

Dirofilaria repens and *Dirofilaria immitis* DNA findings in mosquitoes in Germany: temperature data allow autochthonous extrinsic development

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Abstract After the repeated demonstration of *Dirofilaria repens* infections in German dogs, *D. repens* and *Dirofilaria immitis* DNA was detected in mosquitoes trapped in 2011, 2012 and 2013 in northeastern and southwestern Germany within the framework of culicid monitoring projects. As temperature is the most important factor dictating the extrinsic development of dirofilarial larvae in the potential vector, data of the German Weather Service (DWD) were analysed for the regions where the positive mosquitoes had been collected. Based on the mean daily temperatures recorded by weather stations most closely to the collection sites of the mosquitoes, it can be concluded that the mosquitoes were trapped in time periods that allowed for the completion of the developmental cycle of the worms in the mosquitoes and a subsequent transmission to a vertebrate host. The results of this study confirm the principal climatic suitability of certain German regions for the establishment of natural dirofilarial transmission cycles. Moreover, the theoretical climatic considerations, together with findings of *D. repens* infections in German dogs

and mosquitoes, strongly suggest that the continuing spread of at least *D. repens* from its traditional habitats in the Mediterranean has reached southwestern and northeastern Germany.

Keywords Dirofilariosis · Heartworm · *Dirofilaria repens* · Mosquito · Climate change · Germany

Introduction

The recent reports from Germany, Austria and The Netherlands (Hermosilla et al. 2006; Duscher et al. 2009; Overgaauw and van Dijk 2009) as well as from Hungary, Poland, Slovakia or Ukraine of autochthonous infections of *Dirofilaria repens* (Szénási et al. 2008; Miterpáková et al. 2010; Cielecka et al. 2012; Sałamatin et al. 2013; Víchová et al. 2014), the causative agent of subcutaneous dirofilariosis in dogs and humans, demonstrate the spreading of the parasitic worm from endemic regions in the Mediterranean to northern and eastern Europe. Although less frequently, *Dirofilaria immitis*, the agent of canine heartworm disease, has also repeatedly been diagnosed in eastern Europe, with the first case of an autochthonous infection in a dog being reported from Poland in 2012 (Jacso et al. 2009; Iglódyová et al. 2012; Mircean et al. 2012; Świątalska and Demiaszkiewicz 2012; Víchová et al. 2014). *D. immitis* infections have previously been known in Germany from dogs with a travel history to southern Europe only (Pantchev et al. 2011).

While *D. repens* has a low pathogenic potential in dogs, *D. immitis* causes severe cardiovascular disease in canids and felids. As zoonotic pathogens, both *D. repens* and *D. immitis* can also trigger a bouquet of clinical syndromes in humans as aberrant hosts (Simón et al. 2012). However, due to the lack of generalized knowledge of the disease aetiology among physicians, associated with the problem that reliable diagnostics do

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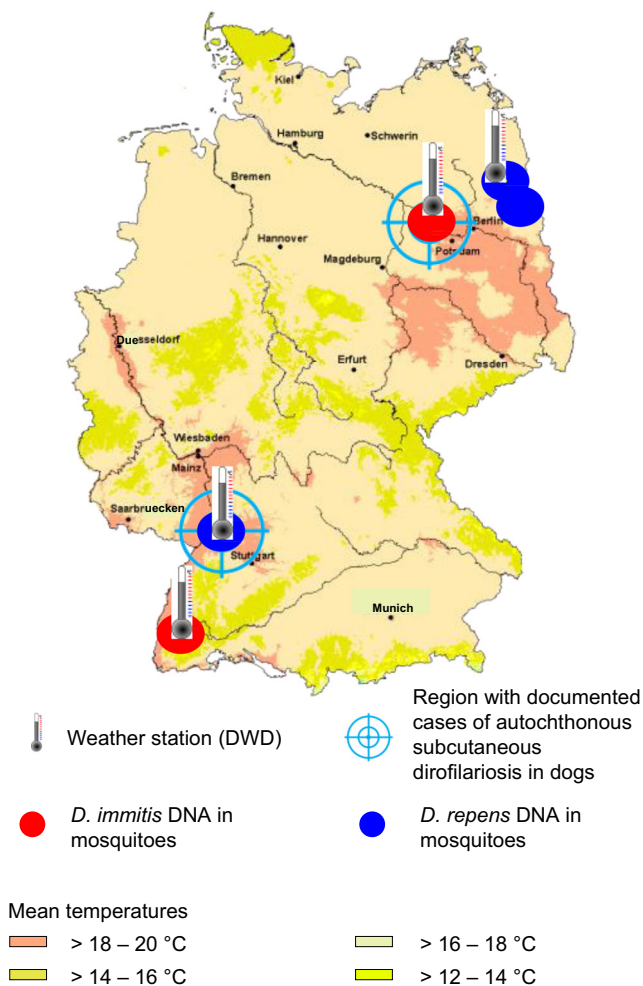


