Parasitology International 73 (2019) 101960

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Human dirofilariosis of buccal mucosa – First molecularly confirmed case and literature review

Stefan Momčilović[a,b,*], Simona Gabrielli[c], Milan Golubović[d], Tanja Smilić[e], Miljan Krstić[f], Saša Đenić[d], Marina Ranđelović[d,g], Suzana Tasić-Otašević[d,g]

[a] Plastic and Reconstructive Surgery Clinic, Clinical Center Niš, Blvd Zorana Djindjica 48, Niš 18000, Serbia
[b] Faculty of Medicine, University of Niš, Blvd Zorana Djindjica 81, Niš 18000, Serbia
[c] Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, Piazza le Aldo Moro 5, Rome 00185, Italy
[d] Department of Microbiology and Immunology, Faculty of Medicine, University of Niš, Blvd Zorana Djindjica 81, Niš 18000, Serbia
[e] Center for Radiology, Clinical Center Niš, Blvd Zorana Djindjica 48, Niš 18000, Serbia
[f] Department of Pathology, Faculty of Medicine, University of Niš, Blvd Zorana Djindjica 81, Niš 18000, Serbia
[g] Center of Microbiology and Parasitology, Public Health Institute Niš, Blvd Zorana Djindjica 81, Niš 18000, Serbia

ARTICLE INFO

Keywords:
Human dirofilariosis
Submucosal infection
Diagnosis

ABSTRACT

When presented in unusual manner, human dirofilariosis is hard to recognize and often leads to misdiagnosis and unjustified use of various medications. Herein, we report a rare case of *Dirofilaria* infection localized in buccal mucosa.

A 45-year-old male, residing in the Central Serbia, developed striking edema of buccal mucosa followed by leucocytosis, with both neutrophylia and eosinophylia. Thirty days after first symptoms occurred, nodule was formed along the lateral edge of right maxilla. Therefore, patient underwent surgical extirpation and subsequent histopathological analysis of the specimen revealed the presence of a nematode. Based on morphological characteristics of detected parasite, the diagnosis of submucosal *Dirofilaria repens*-like infection was made. The diagnosis was confirmed, being the first case in the world analyzed by molecular methods.

So far, only 13 such cases have been reported worldwide and our paper brings attention to rare form of this zoonosis and updates the data about human dirofilariosis in buccal mucosa.

1. Introduction

Dirofilariosis is vector-borne zoonosis caused by roundworms of genus *Dirofilaria (D.)*. More than 40 species are described in this genus [1], while *D. immitis* and *D. repens* have adapted to animal (canine and feline) and human hosts with distinct biological and clinical implications [2,3]. In both of these species, biological vectors are mosquitoes usually belonging to *Aedes*, *Culex*, *Anopheles* or *Mansonia* genera [3] with *Aedes vexans*, *Culex pipiens*, and *Aedes albopictus* being the main vectors in Europe [4]. During the blood meal, these transient hosts transmit infectious, third-stage, filarial larvae to permanent hosts, mainly dogs [5]. Maturation of larvae is then continued inside definitive host until adult parasites are formed. Life cycle is finished when pregnant female produces microfilariae that enter the peripheral blood, where they can be ingested by mosquitoes [3].

Humans can become accidental hosts in the same way. However, further development of parasites in infected person is normally stopped since these nematodes are only partially adapted to humans as hosts [3]. Still, there are cases of adult parasites and microfilariae [6] found at lesion site and even some reports of microfilariae present in peripheral blood [7,8].

Human dirofilariosis manifestations depend on species involved and site of mosquito bite. Most frequently, it manifests in the form of subcutaneous nodules. These tumor-like lesions usually appear on head, arms and trunk, but even breasts or male genitals can be affected [5]. Ocular forms of dirofilariosis are described separately because of their specific localization and possibility of severe complications [9]. Almost all periorbital and orbital structures can be afflicted, but most often this infection is presented as subcutaneous nodule in the eyelid or as subconjunctival or subtenonian space affliction. Both subcutaneous and ocular forms are referred to as superficial and in most of the cases they are caused by *D. repens*. In addition, intramuscular *D. repens* infection has also been reported [10]. This species is the most prevalent from genus *Dirofilaria* in Europe, especially in Mediterranean regions and in

