., Cantarelli V.V.: Multiplex SYBR® green-real time PCR (qPCR) assay for the detection and differentiation of *Bartonella henselae* and *Bartonella clarridgeiae* in cats. Rev Inst Med Trop Sao Paulo 2014, 56, 93–95, doi: 10.1590/S0036-46652014000200001.
- Stoimenov G.M., Tchakarova S.: Detection of Leishmania infantum antibodies in stray dogs from nonendemic areas in Bulgaria. Vector Borne Zoonotic Dis 2024, doi: 10.1089/vbz.2023.0062.
- Taber R., Pankowski A., Ludwig A.L., Jensen M., Magsamen V., Lashnits E.: Bartonellosis in dogs and cats, an update. Vet Clin North Am Small Anim Pract 2022, 52, 1163–1192, doi: 10.1016/j.cvsm.2022.06.006.
- Tasker S.: Hemotropic Mycoplasma. Vet Clin North Am Small Anim Pract 2022, 52, 1319–1340, doi: 10.1016/j.cvsm.2022.06.010.
- Teodorowski O., Kalinowski M., Skrzypczak M., Witt K., Madany J., Winiarczyk S., Adaszek Ł. *Babesia gibsoni* infection in dogs in Poland. Pol J Vet Sci 2020, 23, 469–471, doi: 10.24425/pjvs.2020.134694.
- Teodorowski O., Kalinowski M., Winiarczyk D., Dokuzeylül B., Winiarczyk S., Adaszek Ł.: Babesia gibsoni infection in dogs – a European perspective. Animals 2022,12, 730, doi: 10.3390/ani12060730.
- Teodorowski O., Winiarczyk S., Dębiak P., Skrzypczak M., Mazurek Ł., Adaszek Ł.: Clinical course of granulocytic anaplasmosis in hunting dogs. Pol J Vet Sci 2021, 24, 175–181, doi: 10.24425/pjvs.2021.136807.
- Tołkacz K., Kretschmer M., Nowak S., Mysłajek R.W., Alsarraf M., Wężyk D., Bajer A.: The first report on *Hepatozoon canis* in dogs and wolves in Poland: clinical and epidemiological features. Parasit Vectors 2023, 16, 313, doi: 10.1186/s13071-023-05928-5.
- Toz S.O., Nasereddin A., Ozbel Y., Ertabaklar H., Culha G., Sevil N., Ziya Alkan M., Jaffe C.L.: Leishmaniasis in Turkey: molecular characterization of *Leishmania* from human and canine clinical samples. Trop Med Int Health 2009, 14, 1401–1406, doi: 10.1111/j.1365-3156.2009.02384.x.
- Willi B., Meli M.L., Lüthy R., Honegger H., Wengi N., Hoelzle L.E., Reusch C.E., Lutz H., Hofmann-Lehmann R.: Development and application of a universal hemoplasma screening assay based on the SYBR Green PCR principle. J Clin Microbiol 2009, 47, 4049– 4054, doi: 10.1128/JCM.01478-09.
- Willi B., Novacco M., Meli M., Wolf-Jäckel G., Boretti F., Wengi N., Lutz H., Hofmann-Lehmann R.: Haemotropic mycoplasmas of cats and dogs: transmission, diagnosis, prevalence and importance in Europe. Schweiz Arch Tierheilkd 2010, 152, 237–244, doi: 10.1024/0036-7281/a000055.
- 54. Zygner W., Gójska O., Rapacka G., Jaros D., Wędrychowicz H.: Hematological changes during the course of canine babesiosis caused by large Babesia in domestic dogs in Warsaw (Poland). Vet Parasitol 2007, 145, 146–151, doi: 10.1016/j.vetpar.2006.11.008.
- Zygner W., Górski P., Wędrychowicz H.: Detection of the DNA of Borrelia afzelii, Anaplasma phagocytophilum and Babesia canis in blood samples from dogs in Warsaw. Vet Rec 2009, 164, 465–467, doi: 10.1136/vr.164.15.465.
- Zygner W., Gójska-Zygner O., Górski P., Bartosik J.: Over 20 years of research on canine babesiosis at the Faculty of Veterinary Medicine of WULS-SGGW in Warsaw (in Polish). Med Wet 2017, 73, 606–612, doi: 10.21521/mw.5792.