

## Acknowledgments

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## About the Author

Dr. Vieira is a research associate at the Vector-borne Diseases Laboratory, Universidade do Paraná, Curitiba, Brazil, and specializes in tickborne diseases. Her research is focused on molecular characterization of Anaplasmataceae in Brazil.

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Address for correspondence: Thállitha S.W.J. Vieira, Departamento de Medicina Veterinária, Campus Agrárias, Universidade Federal do Paraná. R. dos Funcionários, 1540, Juvevê, Curitiba, PR, 80035-050, Brazil; email: thallitha.vieira@ufpr.br

## *Dirofilaria immitis* Pulmonary Dirofilariasis, Slovakia

Martina Miterpáková, Daniela Antolová, Jana Rampalová, Miroslava Undesser, Tomáš Krajčovič, Bronislava Víchová

Author affiliations: Institute of Parasitology of Slovak Academy of Sciences, Košice, Slovakia (M. Miterpáková, D. Antolová, B. Víchová); Unilabs Slovakia Ltd., Diagnostic Center of Pathology, Bratislava, Slovakia (J. Rampalová, T. Krajčovič); Ambulance of Pneumology and Phthysiology, Dunajská Streda, Slovakia (M. Undesser)

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*Dirofilaria immitis* is a parasite related to pulmonary dirofilariasis in humans, its accidental hosts. We detected an autochthonous case of *D. immitis* infection in a woman from Slovakia. The emergence and spread of this parasite in Europe indicates a critical need for proper diagnosis of infection.

*Dirofilaria immitis* is a filarioid nematode that infects numerous mammalian species. Dogs are the main reservoir and various mosquito species (e.g., genera *Culex*, *Anopheles*, *Aedes*, and *Ochlerotatus*) the infection vectors. The parasite is related to the pulmonary form of dirofilariasis, manifested by the formation of coin lesions or nodules in lung parenchyma in humans, an accidental parasite host (1).

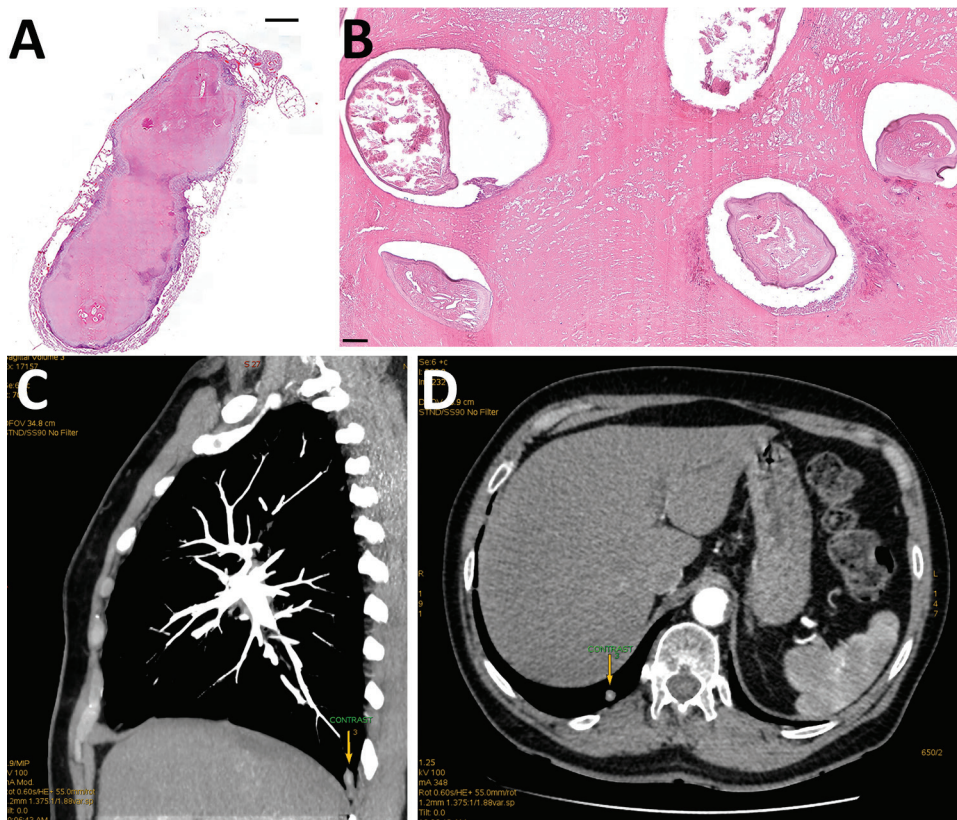
In Europe, the species *D. repens* causes most reported cases of human dirofilariasis. Just over 30 cases of human *D. immitis* infection have been unambiguously diagnosed, compared with >4,000 from *D. repens* (2).

