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RESEARCH ARTICLE

Distribution of *Dirofilaria repens* (Railliet et Henry, 1911) in Southern and Southeastern Russia

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Abstract
The aim of the study was to investigate the current distribution of *Dirofilaria* repens in dogs from the Southern, Volga, and North Caucasian federal districts of European Russia. A total of 6,444 canine blood samples from 100 localities were screened using a species-specific real-time PCR assay. *D. repens* DNA was detected in 485 samples (7.52% prevalence), confirming the parasite's presence across a vast area. Positive cases were identified in the Republics of Bashkortostan, Mari El, Mordovia, Tatarstan, Chuvashia, Karachay-Cherkessia, Chechnya, Adygea, and the Crimean Peninsula, as well as in Perm, Stavropol, Krasnodar, Rostov, Volgograd, Astrakhan, Ulyanovsk, Samara, Penza, Nizhny Novgorod, and Kirov regions. The prevalence varied significantly among regions, ranging from 1.04% in Astrakhan region to 48.39% in the Republic of Mari El. While the lack of travel histories for sampled dogs means that some detected cases could be imported, the high prevalence observed in many cities provides robust evidence of autochthonous transmission. Our findings demonstrate that *D. repens* is now endemic across a much broader territory of European Russia than previously recognized, including lesser-known foci in the Volga Basin and the Western Urals, which has significant implications for veterinary and public health surveillance.

Keywords
Dirofilaria repens, PCR, Russia, canine Dirofilariasis, molecular epidemiology, prevalence, zoonosis

Introduction

The subcutaneous nematode *Dirofilaria repens* (Spirurida: Onchocercidae) is a filarial parasite that primarily cycles between culicid mosquito vectors and canid definitive hosts, including domestic dogs and wild species. A wide range of mosquitoes from genera such as *Aedes*, *Culex*, *Anopheles*, and *Mansonia* can serve as competent vectors (Krivorotova and Nagorny 2016). While canids are main definitive host, the parasite can also infect accidental hosts, such as cats, ferrets, and humans, where it often fails to complete its life cycle. In the definitive host, developing larvae (L3-L4) and pre-adults reside in the subcutaneous tissues, while adult females release first-stage larvae (microfilariae, L1) into the bloodstream.

Accurately mapping the distribution of *D. repens* is complicated by diagnostic challenges. Although necropsy and morphological examination of adult worms remain the gold standard for definitive diagnosis, these methods are impractical for large-scale surveillance. Diagnosing infection in live animals may be associated with several challenges. Serological tests for *D. repens*, unlike those available for *D. immitis*, are not widely accessible. Furthermore, microscopic detection of microfilariae in blood is unreliable for several reasons: it cannot detect prepatent, unisexual, or amicrofilaremic infections, and morphological identification to the species level is often uncertain. While molecular methods like species-specific PCR offer high specificity and sensitivity for detecting parasite DNA in blood, they share the limitation of being unable to identify infections where microfilariae are absent from the bloodstream (Gioia et al. 2010). Consequently, many cases, particularly in accidental hosts, are only confirmed post-surgically following the extraction and morphological analysis of the worm.

D. repens is a pathogen of growing One Health concern, being a primary cause of human Dirofilariasis. The number of reported human cases has risen significantly over the past century, from a few dozen to hundreds globally, indicating an expanding problem (Cancrini et al. 1999; Pampiglione et al. 2009, Pampiglione and Rivasi 2000). This trend is particularly evident across Europe, where the parasite's range now spans from the Mediterranean to Finland, with autochthonous cases reported in numerous countries, including Ukraine, Poland, Hungary, and others (Capelli et al. 2018). The Russian Federation contributes substantially to this burden, with approximately 700 human cases documented to date (Darchenkova et al. 2009; Sergiev et al. 2009; Sergiev et al. 2012).

The nematode *D. repens* is globally distributed, being endemic across Europe, the Middle and Far East, and parts of Africa, while the Americas have historically been considered non-endemic (Simón et al. 2012; Smith et al. 2022). In Europe, it is well-established and expanding northward, with high prevalence reported in countries like Italy, Greece, and Spain (Fioretti et al. 2003; Tarello 2002, 2011; Papazahariadou et al. 1994; Cancrini et al. 2000; Traversa et al. 2010, Scala et al. 2013). The infection is spreading from Portugal to the Southeastern regions of Finland and Siberia, and in some areas its prevalence overlaps that of *D. immitis* (Genchi and Kramer 2020). In Moldova, a study of mosquitoes for *D. repens* larvae revealed a high prevalence, indicating the endemicity of this region (Şuleşco et al. 2016). Its presence in Africa is documented but less understood, with reports from North and South Africa (Genchi et al. 2001; Izenour et al. 2025), and in Asia it is confirmed in countries including India, Iran, Sri Lanka, Israel, Kuweit, Saudi Arabia, Türkiye, and the United Arab Emirates (Megat Abd Rani et al. 2010; Pradeep et al. 2019; Pedram et al. 2019; Izenour et al. 2025, Balendran et al. 2022). Despite the historical absence in the Americas, recent sporadic autochthonous cases in Colombia signal its emerging potential in new regions (Ballesteros et al. 2023).

