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## Stable Transmission of *Dirofilaria repens* Nematodes, Northern Germany

**To the Editor:** Dirofilariosis caused by infection with the filarial nematode *Dirofilaria repens* is considered an emerging zoonosis in Europe (1). The main reservoirs for this parasite are dogs and other carnivores. As is the case for other filarial species, mosquitoes transmit infectious third-stage larvae, which develop into fertile adults in their definitive vertebrate hosts. Humans may become infected as aberrant hosts, and, in most cases, these worms remain infertile (1,2). Infections in humans usually manifest as subcutaneous nodules, which are caused by developing worms that are trapped by immune mechanisms (2). Subcutaneous migration of a worm may result in local swelling. Severe clinical manifestations have also been reported and may affect various organs, including the brain, lung, and eye (2,3). Eye infections are found in particular during the migratory phase of the parasite.

Transmission of *D. repens* occurs in various regions of the Old World, including Europe, Africa, and Asia. The primary areas in Europe to which *D. repens* is endemic are countries of the Mediterranean region, where warm temperatures enable development of infectious third-stage larvae in mosquitoes. However, during the past decade, several autochthonous cases of canine and human dirofilariasis have been reported from countries farther north, including Austria, the Czech Republic, and Poland (4–6). Several factors might be responsible for this spread to the north, including climate change and increased translocation of dogs from southern to central Europe.

Until recently, central Europe, including Germany, was not considered

a region to which *D. repens* was endemic. However, a survey in 44 hunting dogs from the Upper Rhine region showed that 3 animals were positive for *D. repens* microfilariae (7). In addition, testing of a kennel of sled dogs located in the federal state of Brandenburg, near Berlin, found 8 of 28 animals were infected with the parasite (8).

To determine whether local transmission of *D. repens* is taking place in Germany, we retrospectively analyzed 74,547 mosquitoes from a large-scale German mosquito-borne virus surveillance program for the presence of filarial DNA. Mosquitoes were collected during the main animal-trapping seasons (May–September) 2011 and 2012 by using carbon dioxide-baited encephalitis virus surveillance or gravid traps (9). Mosquitoes were collected from 55 trapping sites located in 9 federal states in the southwest and northeastern parts of Germany (Figure), frozen at  $-70^{\circ}\text{C}$ , and transported to the laboratory at the Bernhard Nocht Institute, where they were classified morphologically to the species level. Up to 25 mosquitoes of the same species from individual collection sites were subsequently combined to create a total of 4,113 pools. These pools comprised a broad range of ornithophilic and mammalophilic mosquitoes common in Germany; *Culex* spp. and *Aedes vexans* were the most abundant species.

Nucleic acids were extracted from each mosquito pool, and the presence of filarial DNA was determined by PCR using a set of generic primers that amplify DNA from a broad range of filarial species, including those affecting birds and other animals (9). A total of 1,050 (25.5%) of the 4,113 pools were found to be positive for filarial DNA. To further determine if any of these pools contained DNA from *D. repens*, we analyzed all 1,050 pools by PCR using the *D. repens*-specific primers 5'-GATGGCGTTTCCTCGTG-3' and 5'-GACCATCAACACTTAAAG-3'.



Figure. Origin of mosquito samples analyzed for infection with *Dirofilaria repens*, by federal state, Germany, May–September 2011 and 2012 ( $n = 74,547$ ). Black dots indicate collection locations of mosquitoes that tested positive for *D. repens* DNA.

Four pools were positive for *D. repens* DNA; all of these pools consisted of mammalophilic mosquitoes collected at 2 trapping sites in the federal state of Brandenburg, near Oder Valley, at a latitude of  $52^{\circ}\text{N}$  (global positioning satellite coordinates  $52^{\circ}47'\text{N}$ ,  $14^{\circ}14'\text{E}$ , and  $52^{\circ}51'\text{N}$ ,  $14^{\circ}7'\text{E}$ ). One pool, consisting of *Culiseta annulata* mosquitoes, was collected in August 2011; the other 3 pools, 2 of *Anopheles maculipennis* sensu lato and 1 of *Ae. vexans* mosquitoes, were trapped in July and August 2012. The identification of *D. repens* DNA in all 4 pools was confirmed by DNA sequencing of

a 538-bp fragment of the gene encoding cytochrome oxidase 1 (GenBank accession no. KF410864). Sequence comparisons indicated 99.8% identity to the same gene in a *D. repens* nematode isolate from Italy (accession no. AM749234).