* Corresponding author at: Plastic and Reconstructive Surgery Clinic, Clinical Center Niš, Blvd Zorana Djindjica 48, 18000 Niš, Serbia.
E-mail address: m-stefan@mts.rs (S. Momčilović).

https://doi.org/10.1016/j.parint.2019.101960
Received 23 March 2019; Received in revised form 3 June 2019; Accepted 14 July 2019
Available online 20 August 2019
1383-5769/

the Eastern parts of continent [11]. On the other hand, *D. immitis* is considered as New World pathogen, although its prevalence in Europe is rising [12,13]. This species usually causes visceral disease, most frequently presented as lung nodules. Sometimes, these pulmonary lesions can imitate malignant tumor during radiological examination [13].

Diagnosis of human dirofilariosis is based on analysis of worm, rarely worms, extirpated from nodule during surgical intervention. In this occasion, only formed, mature adults can be identified by parasitologists, based on morphological characteristics. On the contrary, when worm is immature, the determination is only possible by molecular tools which are not affordable to the most laboratories. Recently, reports of in-house serological tests for detection of specific antibodies are promising for indirect diagnosis of this infection in humans [14,15].

From epidemiological point of view, the occurrence of human dirofilariosis in some regions correlates with prevalence of this parasitosis in dogs. Many studies conducted on the territory of Serbia highlighted that this region is endemic for dirofilariosis in asymptomatic dogs with prevalence ranging from 3% in the southern to 8% in the northern regions [16,17].

With emerging of this zoonosis, knowledge about dirofilariosis and consideration of *Dirofilaria* infection in patients presenting with unexplained nodules is going to be important in future.

This paper describes the case of human dirofilariosis of buccal mucosa with the goal to point out a rare manifestation of *Dirofilaria* infection in humans, to update human dirofilariosis data and to compare clinical findings, prognosis and treatment of this case with other documented infections of the same localization.

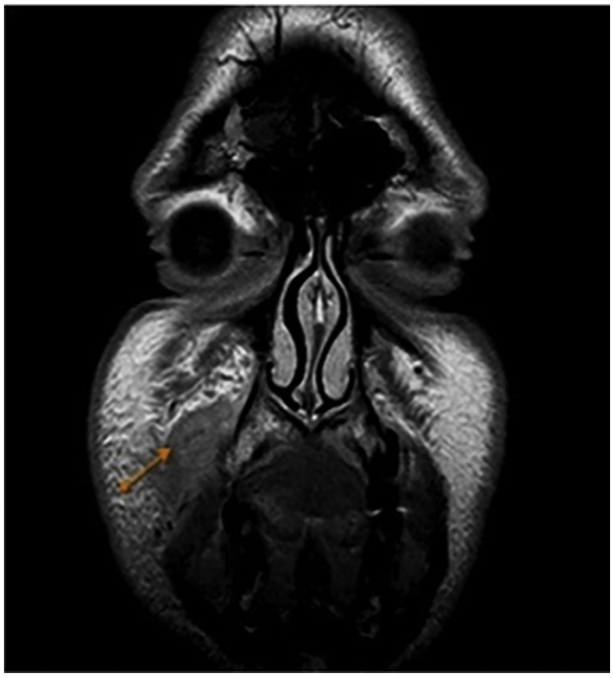

Fig. 2. Coronal T1- weighted MR image displays an inhomogeneous, well defined nodular lesion in the posterior part of the right buccal space, in front of right masseter muscle (arrow). The lesion is isointense to the muscle that is not invaded.

2. Case report

The reported case describes submucosal form of human dirofilariosis, which is, in the light of current knowledge, very unusual and rare. The patient was 45-year-old male office worker with no significant past medical history. In November 2017, the infection started dramatically, with a striking edema of buccal mucosa which caused asymmetrical deformity of the face (Fig. 1). During the collection of anamnestic data, the patient was unable to remember any face injury or insect bite and had no history of traveling abroad.