Despite this widespread and expanding global distribution, detailed and up-to-date epidemiological data for large regions, such as the Russian Federation, remain fragmented. Within Russia, the historical endemic foci for *Dirofilaria* are the warm southern regions. The parasite was first reported in the Rostov region in 1929 (Gurvich 1929), and the Caucasian republics have long been recognized as part of its range (Kozlov 1977), although data from many North Caucasian territories have only emerged recently (Trunova 2008; Kabardiev et al. 2017; Bagaeva et Bocharova 2008). The Lower Volga region is another well-documented area of high prevalence in animals and humans (Kamenov and Shinkarenko 2019; Vedeneev 2004; Romanova et al. 2013; Ermilov et al. 2010; Arkhipova and Arkhipov 2004; Bakina et al. 2013; Nagorny and Krivorotova 2010; Varlamova and Arkhipov 2016; Bogdanova and Boyko 2007; Bogdanova 2008, Sergiev et al. 2012; Arakel'yan et al. 2017). In contrast, surveillance data become scarcer for areas further north and east. Infection rates appear to decrease upstream along the Volga River, and territories to the east, such as the Western Urals, are characterized by sporadic and often poorly documented findings, making their epidemiological status unclear (Kudinov and Annikova 2002; Durnova et al. 2020; Tabakaeva et al. 2015; Shchegolenkova et al. 2014). Although the distribution of *D. repens* in the Asian part of Russia has been thoroughly reviewed recently (Konyaev and Prilepsky 2023), the complete picture of its spread remains unclear.

Most faunistic surveys from the southern and southeastern territories of European Russia concerning the distribution of *Dirofilaria* in domestic carnivores are typically based on small sample sizes and were conducted without highly specific and sensitive methods for species identification. In particular, molecular genetic methods were rarely employed, and species determination was either not performed or based solely on microfilaria morphology. The focus on the more clinically severe *D. immitis* in veterinary practice has also led to the underreporting of *D. repens* cases. The territories of the North Caucasus and the Volga region remain particularly understudied. To address these gaps, we conducted a large-scale molecular survey covering the Southern, North Caucasian, and Volga Federal Districts. The aims of this study were: to accurately map the current distribution of *D. repens* in dogs across southern and southeastern European Russia using species-specific real-time PCR, and to synthesize these new data with previous findings to provide an updated overview of the parasite's range.

Materials and methods

Between 2018 and 2023, a total of 6,444 whole blood samples were obtained from domestic dogs (*Canis lupus familiaris*) from 100 localities across three federal districts of European Russia: The North Caucasian, Volga, and Southern Federal Districts. Samples were collected by collaborating veterinary clinics and diagnostic laboratories. No clinical histories or travel records of the animals were available for this study. Genomic DNA was extracted from 200 μL of each whole blood sample using the RealBest Extraction 100 kit (Vector-Best, Novosibirsk, Russia), strictly following the manufacturer's protocol. The detection of *Dirofilaria repens* DNA was performed using a commercial real-time PCR (rt-PCR) kit for the differential detection of *Dirofilaria immitis* and *Dirofilaria repens* (V-5406, Vector-Best, Novosibirsk, Russia). The prevalence of infection (defined as the percentage of PCR-positive samples) was calculated for each administrative region (federal subject) and for individual cities. The 95% confidence intervals (95% CI) for the prevalence estimates were calculated using the Clopper-Pearson method (Clopper and Pearson 1934). The geographic distribution of positive samples was visualized on a map created using ArcGIS Pro 2.2 (Esri, USA), with final adjustments made in Adobe Photoshop CC.

Results

Screening of 6,444 canine blood samples by species-specific real-time PCR revealed *Dirofilaria repens* DNA in 485 specimens, corresponding to an overall prevalence of 7.52% (95% CI: 6.89–8.20%) across the study area. The parasite was detected in dogs from numerous administrative regions spanning the Southern, North Caucasian, and Volga Federal Districts. The detailed results, disaggregated by locality and aggregated by federal subject, are presented in Table 1.

