**Dirofilaria repens** in canine testicles in Bosnia and Herzegovina

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**Abstract**

In Europe, canine and feline dirofilariosis are primarily caused by *Dirofilaria immitis* and *D. repens*. The present report describes a case of *D. repens* infection of the testicular subcutaneous connective tissue in a dog from northeastern Bosnia. A nine-year-old, outdoor-kept, mixed-breed dog, with occasional paresis of the hind legs, was clinically examined and showed four nodules on testicles, several skin lesions, alopecia and erythematous pruritic rash in ventral abdominal and perineal region. Six adults (length from 8 to 12 cm) were isolated after indicated orchiectomy and identified as *D. repens*. A blood sample was analyzed with a Knott’s technique, acid phosphatase staining, ELISA testing and multiplex PCR assay. Tissue cyst samples were stained with modified Wright technique. The SNAP Heartworm test did not detect *D. immitis* antigen and molecular PCR identification showed that the sequence was *D. repens* with the highest similarity (99%) to the isolate from a dog.

Description of *D. repens* clinical presentation in the present case report suggests that infection should be included in the differential diagnosis in all dogs with nodular subcutaneous lesions and pruritus since the potential spread of parasite within dog population may increase the risk of human infections.

**Keywords:** *Dirofilaria repens*, dog, testicles, Bosnia and Herzegovina

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**Introduction**

The awareness of vector-borne helminths (VBH) is increasing in Europe due to the spread of competent vector species. Currently, 45% of the total human population in Europe, as well as their domestic and companion animals have been exposed to the risk of VBH infection (Otranto et al., 2013; Petrić et al., 2012).

Global changes, including climatic and consequent changes of vectors distribution, their ability to transmit range of vector-borne parasites such as *Dirofilaria* spp. (Genchi et al., 2009). Recent studies have confirmed that dirofilariosis is spreading throughout previously uninfected European areas, particularly in Eastern Europe (Genchi et al., 2005; Trotz-William and Tree, 2003).

Dirofilariosis is a parasitic disease caused by *Dirofilaria* spp. transmitted by mosquitoes of the family Culicidae. In Europe, canine and feline dirofilariosis are primarily caused by *D. immitis* and *D. repens* (Genchi et al., 2009). Both are considered as emerging zoonotic parasites causing dirofilariasiases (Pampiglione and Rivasi, 2000).

Canine dirofilariosis was initially diagnosed in Bosnia and Herzegovina (B&H) in 2009 from the blood of 418 dogs tested by Knott’s test and ELISA. Two different species have been identified with the overall prevalence of 5%: *D. immitis* in 13 dogs (3.1%) and *D. repens* in 8 dogs (1.9%). All but one of the dirofilariosis cases were observed in dogs without history of travelling outside B&H, which implies that canine dirofilariosis is autochthonous in B&H. As for the Tuzla region, infection with *D. repens* was not detected, while *D. immitis* was observed in 4.16% (2/48) of the tested dogs (Zahirović, 2010; Zahirović et al., 2015).

*D. immitis* mostly invades right heart chambers and pulmonary arteries. Most of the infected dogs do not show symptoms for months or years. However, the infection may become fatal if the parasitic load severely damages the arteries and the cardiac chambers.

The infections with *D. repens* are usually without visible clinical signs. If present, they can be classified in two clinical syndromes: multifocal nodular dermatitis and prurigo papularis dermatitis. Other dermatological clinical signs seems to appear with time, such as pruritus in 100% of animals, erythema (79%), papules (62%), focal or multifocal alopecia (55%), hyperkeratosis (18%), crusting (14%), nodules (12%), acanthosis (5%), eczema (3%), pyoderma (3%), and edema (1%) (Tarello, 2011).

There are only a few descriptions of findings *D. repens* in canine testicles. Demiaszkiewicz et al. (2013) described a case of *D. repens* in testicles of an eight-year-old German Shepherd in Poland, while Saevik et al. (2014) described a similar case in a four-year-old dog from Norway, originally imported from Hungary.

The present report describes a rare case of *D. repens* infection of the testicular subcutaneous connective tissue in a dog from northeastern B&H.
Material and Methods

Study area

Tuzla is the largest municipality in northeastern B&H. The climate of municipality is moderately continental with abundant precipitation and several shallow rivers cause often floods in the region, which creates an ideal biotope for insects as potential intermediate hosts for various pathogens.