Clinical presentation and blood analysis that revealed leucocytosis, with both neutrophylia [7.9 × 10^9/L (reference values 1.1–6.5 × 10^9/L)] and eosinophylia [1.1 × 10^9/L (reference values 0.0–0.4 × 10^9/L)], aroused suspicion of bacterial infection followed by allergic reaction. Therefore, the patient was routinely treated with antibacterial, antihistamine and antiedematous therapy. In the first few days of treatment, patient's condition slightly improved. However, four to five days after the initiation of therapy, symptoms and signs recurred, leading to therapy modification. Antibiotics were changed several times and patient received penicillin G procaine (600,000 units/day), gentamicin (250 mg/day), metronidazole (2 g/day), ciprofloxacin (1 g/day) and amoxicillin (1 g/day). Thirty days after first symptoms occurred, solitary, firm and movable, 20 × 15 mm nodule was formed along the

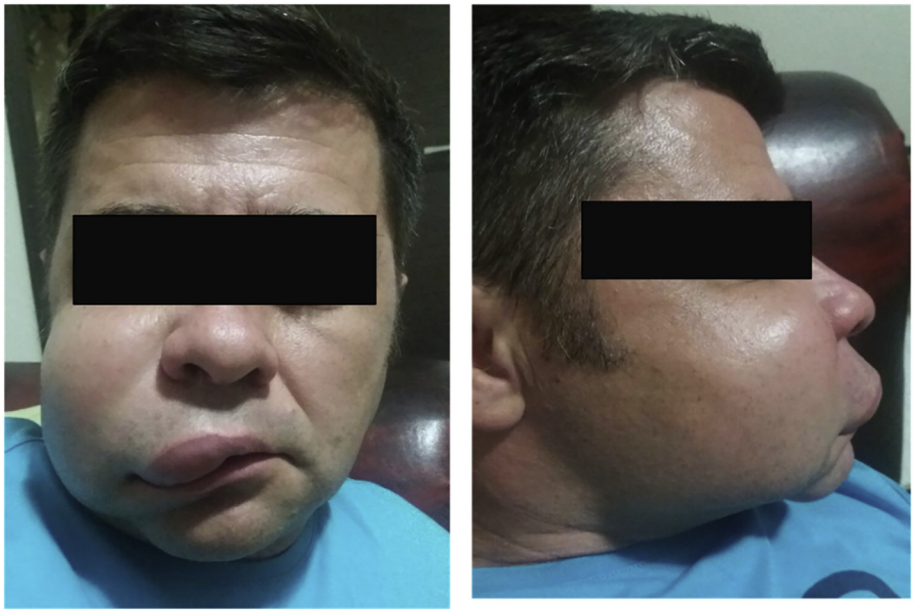

Fig. 1. Striking edema of buccal mucosa which caused asymmetrical deformity of the face in patient – frontal and lateral view.

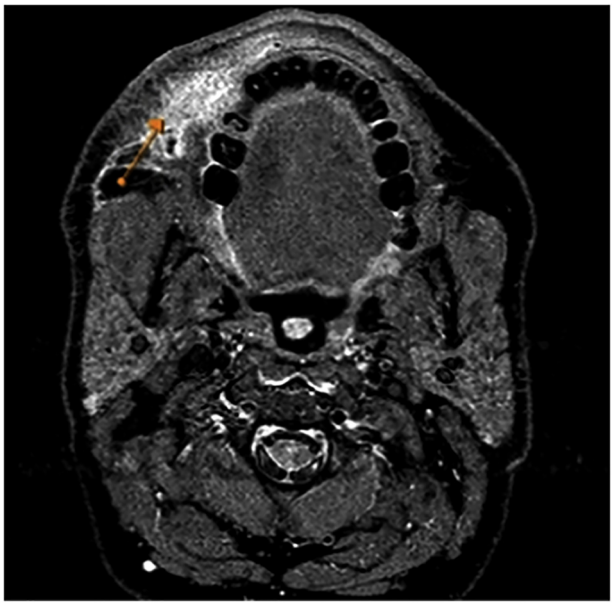

Fig. 3. Axial T2w/STIR MR image shows well-defined lesion with high signal intensity (arrow) and increased signal intensity of surrounding diffuse inflammation.

lateral edge of right maxilla. On the same day, patient was referred to a local clinic for maxillofacial surgery.