Table 1. Infection of dogs with *Dirofilaria repens* in the Southern, North Caucasus and Volga Federal Districts of the Russian Federation

Geographic territory	Prevalence %	Sample size	CI, 95%
Volga federal district	**13.30%**	**1101**	**11.31–15.41**
Republic of Bashkortostan	**10.74%**	**149**	**6.26–16.85**
Blagoveshchensk	12.33%	73	5.8–22.12
Ufa	8.33%	72	3.12–17.26
Republic of Mari El	**48.39%**	**31**	**30.15–66.94**
Yoshkar-Ola	48.39%	31	30.15–66.94
Republic of Mordovia	5.56%	18	0.14–27.29
Saransk	5.56%	18	0.14–27.29

Geographic territory	Prevalence %	Sample size	CI, 95%
Nizhny Novgorod region	**11.71%**	**111**	**6.39–19.19**
Arzamas	20.00%	5	0.51–71.64
Nizhny Novgorod	10.78%	102	5.51–18.48
Penza region	**13.64%**	**22**	**2.91–34.91**
Penza	14.29%	21	3.05–36.34
Perm Territory	**19.23%**	**52**	**9.63–32.53**
Perm	17.65%	51	8.4–30.87
Samara region	**7.39%**	**203**	**4.19–11.89**
Novokuybyshevsk	6.06%	33	0.74–20.23
Samara	9.03%	144	4.89–14.94
Saratov region	**11.49%**	**235**	**7.71–16.27**
Saratov	7.87%	89	3.22–15.54
Engels	13.99%	143	8.76–20.77
Republic of Tatarstan	**20.48%**	**166**	**14.62–27.43**
Kazan	24.09%	137	17.2–32.13
Nizhnekamsk	7.14%	14	0.18–33.87
Ulyanovsk region	**11.36%**	**44**	**3.79–24.56**
Ulyanovsk	11.36%	44	3.79–24.56
Republic of Chuvashia	**12.50%**	**16**	**1.55–38.35**
Cheboksary	12.50%	16	1.55–38.35
Kirov region	**12.20%**	**41**	**4.08–26.2**
Kirov	12.20%	41	4.08–26.2
North Caucasian federal district	**3.39%**	**383**	**1.82–5.73**
Karachay-Cherkess Republic	**22.22%**	**9**	**2.81–60.01**
Cherkessk	22.22%	9	2.81–60.01
Stavropol region	**3.77%**	**212**	**1.64–7.3**
Georgiyevsk	11.11%	18	1.38–34.71
Zmeika	7.69%	13	0.19–36.03
Stavropol	4.03%	124	1.32–9.16
Chechen Republic	**16.67%**	**18**	**3.58–41.42**
Grozny	16.67%	18	3.58–41.42
Southern federal district	**6.57%**	**4960**	**5.9–7.3**
Republic of Adygea	**6.25%**	**64**	**1.73–15.24**
Maykop	6.25%	64	1.73–15.24
Astrakhan region	**1.04%**	**192**	**0.13–3.71**
Astrakhan	1.04%	192	0.13–3.71

Geographic territory	Prevalence %	Sample size	CI, 95%
Volgograd region	**11.06%**	**217**	**7.22–16.01**
Volgograd	10.49%	162	6.23–16.27
Volzhsky	13.73%	51	5.7–26.26
Krasnodar region	**7.83%**	**1865**	**6.65–9.14**
Anapa	5.71%	245	3.16–9.4
Armavir	4.17%	48	0.51–14.25
Vesely	30.00%	10	6.67–65.25
Gelendzhik	13.33%	30	3.76–30.72
Yeysk	16.67%	6	0.42–64.12
Korenovsk	20.00%	10	2.52–55.61
Krasnodar	7.55%	715	5.72–9.74
Novorossiysk	2.84%	141	0.78–7.1
Seversk	16.00%	25	4.54–36.08
Sochi	10.53%	285	7.22–14.69
Temryuk	9.80%	102	4.8–17.29
Yablonovsky	18.42%	38	7.74–34.33
Republic of Crimea	**4.94%**	**688**	**3.45–6.84**
Kerch	9.38%	64	3.52–19.3
Sevastopol	5.17%	290	2.92–8.39
Simferopol	4.74%	211	2.3–8.54
Yalta	4.55%	66	0.95_12.71
Rostov region	**6.00%**	**1934**	**4.98–7.15**
Azov	3.03%	66	0.37–10.52
Aksay	8.70%	23	1.07–28.04
Bataysk	5.36%	56	1.12–14.87
Volgodonsk	10.77%	65	4.44–20.94
Kamensk-Shakhtinsky	4.69%	64	0.98–13.09
Kuleshovka	7.23%	83	2.7–15.07
Novocherkassk	8.33%	384	5.77–11.56
Rostov-on-Don	5.27%	911	3.91–6.93
Samarskoye	12.20%	41	4.08–26.2
Taganrog	6.82%	88	2.54–14.25
Shakhty	1.47%	136	0.18–5.21
Total	**7.52%**	**6444**	**6.89–8.20**