In Slovakia, human autochthonous dirofilariasis has occurred since 2007. Meanwhile, 24 cases have been confirmed, all caused by *D. repens* nematodes (3,4; M. Miterpáková, unpub. data).

In January 2020, a 66-year-old woman from southwestern Slovakia was admitted to the Ambulance of Pneumology and Phthysiology reporting chest pain, cough, and asphyxia. The patient, who had a long-term history of smoking, had been treated for bronchial asthma and chronic obstructive pulmonary disease since 2012. She had not been abroad for >5 years. Results of hematologic and biochemical examinations were within the physiologic ranges; pulmonary function tests revealed moderate obstructive pulmonary

disorder: a decrease of vital capacity to 77% (reference range >80%) and forced expiratory volume during the first second to 63% (reference range  $\geq 80\%$ ). A chest radiograph showed bilaterally hyperlucent lungs with coarse bronchovascular markings; therefore, emphysema was suspected. Subsequent computed tomography confirmed bilateral paraseptal emphysema and numerous nonspecific lesions,  $\approx 5$  mm in diameter, in the S2 segment of the right lung and solitary nodules in the S8/9 segment of the left lung. Because of suspected malignancy, the patient was regularly monitored. After 12 months, the cancer markers had elevated. A control computed tomography examination showed a subpleural focal lesion in the S10 segment of the right lung; positron emission tomography/computed tomography confirmed hypermetabolic activity of the lesion. Biopsy was recommended because of a suspected tumor.

In May 2021, a wedge surgical resection of the nodule was performed. Histologic examination of resected tissue revealed a well-circumscribed necrotic nodule containing small irregularly shaped tubular formations affected by massive degenerative changes. The edge of the nodule consisted of nonspecific fibrotic and inflammatory granulations (Figure). The final pathology report suggested the



**Figure.** Histologic examination of resected tissue from a 66-year-old woman from southwestern Slovakia. A, B) Cross section showing *Dirofilaria immitis* nematodes embedded in necrotic material obtained from well-defined pulmonary nodule. Hematoxylin and eosin staining; original magnification  $\times 20$  for panel A,  $\times 100$  for panel C, D) Chest computed tomography scan showing a subpleural focal lesion in the S10 segment of the right lung (arrows).

presence of massively degenerated fragments of a nonvital parasite, with *Dirofilaria* spp. suspected.

We amplified the mitochondrial *cox1* gene fragments of *D. repens* (209 bp) and *D. immitis* (203 bp), in accordance with Rishniw et al. (5). The analyzed tissue was positive for *D. immitis* and subsequent sequencing and BLAST analysis of the sequence (GenBank accession no. MZ438680) revealed 100% identity within the region overlapping other homologous *D. immitis* sequences from GenBank (e.g., accession nos. KC985239, NC005305).

Pulmonary dirofilariasis is still very rare in Europe, and many cases are evaluated as imported. Even cases published as autochthonous are still under discussion, and no final decision has been reached. For instance, Pampiglione et al. (6) published an analysis of 28 human cases diagnosed in Europe and attributed to *D. immitis* parasites or a species other than *D. repens*. In this analysis the researchers excluded *D. immitis* parasites as a causative agent in all the reviewed cases (6). A non-specific localization of *D. repens* infection in lung tissue was recently reported in several patients from Russia, and 1 case was diagnosed in Slovakia (3,7). Recent data from several European countries, including Slovakia, indicate dramatic increase of *D. immitis* infections in the canine population (4,8,9), which may cause a rise in human cases in the near future.

Human pulmonary dirofilariasis is characterized by the formation of typical nodules (coin lesions) around immature adult worms located mainly on the lung periphery (6). Differential diagnosis of the nodules is important because >20 other pathologic conditions manifests by coin lesions, including tumors, cysts, and inflammatory granulomas. Coin lesions observed in patients with pulmonary dirofilariasis are spherical, not pyramidal as embolic infarct, and generally range from 1 cm to 4.5 cm in diameter (10). Few patients with pulmonary dirofilariasis show clinical symptoms. When symptoms are present, they are nonspecific and include thoracic pain, cough, and purulent sputum. These symptoms imitate pneumonitis, and patients are often treated incorrectly with antimicrobial drugs (1).