2.1. Magnetic resonance (MR) analyses

Prior to the surgical intervention, magnetic resonance imaging (MRI) examination was performed. Coronal T1-weighted MRI scans displayed well defined nodular lesion isointense to the muscles in the right buccal space (Fig. 2). On the other hand, this node had high signal intensity on T2-weighted and STIR (fat suppression technique) MR images (Fig. 3). The lesion was surrounded by a diffuse inflammation that exhibited low signal intensity on T1w and high signal intensity on T2w and STIR which is typical for granuloma. The differential diagnosis included sarcoidosis, foreign body reactions, tuberculous lymphadenitis, sarcoma and myxoma.

2.2. Histopathological examination

After surgical extirpation, the microscopic observation of the histological sections revealed the presence of a nematode, enclosed in the nodule, showing morphological features of a filarioid parasite. Surgically resected mucosal specimen was a gray-pink nodular fragment, measuring 15 mm in diameter with a small cystic space on gross examination. The specimen was thoroughly reviewed in multiple histological sections stained with haematoxylin and eosin (H&E). Histopathological examination revealed sections of fibrous connective tissue and fascicles of skeletal muscle fibers with a pseudocystic cavity lined by intense inflammatory cell infiltrate composed of numerous plasma cells and occasional neutrophils, eosinophils and lymphocytes. This cavity contained transversal sections of the parasitic worm immediately surrounded by granular and amorphous lipoprotein debris. The sections of a helminth disclosed thick multilayered cuticle with longitudinal ridges on the circumference, as well as intestinal and reproductive tubes enveloped by well-developed muscular layer (Fig. 4). Based on morphological characteristics of detected parasite and epidemiological data, the diagnosis of submucosal *Dirofilaria repens*-like infection was made.

2.3. Blood analyses

The modified Knott's technique was applied to concentrate and to detect blood circulating microfilariae. One ml of EDTA-anticoagulated, peripheral blood of patient was mixed with 10 ml of 2% formalin in a 15-ml tube and centrifuged for 5 min at 1500 ×*g*. The supernatant was discarded and a drop of 0.1% methylene blue stain was added to the sediment. The sediment was transferred onto the center of slide and examined as a wet mount under the light microscope. Parasitological analyses of peripheral blood using a modified Knott test showed no circulating microfilariae.

2.4. Molecular analysis

For the identification to species level, DNA was extracted from the paraffin block using the QIAamp DNA FFPE Tissue Kit (Qiagen) following the manufacturer's instructions. The extracted DNA was dissolved in 50 µl of double distilled water and submitted to molecular amplification using the primers COIintF (5′-TGATTGGTGGTTTTGG TAA-3′) and COIintR (5′- ATAAGTACGAGTATCAATATC-3′), which amplified a fragment of about 650-bp from the filariod-*cox1* gene

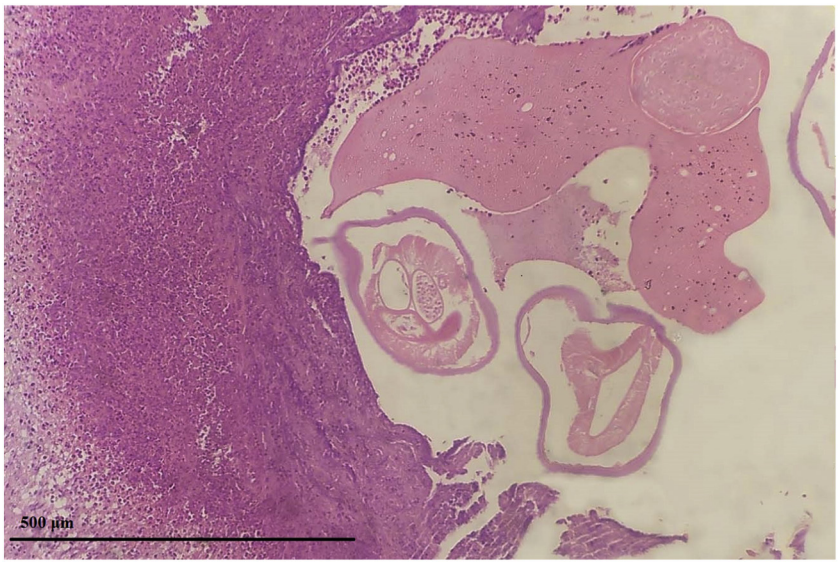

Fig. 4. Transverse section of parasite surrounded by inflammatory cell infiltration stained with H&E (100× magnification).