To ensure statistical reliability and prevent misinterpretation of data from very small sample sets, localities that provided fewer than five samples (Orenburg region and Republics of Udmurtia, Dagestan, Ingushetia, Kabardino-Balkaria, North Ossetia-Alania, and Kalmykia), as well as those with no positive results (Luhansk and Donetsk People's Republics, Zaporozhie and Kherson regions and the Republic of Ingushetia), are included in the regional totals but are not listed as separate entries in the table. The spatial distribution of all localities from which *D. repens*-positive samples were identified is further illustrated in Fig. 1.

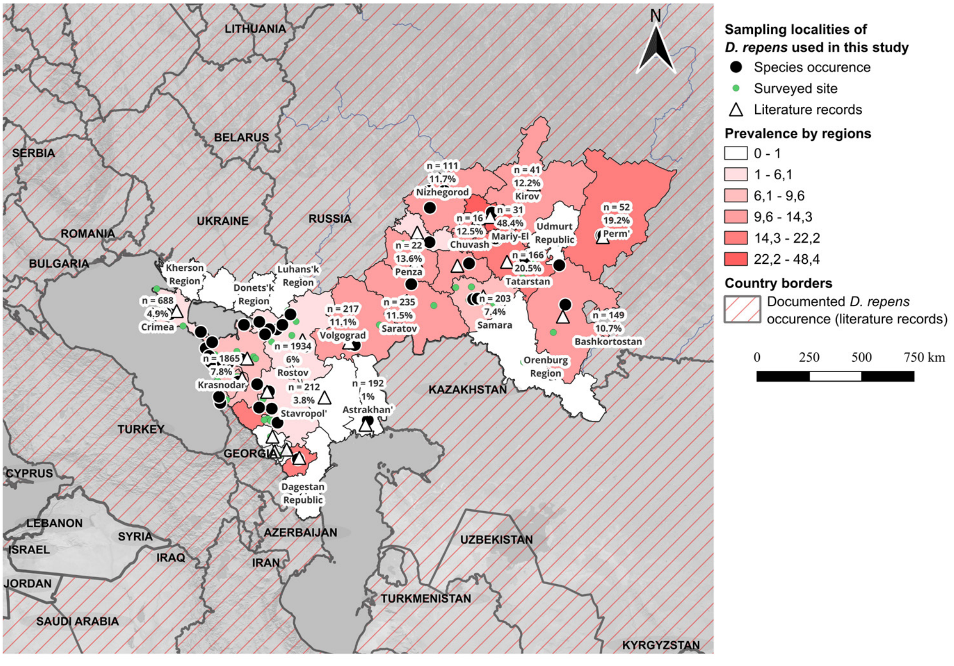

Figure 1. Distribution of *D. repens* in the Southern and Southeastern Russia.

Discussion

Southern Russia is a recognized endemic area for both *Dirofilaria* species; however, while the prevalence of *D. immitis* is well-documented, data on *D. repens* are comparatively scarce. Existing records are often limited to reports of microfilaremia without definitive species identification (Garkavi and Mikhno 2002; Garkavi and Medvedev 2004; Medvedev 2007; Temichev 2016). The principal finding of our study is the unequivocal molecular confirmation that *D. repens* is endemic not only in the historical southern foci of Russia but has established stable populations in the Volga River basin and the foothills of the Western Urals.

The Crimean Peninsula has been recognized as part of the *Dirofilaria* range since 1956 (Kadenatsii 1958). Our data confirm a high prevalence of *D. repens* in dogs across the peninsula, with infection rates ranging from 4.55% to 9.38% in Kerch, Sevastopol, Simferopol, and Yalta. These results align with earlier monitoring that reported a similar prevalence range (6.62–7.37%) in local dogs, although species-level identification was not performed in that study (Volozhaninova and Zhurbenko 2014). The presence of the parasite is further supported by a documented autochthonous human case in the region (Korolev and Romashova 2005).