Case description

A nine-year-old, outdoor-kept, mixed-breed dog from Tuzla was clinically examined. In the anamnesis the owner described occasional paresis of the hind legs, subcutaneous masses and nodules on testicles and abdominal wall.

Clinical diagnostics included standard hematological and biochemical blood testing, ECG and urine examination. Standard bilateral orchiectomy was carried out.

Parasites collected during orchiectomy were washed in standard saline solution and conserved in an alcoholic solution of 70% v/v ethanol and 5% v/v of glycerol. Adult parasites and microfilariae were preliminary identified based on their morphological characteristics and morphometric analysis.

A blood sample (10 mL) was drawn from the dog’s cephalic vein in tubes with added EDTA and analyzed using the Knott’s technique, acid phosphatase staining, ELISA testing (PetChek HTWM®, IDEXX) and PCR assay.

For the PCR identification, the parasitic DNA was extracted using commercial kit (Blood and Tissue Kit®, QIAGEN) according to the manufacturer’s instructions. PCR identification of the parasite was conducted using DIDR-F1 (5’ – AGT GCG AAT TGC AGA CGC ATT GAG - 3’) and DIDR-R1 (5’ – AGC GGG TAA TCA CGA CTG AGT TGA - 3’) primers and GoTaq® G2 Hot Start Colorless Master Mix (PROMEGA). Amplification was carried out according to Rishniw et al. (2006). The primers were used to amplify the Internal Transcribed Spacer Region 2 (ITS2) of D. immitis (542 bp), D. reconditum (578 bp) and D. repens (484 bp). The amplification products were analyzed by capillary electrophoresis (QIAxcel System®, QIAGEN) and purified (ExoSAP-IT®, USB Corp., Cleveland, United States). The sequencing was performed by Macrogen Europe in both directions. Sequences were assembled using the SeqMan Pro software, edited with EditSeq of the Lasergene software (DNASTAR, Madison WI, USA) and compared with available sequences using BLAST.

Tissue samples for cytological imprints were collected from cysts, smeared on slides, air-dried and stained with modified Wright technique.

Results

During routine clinical examination dog showed normal body temperature (38.9°C), while respiratory rate (n=33) and heart rate (n=138) were increased. Several skin lesions, alopecia and erythematous pruritic rash were observed in ventral abdominal and perineal region. Four nodules on testicles and one nodule on the ventral abdominal wall were noted, with slightly enlarged lymph nodes.

Hematocrit (HCT) 32.2 % (37.0-55.0) and hemoglobin (HGB) concentration 11.1g/dL (12.0-18.0) were decreased, while white blood cells (WBC) 17.8×10 9(5.50-16.90), neutrophils (NEU)13.4×10 4(2.00-12.00) and eosinophils (EOS) 2.0×10 4(0.10-1.49) were increased. Alkaline phosphatase (ALKP) value was increased 217 U/l (23-212). Urine examination did not detect any abnormalities.

After the orchiectomy, six elongated parasites (length from 8 to 12 cm) were found, either freely or encapsulated in cysts (diameter up to 3 cm) filled with transparent fluid. Based on the morphological and morphometric characteristics, all the specimens were identified as D. repens adults (Figure 1).

![Figure 1. Dirofilaria repens in canine testicles](image-url)

In blood sample 1550 mf/ml were detected by the Knott technique with typical morphologic characteristics for D. repens (obtuse cephalic ends, and sharp, filiform umbrella-shaped tails). The measured mean body length and width in 50 microfilariae was 300.2-360.5±6.5×8.2μm, corresponding to D. repens microfilariae. Histochemical staining of microfilariae with acid phosphatase showed only one red spot at the anal pore characteristic for D. repens. The SNAP Heartworm test (IDEXX) blood testing did not detect D. immitis antigen.

On the tissue imprints (n=8) high counts of erythrocytes, eosinophils and macrophages were observed, while plasma cells and mast cells were scarcely present. Some of the cyst walls displayed pyogranulomatous inflammation and presence of tumor cells. Additionally, numerous larvae were detected in tumorous tissue, which exhibited an obtuse cephalic end with nuclei and a sharp and filiform tail.

The PCR fragment characteristic for D. repens (approximately 480 bp) was sequenced. A search against
the GenBank database using the BLAST algorithm revealed that the sequence was *D. repens* and had the highest similarity (99%) with the isolate from a dog (GenBank Accession No. AY693808).