Table 1
Human dirofilariosis of buccal mucosa - summary of cases reported in the literature.

No.	Country	Sex	Age	Symptoms	Physical examination of change	Relevant history/ traveling	Size of nodule	Side	Parasito-logical analyses/species	Molecular analysis	Endemic/Non-endemic area for *D. repens* infection	Eosinophilia (%)	Ref.
Europe													
1	Bulgaria	M	37	Spontaneously changing edema present for 4 months	Non-tender, firm nodule. In addition two enlarged lymph nodes in the left sub-mandibular region	Did not travel	1.5 cm × 1 cm	Left	*D. repens*	No	Endemic	Yes (31%)	[23]
2	Serbia	M	45	Acute edema, with node formation after a month	Asymmetrical deformation. Movable, firm nodule	Did not travel	2 cm × 1.5 cm	Right	*D. repens*	Yes	Endemic	Yes	Our case
North America													
3	Texas, USA	M	79	Slowly growing mass	NA	Chronic bronchitis and DM	NA (infilt-ration)	Right	*Dirofilaria* sp., dead and degenerated	No	Non-endemic	Yes	[24]
South America													
4	Brazil	F	65	Swelling and pain	Movable tender mass	Good health	NA	Right	*Dirofilaria* spp.	No	Non-endemic	NA	[25]
Asia													
5	Hong Kong	F	42	Acute exacerbation of mass previously present for 6 months	Firm mildly tender nodule	New immigrant to Hong Kong from southern China	1.5 cm × 1 cm	Right	*D. repens*	No	Non-endemic	No (< 1%)	[26]
6–8	India 3 cases	M	32	Painless mass	Movable, firm nodule	No significant history	1 cm × 2 cm	Right	*D. repens*	No	Western India, Non-endemic	No	[27]
		F	54	Intermittent swelling (4–5 times in 8 months) and itching	Firm, mildly tender node	Hyper-tension, dys-lipidemia	1 cm × 0.5 cm	Left	*D. repens*	No	Southern India, Endemic	No	[28]
		F	19	Painless, intermittent swelling	Soft nodule	NA	1 cm × 1.5 cm	Left	*D. repens*	No	Southern India, Endemic	35%	[29]
9–14	Sri Lanka 6 cases	F	4, 26, 28, 40, 52, 80	Mild pain and tenderness	Firm nodule, with normal overlying mucosa	NA	2 cm × 3 cm in 1 case; NA for others	NA	*D. repens* (5 cases) / *D. repens* like (degenearated) (1 case)	No	Endemic	Present in 3 cases; NA for others	[30]

*M – male; F – female; NA – not applicable.