Our study also confirmed *D. repens* infections along the Black Sea and Azov Sea coasts, specifically in Krasnodar and Rostov regions. The prevalence in Krasnodar region was 7.63% (n=1,822), and in the Republic of Adygea, it reached 11.06% (n=217). While previous studies in this area have reported Dirofilariasis in dogs, jackals, and cats, these findings were typically based on microfilaremia without species confirmation. A report of subcutaneous *Dirofilariae* in a badger (Kravchenko 2015) requires cautious interpretation, as the morphologically similar nematode *Filaria martis* is more characteristic of this host and no molecular data were provided. Our molecular findings are consistent with a recent PCR-based confirmation of *D. repens* in dogs from Sochi (Volgina et al. 2022).

Human Dirofilariasis cases have also been reported in the Donbas region, although detailed epidemiological information – such as specific years, case numbers, and confirmation of autochthonous transmission – is often lacking, as data are typically aggregated for Ukraine as a whole. For instance, *D. repens* has been documented in Dnipropetrovsk region since 2003 (Petlenko et al. 2010; Nagorny and Krivorotova 2010), and several local human cases have been described (Bityukov and Chernov 2020; Zhurilo et al. 2012), with an estimated incidence of 4 cases per million people per year (Antonova et al. 2022). However, no large-scale studies using molecular methods or systematic necropsy data from animals have been conducted in this area. Given that these territories border known endemic areas (Rostov and Krasnodar regions) and share similar climatic conditions, it is highly likely that they constitute part of the range for *Dirofilaria* spp., particularly *D. repens*.

Rostov region is a historically significant and recognized endemic focus for *Dirofilaria*; the first record of a subcutaneous filariid in Russia was reported from this region by Gurvich in 1929. Contemporary studies confirm a high prevalence of both *D. immitis* and *D. repens* in the region (Nagorny et al. 2012; Konyaev 2019b). For example, one study found an infection rate of 31.5% in domestic dogs in the Tsimlyansky District, although the microfilariae were identified only to the genus level (Nagorny and Krivorotova 2016). Another study used morphometry to identify *D. repens* microfilariae in cats (Krivorotova and Nagorny 2015), highlighting the parasite's presence in other hosts. Our results, based on species-specific PCR, provide molecular confirmation of endemic *D. repens* transmission, with 116 positive samples (6.0%) detected across 16 localities in Rostov Region.

The North Caucasian republics such as Abkhazia, Armenia, Azerbaijan, and Dagestan, have historically been considered part of the *Dirofilaria* range (Kozlov

1977). Our molecular data, while based on limited sample sizes in some areas, provide new insights into the current distribution. We confirmed *D. repens* DNA in two of nine dogs from Cherkessk (Karachay-Cherkessia) and in eight samples from Stavropol region (cities of Georgievsk, Stavropol, and Zmeika). In contrast, no positive samples were detected in our analyses from North Ossetia (n=112) or the Republic of Ingushetia, despite the latter being previously listed as endemic without supporting evidence (Durnova et al. 2020). Our findings in Stavropol region are consistent with earlier reports of the parasite in the region during examinations of animals (Kolchenko 2009; Orobets et al. 2013; Mescheryakov 2008, Kudinov and Annikova 2002; Medvedev 2007; Temichev 2016, Kolesnikov and Popov 2012).

In Chechnya republic, three of eighteen canine blood samples were PCR-positive, corroborating local reports of numerous infected dogs (Baisarova 2021). For Dagestan republic, while we did not test samples, multiple necropsy-based studies have reported a high prevalence of *D. repens* (up to 20%) in both wild canids and domestic dogs (Trunova 2008; Kabardiev et al. 2017, Gadzhiev et al. 2010, Gadzhieva et al. 2021), supported by a human case (Morokov 2017). Similarly, in Kabardino-Balkaria, our PCR screening of 32 dogs was negative, but necropsy studies have repeatedly identified subcutaneous *Dirofilariae* in a significant proportion of dogs (Zhuravlev 2009; Gazayev et al. 2020). A previous larvoscopy study in Karachay-Cherkessia also yielded negative results (Krivorotova 2015). In Vladikavkaz (North Ossetia), a high prevalence of Dirofilariasis (28%) was reported in dogs, though species identification was not confirmed, and local human cases attest to autochthonous transmission (Bagaeva and Bocharova 2008; Bagaeva and Khatkhakumova 2016).