**Discussion and conclusions**

*D. repens* infections has been usually considered as non-pathogenic since clinical signs are frequently absent or if present, are usually non-specific. In the current case, the dog showed skin lesions, such as alopecia and erythematous pruritic rash in the ventral abdominal and perineal region, characteristic for *D. repens* clinical presentation (Tarello, 2011). In most of the cases, *D. repens* is localized in cutaneous/subcutaneous tissue and muscles of infected animals, characterized by prominent subcutaneous nodules, often without visible clinical symptoms. In the current case, *D. repens* adults were detected on tumors testes during surgery. The diagnosis of canine subcutaneous dirofilariosis may be challenging due to the lack of appropriate antigen or antibodies detection tests as for *D. immitis*.

A long incubation period, frequent non-specific clinical signs and inapparent infections, as well as the lack of diagnostic experience, may lead to further spread of the infection to other locations. Usually, veterinary practitioners consider that infection with *D. repens* does not need treatment or protection since clinical signs are lacking or are not typical as observed in this case. Veterinarians should be aware of canine infections caused by *D. repens*, particularly in countries where the parasite is not considered as endemic, as was the case in current study. Also, the *D. repens* infection should be included in the differential diagnosis in all dogs with observed nodular subcutaneous lesions or any skin involvement with pruritus. This case raises a question on *D. repens* prevalence in B&H since high prevalence of canine infection with *Dirofilaria* in neighboring Serbia The microfilariae detected in 21/122 dogs (17.2%) were identified as *D. repens* (n=21) and *D. immitis* (n=2). (Tasić et al., 2012). Appropriate treatment and control of *D. repens* infections is also important to prevent the spread of the infection. Use of the prolonged selamectin or moxidectin administration is an appropriate choice to treat canine *D. repens* infections (Otranto et al., 2013). Currently, the only macrocyclic lactone that has shown in experimental studies 100% preventative efficacious and >95% efficacy as an adulticide against *D. repens* is moxidectin (2.5%) in combination with imidacloprid (10%) (Genchi et al., 2013; Petry et al. 2015).

We hope our report together with limited general data on canine dirofilariosis in B&H, will encourage researchers to focus further on the epidemiology of dirofilariosis in the region. Climatic changes, frequent travelling with pets and lack of dog protection due to lower standard favor maintaining and the spread of the parasite within the dog population and consequently may increase the risk of human infections.

**References**

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Ethical standards
The manuscript does not contain the clinical studies or patient data. Everything was done in compliance with current national laws and regulations.

Conflict of interest
The authors declare that they have no conflict of interest.

Dirofilaria repens u testisima psa u Bosni i Hercegovini

Sažetak
U Evropi dirofilarioza pasa i mačaka prvenstveno je uzrokovana *Dirofilaria immitis* i *D. repens*. Prezentirani slučaj opisuje infekciju sa *D. repens* u potkožnom vezivnom tkivu testisa psa iz sjeveroistočne Bosne. Kliničkim pregledom devetogodišnjeg pasa sa povremenim parezama stražnjeg dijela tijela ustanovljene su četiri izrasline na testisima, nekoliko kožnih lezija, alopecija i pruritni eritematozni osip na abdomenu i perinealnoj regiji. Nakon indicirane orhidektomije psa izolirano je i determinirano šest adulta *D. repens* (8 do 12 cm), a dodatno su analizirani uzorci krvi Knott metodom, bojenjem kiselom fosfatazom, ELISA metodom i PCR metodom. Ciste u potkožnom tkivu obojene su modificiranom Wright tehnikom. SNAP Heartworm Test nije otkrio antigen *D. immitis*, a PCR identifikacija je pokazala 99% sličnosti izolata iz psa sa sekvencom *D. repens*. Isto tako, Knott metodom i bojenjem kiselom fosfatazom ustanovljene su mikrofilarije *D. repens*.

Klinička prezentacija i opis *D. repens* u ovom slučaju sugeriira da dijagnostika dirofilarioze treba biti uključena u diferencijalnoj dijagnozi kod svih pasa sa potkožnim nodularnim lezijama i svrbežom. Isto tako, potencijal širenje parazita u psa može povećati rizik za oboljenja kod čovjeka.