In conclusion, we found mosquitoes that were positive for *D. repens* DNA at a latitude of  $52^{\circ}\text{N}$  in northern Germany; these mosquitoes, from 3 species, were collected at 2 locations in 2011 and 2012. Our findings support recent projections that have suggested increases in average temperatures could affect climatic conditions

during summer sufficiently to enable development of *D. repens* larvae in central Europe at latitudes of up to  $56^{\circ}\text{N}$  (10). In the federal state of Brandenburg in Germany, conditions for larval development were predicted to be sufficient in each of the past 12 years. These projections, together with our finding of mosquitoes carrying *D. repens* in Brandenburg in 2 successive years and the recurrent detection of infected dogs in this area (8), present a strong case for the existence of stable local transmission of *D. repens* in this region of Germany.

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## Rabies in Henan Province, China, 2010–2012

**To the Editor:** Rabies is considered a reemerging zoonosis in China because many cases have been reported in recent years (1). The first case of rabies in Henan Province was reported in 1951. No more than 10 cases were reported per year during 1995–2001. However, beginning in 2002, the number of cases increased exponentially each year, and reached >100

in 2005 (2). To identify the epidemic characteristics of rabies in Henan Province, we examined the archived data of cases during 2010–2012. The surveillance data were collected by the Henan Center for Disease Control and Prevention (CDC) through systematic reporting and reports from sentinel hospitals.

Henan Province is situated in the mid-eastern region of China between northern latitudes 31°23′–36°22′ and eastern longitudes 110°21′–116°39′. The climate zone spans from warm temperate to subtropical, is humid to semi-humid with risk for monsoons, and has average annual temperatures ranging from 12°C to 16°C. The province occupies an area of 165,994 km<sup>2</sup> divided into 18 municipalities, which are subdivided into 159 county-level divisions. Its population was reported to be ≈94 million in 2010 (3).

During 2010–2012, a total of 94 cases of rabies in humans were reported in Henan Province. Rabies was diagnosed in almost all of those cases in sentinel hospitals on the basis of clinical features of the disease. Rabies was

# etymologia

## *Dirofilaria* [di-ro-fī-lar'e-ə]

From the Latin *dīrus* (“fearful” or “ominous”) + *filum* (“thread”), *Dirofilaria* is a genus of nematodes of the superfamily Filarioidea. The first known description of *Dirofilaria* may have been by Italian nobleman Francesco Birago in 1626 in his *Treatise on Hunting*: “The dog generates two worms, which are half an arm’s

length long and thicker than a finger and red like fire.” Birago erroneously identified the worms as a larval stage of another parasite, *Diectophyme renale*. The dog heartworm was named *Filaria* by American parasitologist Joseph Leidy in 1856, and the genus was renamed *Dirofilaria* by French parasitologists Railliet and Henry in 1911.

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# Stable Transmission of *Dirofilaria repens* Nematodes, Northern Germany

## Technical Appendix

Technical Appendix Table. PCR analyses of mosquitoes from various federal states of Germany for the presence of filarial DNA

Federal State	No. mosquitoes analyzed	No. pools analyzed	No. (%) pools positive for filarial DNA*	No. (%) pools positive for <i>Dirofilaria repens</i> DNA†
Baden-Wuerttemberg	19,007	1,231	215 (17.5)	0
Bavaria	6,555	358	99 (27.7)	0
Brandenburg	3,124	145	18 (12.4)	4 (2.8)
Hesse	743	45	15 (33.3)	0
Mecklenburg–West Pomerania	17,271	746	35 (4.6)	0
Rhineland-Palatinate	25,208	1,377	625 (45.4)	0
Saxony-Anhalt	1,333	71	22 (31.0)	0
Schleswig-Holstein/Hamburg	1,306	140	21 (15.0)	0
Total	74,547	4,113	1,050 (25.5)	4 (0.1)

\*PCR was performed according to (1).

†PCR was performed by using primers 5'-GAGATGGCGTTTCCTCGTG-3' and 5'-GACCATCAACACTTAAAG-3'.

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