The Lower Volga Region, encompassing Volgograd and Astrakhan Regions and the Republic of Kalmykia, is described in the literature as part of the *Dirofilaria* range. In Volgograd and Volzhsky, we found a prevalence of 11.06% among 217 canine blood samples. Several studies in Volgograd Region have reported *D. repens* infections in dogs, with prevalence ranging from 0.46% to 10.9% (Ignatova 2007; Kamenov and Shinkarenko 2019; Vedeneev 2004; Romanova et al. 2013; Ermilov et al. 2010). A local human case has been registered (Faizrakhmanov et al. 2009). In Kalmykia, studies detected *D. repens* microfilariae with prevalence reaching 33.3% (Arkhipova and Arkhipov 2004; Bakina et al. 2013; Nagorny and Krivorotova 2010; Varlamova and Arkhipov 2016). In Astrakhan Region, we detected *D. repens* DNA in 1.04% of samples (n=192), whereas other studies reported microfilaremia reaching 92.2% in Astrakhan city and 7.8% in Kamyzyaksky District, though species identification was not performed (Arakel'yan et al. 2017). *D. immitis* and *D. repens* were found during necropsies of large dog breeds (Bogdanova and Boyko 2007) and in dogs and cats (Bogdanova 2008). At least 81 human cases have been reported (Arakel'yan et al. 2021). The discrepancy with our data is likely explained by the higher local prevalence of *D. immitis* – our unpublished data show 25.5% of dogs in Astrakhan city are infected with *D. immitis*.

Further upstream the Volga River, in Saratov Region, 27 of 235 samples were PCR-positive. *D. repens* was first registered here in 2002 (Kudinov and Annikova 2002) and was noted in a human case (Durnova et al. 2020). In Ulyanovsk Region, microfilaremia was attributed to *D. immitis* without species confirmation (Shchegolenkova et al. 2014). We found positive samples in Penza (3/22), Samara (15/203), and Ulyanovsk (5/44) Regions. Human cases are reported in Penza (State report 2018), and microfilaremia was noted in dogs (Tabakaeva et al. 2015). Samara Region had not been previously investigated for *Dirofilaria* distribution.

Rare but regular occurrences are documented in the eastern and northeastern parts of the Russian Plain, specifically in Nizhny Novgorod and Kirov Regions, as well as the Republics of Mordovia, Mari El, Chuvashia, and Tatarstan. In Nizhny Novgorod Region, PCR analysis detected *D. repens* DNA in 13 of 111 canine blood samples. The presence of both *Dirofilaria* species in mosquitoes from this region had been previously established (Rakova 2013). One documented case likely involved the importation of *D. repens* into Nizhny Novgorod via service dogs that had been stationed in the Chechen Republic; the parasites were identified during necropsy, though the possibility that these animals had visited other regions cannot be ruled out. In the Republic of Mordovia, only one of 18 dog blood samples from Saransk was PCR-positive. This single finding, like a previous sample from the same city (Konyaev 2019a), is insufficient to confirm the stable presence of *D. repens* in the region, as travel history for the animals was unavailable. In the Mari El Republic, sample analysis from Yoshkar-Ola revealed a high prevalence of subcutaneous Dirofilariasis reaching 48.39% (n=31). While *Dirofilaria* spp. have been reported in dogs from Mari El, the publication in question does not specify the species or clearly state whether the service dogs had traveled to other endemic zones (Malinin et al. 2014). Since 2009, nine autochthonous human cases of subcutaneous Dirofilariasis have been reported in the republic (Bulatova et al. 2017). In the Chuvash Republic, two of 16 samples from Cheboksary tested positive in species-specific PCR. *Dirofilaria* spp. are noted in the parasitic fauna of dogs in Cheboksary, but again, the travel history of these animals was not provided (Kosyaev and Farkhutdinova 2012). Isolated cases of human infection with the subcutaneous species have also been recorded in Chuvashia (Nikolaeva et al. 2022). In Tatarstan, our analysis of samples from Kazan (n=166) demonstrated a high prevalence (20.48%) of dogs with *D. repens*. Previously, only *D. immitis* had been listed in the parasitic fauna of dogs in Tatarstan (Timerbaeva et al. 2012; Konyaev 2019b). However, local human cases of subcutaneous Dirofilariasis have been described by physicians at a neurosurgical clinic in Kazan (Pichugin et al. 2022). In Kirov Region, both *Dirofilaria* species have been regularly identified in wild and domestic canids (Maslennikova and Perletskaya 2012; Byakova and Pilip 2013; Byakova et al. 2017; Rassokhin et al. 2018; Bakina et al. 2013). These were reported as isolated cases, and the animals' travel history was either unknown or they were known to have been imported. Local human cases have also been reported (Belousova et al. 2012). In our study, five of 41 blood samples from dogs in the city of Kirov were PCR-positive.