Fig. 1 Map of Germany showing the geographic origin of mosquitoes tested positive for *D. repens*/*D. immitis*, the regions with documented cases of autochthonous subcutaneous dirofilariosis in dogs and the locations of the weather stations utilized in this study. The background colour of the map displays the long-term average for mean daily temperatures of the months of July (30-year time period from 1961 to 1990) generated by the DWD (Deutscher Klimaatlas)

not exist, cases of human dirofilariosis are often misdiagnosed as other disease entities (Genchi et al. 2011b; Simón et al. 2012).

Dirofilariae are transmitted by various mosquito species (Diptera, Culicidae) widely distributed across Europe. In 2011, 2012 and 2013, *D. immitis* and *D. repens* DNA was found in mosquitoes collected in Germany (Czajka et al. 2014; Kronefeld et al. 2014) while *D. repens* DNA was recently demonstrated in mosquitoes in Slovakia (Bocková et al. 2013). In all cases, it is unclear whether the sources of infection were dogs imported from endemic areas in the Mediterranean or native host species, the latter suggesting a local transmission cycle.

As the development of the worms in the arthropod vector is strictly temperature-dependent, transmission is only possible under appropriate climatic conditions. Analyses of the climate

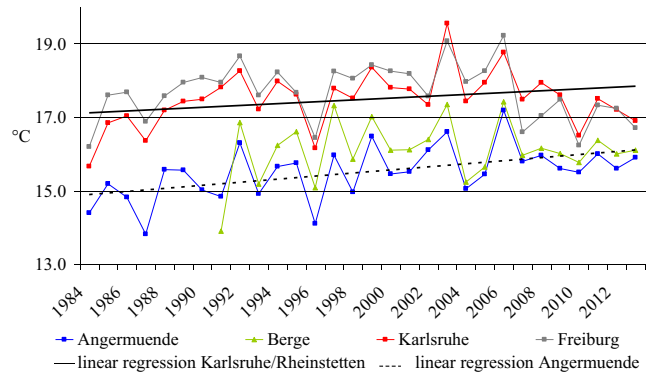


Fig. 2 Daily mean temperatures (°C) from May 1 to October 15, 1984 to 2013, for the four weather stations analysed

in the context of global warming have demonstrated that these conditions may apply in Europe as far north as latitude 56° N and as far east as longitude 39° E, which fully includes Germany (Genchi et al. 2011a; Sassnau and Genchi 2013).

It has been shown that the development of *D. immitis*/*D. repens* in mosquitoes requires 8–13 days at 28–30 °C, 10–11 days at 26 °C and 16–20 days at 22 °C (Webber and Hawking 1955; Fortin and Slocombe 1981; Genchi et al. 2009). According to Fortin and Slocombe (1981), the temperature-dependent extrinsic incubation period can be described by *Dirofilaria* development units (DDU), which calculates as the daily sum of degrees centigrade above a 14 °C threshold (for $T_{\text{mean}} \geq 15$, $DDU = T_{\text{mean}} - 14$). Extrinsic development is completed with the presence of infective larvae in the mosquito if the sum is at least 130 DDU within 30 days following infection [$\sum DDU$, for $i=(i_1, i_2, \dots, i_{30})$], corresponding to the mosquito life span maximum (hereafter referred to as ‘130 DDU₃₀’) (Fortin and Slocombe 1981; Genchi et al. 2005, 2009, 2011b) and irrespective of temperature fluctuations below the 14 °C threshold during this period (Venco et al. 2011).

To examine to what extent the temperature may have influenced the development of *D. immitis* and *D. repens* in those mosquitoes that were recently trapped in Germany and

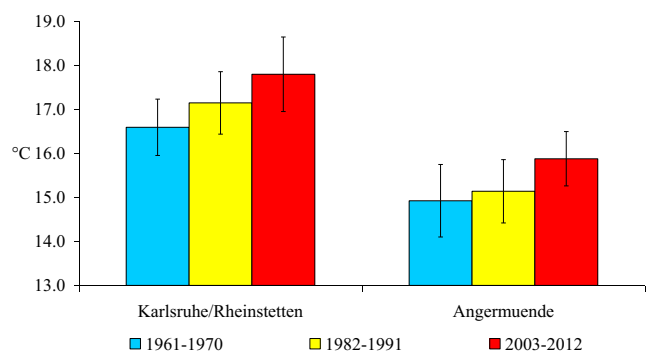


Fig. 3 Ten-year averages of mean daily temperatures for the time period May 1 to October 15, 1961–1970, 1982–1991 and 2003–2012 for the weather stations Karlsruhe/Rheinstetten and Angermuende