Long-term experience from dirofilariasis-endemic areas confirms that diagnosis is key and still a great challenge in the successful encompassment of human pulmonary dirofilariasis. Given the lack of specific and sensitive serologic tests, the only way for correct presurgical diagnosis appears to be the use of medical imaging. According to the European Society of Dirofilariasis and Angiostrongy-

losis (2), the combination of ultrasound and color Doppler charting, which offers findings of well-defined characteristics of *D. immitis* nodules (e.g., regular oval shape, hypoechoic inner content, no signs of polar vascularity) enable attribution to helminthic origin.

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The patient agreed with all examinations and publication of case report and signed the informed content. No identifying data are presented in the paper. The study was performed in accordance with the ethical standards as laid down in the Declaration of Helsinki of 1975, as revised 2013.

### About the Author

Dr. Miterpáková is a senior researcher and deputy director at the Institute of Parasitology of the Slovak Academy of Sciences in Košice, Slovakia. Her primary research interests include epidemiology, ecology and diagnosis of parasitic diseases; zoonoses; and vectorborne diseases.

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Address for correspondence: Daniela Antolová, Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovakia; email: antolova@saske.sk

## Fatal Case of Mediterranean Spotted Fever Associated with Septic Shock, Iran

Saber Esmaeili, Mina Latifian, Mohammad Khalili, Mehrdad Farrokhnia, John Stenos, Mehdi Shafiei, Ehsan Mostafavi

Author affiliations: Pasteur Institute of Iran, Tehran, Iran (S. Esmaeili, M. Latifian, E. Mostafavi); Pasteur Institute of Iran, Akanlu, Iran (S. Esmaeili, M. Latifian, E. Mostafavi); Shahid Bahonar University of Kerman, Kerman, Iran (M. Khalili); Kerman University of Medical Sciences, Kerman (M. Farrokhnia, M. Shafiei); University Hospital Geelong, Geelong, Victoria, Australia (J. Stenos)

A fatal case of Mediterranean spotted fever associated with septic shock was reported in a 61-year-old man living in a village in southeastern Iran. The patient had a history of tick bite a few days before symptom onset. Phylogenetic analysis confirmed infection by *Rickettsia conorii* subspecies *israelensis*.

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Mediterranean spotted fever (MSF) is a zoonotic disease caused by *Rickettsia conorii*. The main vector of this bacterium is the *Rhipicephalus sanguineus*

tick (1); the main hosts of these ticks are domestic dogs, and humans are incidental hosts (2). MSF is endemic to the Mediterranean, Europe, Africa, western Asia, and India. The case-fatality rate is 3%–7% in hospitalized patients (3,4).

In 2017, human cases of MSF were reported in Kerman province in southeastern Iran (5). No data are available on the epidemiology of MSF in Iran; we report a fatal case of MSF associated with septic shock.

The patient was a 61-year-old man with a 10-year history of hypertension and rheumatoid arthritis who lived in a village in proximity to Bam County, Kerman province, Iran. He was a farmer, had no history of domestic animal-keeping, and reported contact with livestock and a tick bite a few days before symptom onset. The initial clinical signs of the disease appeared on September 6, 2019, and the patient was admitted to a hospital in Bam on September 9; symptoms were fever, nausea, vomiting, myalgia, urinary retention, and flank pain. The patient had scleral icterus, and a black skin eschar at the tick bite site and skin rash were visible on his left leg.

When the patient's condition deteriorated, he was transferred to a hospital in Kerman on September 15. At admission, symptoms were septic shock, tachycardia, tachypnea, fever, and hypotension (85/50 mm Hg); he immediately began treatment with ceftriaxone, metronidazole, and parenteral hydration. Maculopapular skin rash was visible on the left leg. The patient had thrombocytopenia, and an increase was observed in leukocyte counts, renal factor levels (urea and creatinine), liver enzyme levels (aspartate aminotransferase, alanine transferase, and alkaline phosphatase), partial thromboplastin time of coagulation, and bilirubin levels (Table). Hemoglobin and hematocrit levels decreased, and the patient experienced hematuria and proteinuria; calcium oxalate and amorphous urate crystals were further reported in microscopic examinations. Treatment of prednisolone, heparin, doxycycline, and vancomycin was initiated.

On September 16, the patient was transferred to Afzalipour Hospital in Kerman (Referral Center for Infectious Diseases, Kerman Province). At the time of admission, the patient was conscious, his condition was stable, and his temperature was 37.6°C. No abnormalities were observed in clinical examinations of the heart, chest, and abdomen, but we noted bilateral lower extremity edema and left leg skin lesions (rash and eschar). The results of laboratory tests of blood and urine samples were