The regions of the Western Urals – the Republics of Bashkortostan and Udmurtia, Perm region, and Orenburg Region – appear to lie within the current range of *D. repens* and are most likely also subject to regular introduction of cases imported from other regions. In Bashkortostan, the prevalence of dogs was 10.74% (n=149; samples from Blagoveshchensk, Ishimbay, Neftekamsk, and Ufa). In Orenburg Region, neither of the two samples obtained tested positive. Previously, *Dirofilaria* was detected in two humans in Orenburg Region, though the patients' travel history is unknown (Han et al. 2022). According to data kindly provided by Vitaly Vladimirovich Paramonov, dogs infected with *D. repens* arrived in Bashkortostan from multiple locations, including Perm, Nizhnevartovsk, Chelyabinsk, Samara regions, Oryol, Saransk (Mordovia), Astrakhan, Langepas (Khanty-Mansi Autonomous Okrug), Serov (Sverdlovsk Region), Saraktash (Orenburg Region), Yakutsk, Ulyanovsk, Arzamas, Tambov, Izhevsk, and Kostroma. Infections were also detected in local animals with no history of leaving the republic. Part of this information is presented in his dissertation (Paramonov 2014). The cities of Perm and Kondratovo are characterized by a high prevalence of subcutaneous Dirofilariasis in dogs, reaching 19.23% (n=52). In Perm region, cases of both *D. repens* (n=15) and *D. immitis* (n=7) were registered among 562 service dogs, confirmed by serological methods and microscopy with Romanowsky-Giemsa staining; however, these animals were likely taken to endemic foci (Sogrina 2017). *D. repens* nematodes were discovered during the pathological autopsy of three dogs, though their origin was not specified in the publication (Ivanov et Sivkova 2022). Sivkova T.N. and Zimenkov V.A. described one case of subcutaneous *Dirofilaria* detection in a badger during a helminthological examination of the carcass (Sivkova et Zimenkov 2016). However, this finding was not accompanied by a morphological description or other confirmatory data and could pertain to *F. martis*. None of the 11 canine blood samples we examined from Udmurtia tested positive for *D. repens* via PCR. Nevertheless, no fewer than 16 cases of *Dirofilaria* detection in humans have been reported in this region (Bochkarev 2014).

In reviews concerning the distribution of both cardio-pulmonary and subcutaneous Dirofilariasis, the territory of Russia is frequently depicted as a continuous endemic zone (Capelli et al. 2018; Noack et al. 2021). This approach does not reflect the actual situation, given the country's vast area and the diversity of its ecological conditions. Our findings significantly refine the distribution of *D. repens*, demonstrating its stable presence not only in the southernmost regions, but also in the Volga Region and the Southern Urals. The prevalence of subcutaneous Dirofilariasis in dogs in Perm and Tatarstan territory is comparable to rates in Southern European countries like Italy, Spain, and Greece (Fioretti et al. 2003; Tarello 2002, 2011; Papazahariadou et al. 1994; Cancrini et al. 2000; Traversa et al. 2010, Scala et al. 2013). A significant prevalence is also noted in the Southern federal district, where its level is similar to that in some countries of the Middle East and North Africa (Izenour et al. 2025). For a complete understanding of the *D. repens* range, it is necessary to consider the situation in neighboring countries. In Ukraine, the issue has not been studied sufficiently; however, a significant number of human cases are reported (Bajer et al. 2016). According to the latest large-scale study, the prevalence in dogs reaches 16–17% in cities such as Berdychiv, Lviv, Kharkiv, Sumy, and Zvenyhorodka (Pękacz et al. 2025). Similar canine prevalence rates were observed by us in regions of the Volga Region, specifically Perm Krai, and in some cities of Krasnodar Krai, whereas on the Crimean Peninsula, the prevalence in dogs is substantially lower. In Kazakhstan, in the city of Uralsk, located in the Caspian Depression near the border with Russia's Volga Region, a high prevalence of *D. repens* in dogs (29.4%) has been recorded (Nametov et al. 2025), which significantly exceeds the rates in the cities of the Volga Region. Interestingly, data for another city in the Caspian Depression, Astrakhan, vary; a *D. repens* prevalence of only 1% was reported there. The countries of the South Caucasus region remain understudied regarding the distribution of *D. repens*. In Armenia, only cases of *D. immitis* with high prevalence (31.2%) are reported, which has been the sole species detected in recent years (Kryazhev and Slobodyanik 2019; Zykova et al. 2023). For Azerbaijan, there are also reports only of the presence of *D. immitis*; however, these studies utilized the Knott's method, and differential diagnosis was performed solely for *Acanthocheilonema reconditum* based on microfilariae size, which is not a reliable method for distinguishing *D. immitis* from *D. repens* (Javadi et al. 2011). Further south, *D. repens* is found in Iran, where the prevalence in dogs reaches 26% (Pedram et al. 2019), as well as in Iraq, Israel, Kuwait, Saudi Arabia, Turkey, and the United Arab Emirates (Izenour et al. 2025). Thus, the range of *D. repens* in Europe extends from the countries of Southern and Eastern Europe across the European part of Russia to the Southern Urals. In conclusion, our study fundamentally refines the map of *D. repens* distribution in Eurasia. We provide robust molecular evidence of its entrenched sustained presence throughout the European part of Russia, from the Black Sea to the Urals. The high prevalence in the Volga Region, comparable to some Mediterranean countries, underscores its significant zoonotic potential in these newly established foci. Furthermore, our synthesis of data from neighboring countries suggests that the Greater Caucasus Mountain Range acts as a major biogeographical barrier, delimiting the southern boundary of the parasite's continuous European range. Future studies should focus on monitoring the dynamics of this expansion and the environmental drivers facilitating it.