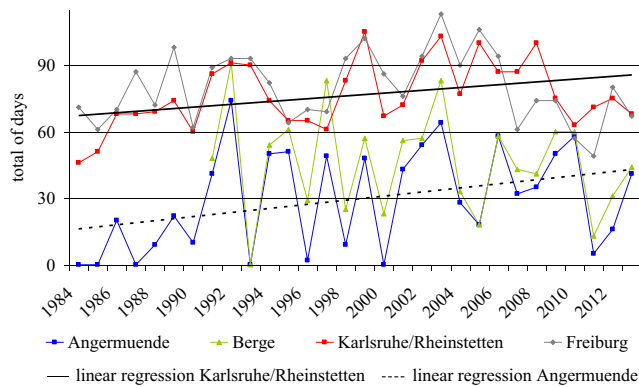


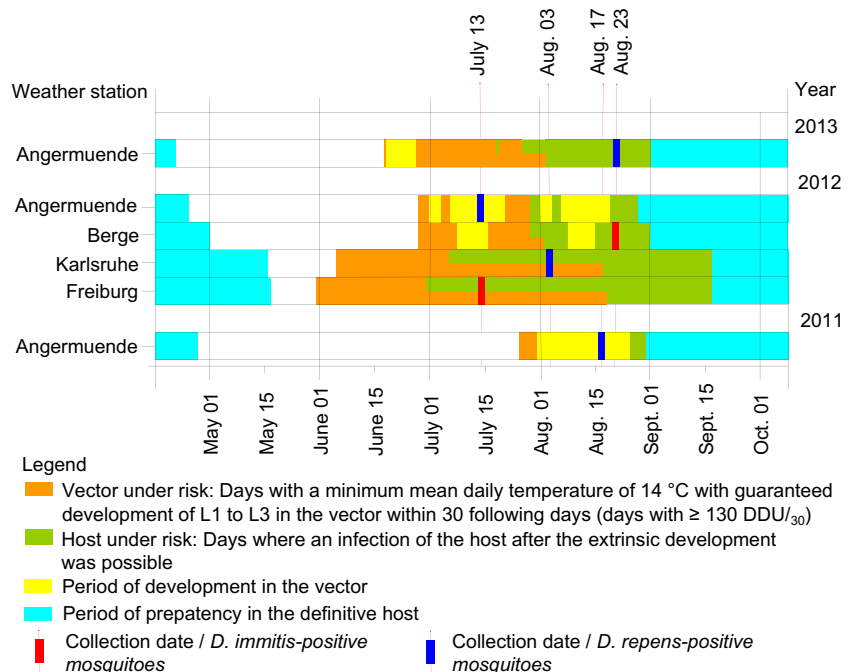
Fig. 4 Total of days with at least 130 DDU₃₀ from 1984 to 2013

whether the temperature conditions were appropriate to allow an establishment of the dirofilarial species in Germany, local temperature data were correlated to the extrinsic incubation period of the worms.

Materials and methods

Average daily temperature data were obtained from the German Weather Service (DWD, www.dwd.de) weather stations Angermuende [53.0° N, 13.6° E] (1984–2013) and Berge [52.4° N, 12.5° E] (1991–2013) in the German federal state of Brandenburg (northeastern Germany) and from the weather stations Karlsruhe/Rheinstetten [49.0° N, 8.2° E] (1984–2013) and Freiburg [48° N, 7.8° E] (1984–2013) in the federal state of Baden-Wuerttemberg (southwestern Germany). These weather stations are operated close to the

Fig. 5 Dates of trapping of the *Dirofilaria*-positive mosquitoes and potential development of different stages of *D. immitis* and *D. repens* in the vector and the host



locations where the *Dirofilaria* DNA-positive mosquitoes were collected in 2011, 2012 and 2013 (Fig. 1). The mean daily temperatures from May 1 throughout October 15, assumed as the potential local mosquito activity season, were used to simulate the temperature conditions at the mosquito-collection sites and to calculate the DDU.

The sum of days per year allowing completion of extrinsic development in a 30-day period (days guaranteeing at least 130 DDU₃₀) were determined for each weather station and plotted in a line plot. Linear regression lines for the total of days with at least 130 DDU₃₀ were drawn for the minimum and maximum lines only.

Pearson’s two-tailed correlation test (SPSS 11.8 for Windows) was used to analyse the weather station data with respect to the time period guaranteeing at least 130 DDU₃₀. Two-tailed Pearson’s levels of significance ($p < 0.05$, $p < 0.01$ and $p < 0.001$) were calculated for the correlations. The descriptive statistical analysis was performed using Microsoft Excel® for Windows (Microsoft Corp., Redmont, USA).