A key limitation of this study stems from the lack of accompanying clinical history for the submitted blood samples. Without data on the dogs' travel history within the country, some of the *Dirofilaria* infections detected could have been acquired in regions other than where the samples were collected. The sampling methodology itself, reliant on veterinarians submitting samples introduces a potential selection bias. The PCR methodology, while chosen for its high specificity, also presents inherent constraints for prevalence studies. Its success is contingent on a sufficient concentration of microfilariae in the bloodstream (at least 1 per 200 μL of blood). It is acknowledged that other ante-mortem detection methods have significant drawbacks; the Knott's test lacks species specificity and is less sensitive than PCR for low-level infections. Therefore, despite its limitations, PCR remains a suitable and powerful tool for large-scale surveillance of subcutaneous Dirofilariasis, offering a balance of sensitivity, specificity, and throughput that is currently unmatched by alternative approaches. However, these limitations are offset primarily by the large sample sizes used in this study, which exceed those of comparable research (e.g., studies focusing on the United States (Smith et al. 2022) and essentially miss only a certain percentage of infection. It is important to note that, in this context, the emphasis is on confirming presence rather than asserting absence. The detection of parasite DNA in 7.52% of samples, with notably high prevalence in major urban centers strongly suggests these areas are endemic for the parasite.

Despite the substantial body of research on the distribution of subcutaneous *Dirofilaria* in Southern and Southeastern Russia, knowledge gaps persist in certain districts. Special focus should be placed on understudied regions, particularly those bordering known high-risk areas, where previous research may not have utilized molecular genetic techniques for definitive species identification. The methodology for sampling definitive hosts must be designed to systematically collect data on animal travel history. Sensitive molecular methods, such as species-specific PCR, should be an integral part of future research. While morphometric analysis of larvae is highly dependent on researcher expertise and suffers from challenges in standardizing preparation techniques, and while sequencing of marker genes offers a more objective, sensitive, and specific alternative, the latter remains costly. Therefore, species-specific PCR represents a sensitive and cost-effective alternative, with less stringent sample quality requirements.

Conclusion

The present study, through extensive species-specific PCR-screening, demonstrates that the endemic range of *Dirofilaria repens* in Russia is significantly more expansive than previously recognized. We confirm the parasite's stable establishment not only in historical southern foci (Krasnodar Krai, Rostov Oblast, Republic of Crimea) but also reveal its entrenchment in the Volga River basin (Volgograd, Astrakhan, Tatarstan) and the Southern Urals. This pattern signifies a substantial northward and eastward range expansion, likely facilitated by climate change and increased anthropogenic movement of hosts.

Our findings also highlight critical surveillance gaps. Regions such as Donbas, Kherson, Zaporozhie, and the Republic of Ingushetia possess suitable ecological conditions yet lack comprehensive data, underscoring an urgent need for targeted studies there. Future efforts to map the parasite's distribution must integrate veterinary surveillance, medical case reporting, and entomological surveys, employing species-specific molecular methods to avoid misidentification.

Beyond mapping, our work establishes *D. repens* and *D. immitis* as exemplary models for studying parasite range dynamics in the 21st century. We propose that future research should focus on molecular genetic analyses to characterize the haplotype diversity of these parasites. Such studies will be pivotal in reconstructing the routes of dispersal, identifying the origins of new foci, and understanding the evolutionary forces shaping their expanding ranges. This knowledge is crucial for developing predictive models and proactive public health strategies against this and other vector-borne zoonoses in a changing world.

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