‘Risk periods’, where infections of the vector and the host, respectively, would have been possible, were identified. The results were plotted in a modified stacked bar-horizontal plot for the years and weather stations, deputizing the mosquito-collecting-years and places only.

Results

Analyses of weather data from 1984 to 2013 revealed a rising trend for the mean daily temperatures at all four weather stations examined, with the temperatures of the Baden-Wuerttemberg

weather stations Karlsruhe/Rheinstetten and Freiburg being constantly higher by about 2 °C as compared to the Brandenburg stations Angermuende and Berge (Fig. 2). The long-term average mean daily temperatures for Angermuende show a rise from 14.9 °C for the period 1961–1970 to 15.9 °C for the period 2003–2012. Similarly, an increase of 1.2 °C (from 16.6 to 17.8 °C) is observed during the 10-year period for the Karlsruhe/Rheinstetten weather station (Fig. 3).

As a result of elevation in temperatures over time, the total number of days per year with at least 130 DDU₃₀ also increased between 1984 and 2013 (Fig. 4). A high level of two-tailed significance ($p < 0.001$) was given when the graphs of the Brandenburg weather stations ($r = 0.92$) and the Baden-Wuerttemberg weather stations ($r = 0.69$) were compared.

In Karlsruhe/Rheinstetten, as a representative of southwestern Germany, there was no single year with less than 130 DDU₃₀ during the 30-year observation period. Likewise, the requirement of at least 130 DDU₃₀ was fulfilled in 25 out of 30 years in Angermuende as a representative of northeastern Germany (Fig. 4).

The *Dirofilaria*-positive mosquitoes found in Germany between 2011 and 2013 were all trapped in July or August of the various years. According to the weather station data, the temperature conditions of the respective days of trapping were in a time period allowing the extrinsic development of *Dirofilaria* larvae in the arthropod vector (Fig. 5).

Discussion

The results of this study demonstrate that the mean daily temperatures in the considered areas were suitable for completion of the extrinsic development of *D. repens* and *D. immitis* in the years and locations when and where the infected mosquitoes were trapped (Fig. 5). In two cases, Angermuende 2013 and Berge 2012, the larvae could have been in the infectious stage, while in two other cases, Karlsruhe 2012 and Freiburg 2012, the larvae could have still been developing or already in the infectious stage. A completion of development was possible under these circumstances in all cases. However, in two other cases, Angermuende 2011 and 2012, the larvae probably would not have been fully developed. These theoretical data cannot be correlated with experimental data since the procedure of dirofilarial examination of the mosquitoes only allowed a statement on presence or absence of larvae and was not targeted at discerning the developmental stage.

Notwithstanding, the repeated findings of autochthonous *D. repens* infections in the same kennel of dogs in 2007 and 2012 (Sassnau et al. 2009; Sassnau et al. 2013) together with the finding of *D. repens*-positive mosquitoes (Czajka et al. 2014) in three subsequent years in the same region of north-eastern Germany suggest that a natural transmission cycle has

already established in parts of the federal state of Brandenburg.

Likewise, four autochthonous cases of canine cutaneous dirofilariosis were reported from dogs in 2007 near Karlsruhe in southwestern Germany (Hermosilla et al. 2006; Pantchev et al. 2009). The finding of dirofilarial DNA in the collected mosquitoes in the same region indicates that an endemisation might also have taken place in parts of southwestern Germany.

Besides continuously increasing mean temperatures (DWD 2013), pet travelling and import of pets from endemic areas are important factors contributing to the spread of *D. immitis* and *D. repens* into areas previously free of dirofilariae (Genchi et al. 2011a; Morchón et al. 2012). In the scenario of a spread, the difficulties in correct diagnosis, the usual lack of symptoms in the case of a *D. repens* infection and the long patency period may facilitate an unrecognized accumulation of dirofilariae in the vertebrate host population and establishment of the pathogens as probably has recently happened in Germany.

Conclusions

This study suggests that *D. immitis* and *D. repens* might probably have established in several areas in Germany which are now at risk of endemisation of canine and human dirofilariosis. To further elucidate the situation epidemiologically, i.e. to confirm or reject establishment and, as the case may be, to determine distribution areas, further serological studies in dogs are needed. A surveillance of the prevalence of dirofilariosis in wild carnivores, serving as worm reservoirs, could be helpful in the understanding of the spread of this emerging zoonosis.

With regard to human and veterinary health, physicians, veterinarians and public health personnel should be sensitized against autochthonous *Dirofilaria* infections, and recommendations for diagnostic, therapeutic and prophylactic procedures have to be in place.

Competing interests The authors declare that they have no competing interests.

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