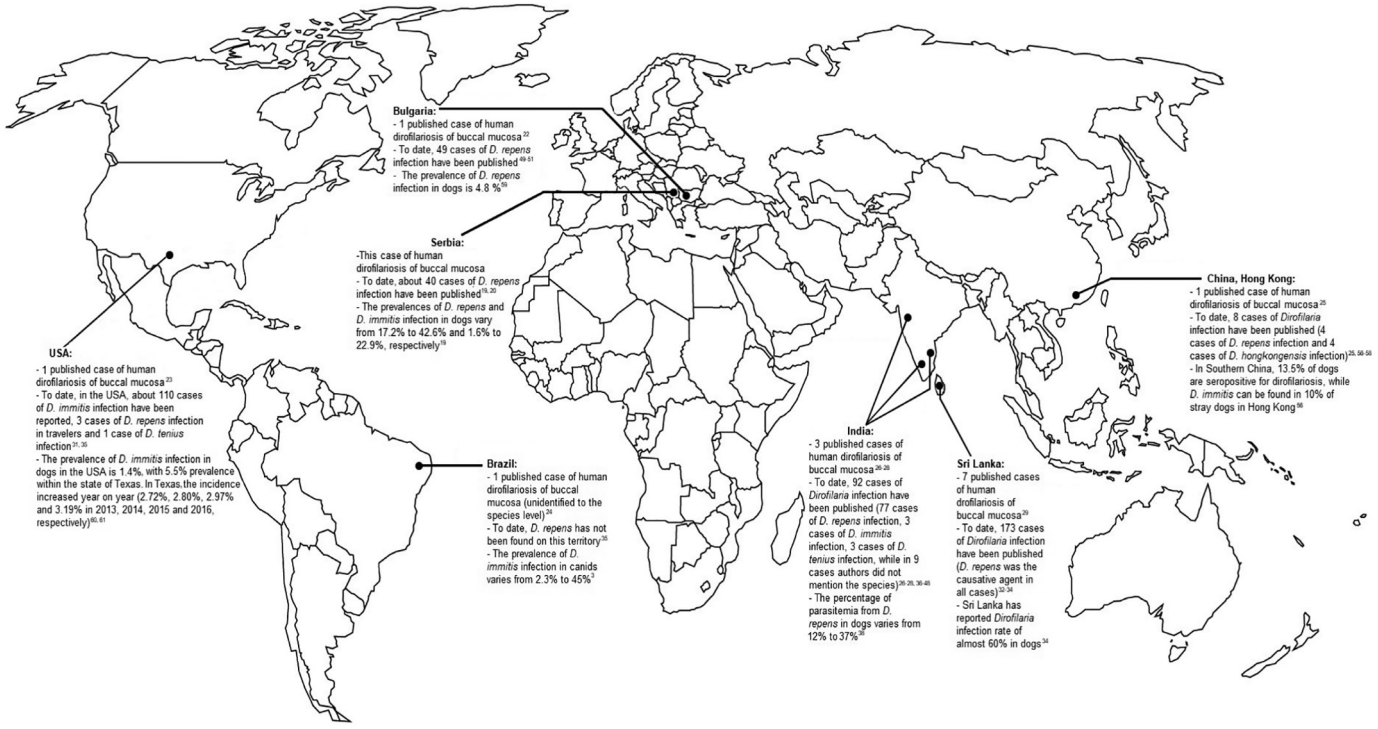

Fig. 5. Distribution of human and canine dirofilariosis among countres in which dirofilariosis of buccal mucosa has been reported.

fragment [18]. Conventional PCR was performed in a final volume of 25 µl under the following final conditions: 10× buffer including 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 1 mM each of forward and reverse primers, and 1 unit of polymerase (BIOTAQ, Bioline, UK). To test the specificity of the reaction, 2 ml of DNA extracted from *Dirofilaria immitis* and equivalent volume of double distilled water were included in each PCR run as positive and negative controls, respectively. The amplification was performed in a thermocycler (BIO-RAD, USA) using the following cyclic profile: initial denaturation at 94 °C for 10 min, followed by 5 cycles of further denaturation at 94 °C for 30 s, annealing at 52 °C for 45 s and an extension at 72 °C for 1 min, followed by 30 cycles of further denaturation at 94 °C for 30 s, annealing at 54 °C for 45 s and an extension at 72 °C for 1 min, followed by a final extension for 7 min at 72 °C.

The PCR final products were separated by stained (SafeViewBiologicals, UK) 1.2% agarose gel electrophoresis at 100 V for 45 min and visualized on a UV transilluminator (BIO-RAD, USA). Amplicons of expected size were then purified and sequenced (BioFab Research, Italy).

The resulting chromatograms were analyzed and edited in the computer software Chromas version 2.33 (Technelysium Pty Ltd., Australia). The sequences obtained were compared to sequences previously deposited in GenBank and available at the website (http://www.ncbi.nlm.nih. gov/genbank/) by using the BLAST application.

The sequence analysis showed a match of 100% with the sequence from the same gene of the species *D. repens*, deposited in GenBank (Accession number DQ358814.1).

Twenty days after surgical intervention, patient was fully recovered and haematological parameters were completely normalized. Also, during a 12-month follow-up period, the patient did not present any clinical evidence of recurrence.

3. Discussion

In Europe [11,19–21], *D. repens* is the dominant species and it mainly causes subcutaneous and ocular forms of human dirofilariosis.

Human dirofilariosis of the buccal mucosa is very unusual and rare. So far 13 cases of this form are reported in the whole world, with only a few of them confirmed to the level of species (Table 1). This species identification was based only on parasitological examination and it showed *D. repens* domination [22–30]. Besides these 13 reports, several cases of subcutaneous *Dirofilaria* infection on cheek were misidentified as oral dirofilariosis [31,32]. Although these two conditions are difficult to distinguish clinically, it does not have an impact on the patient's treatment.

From the epidemiological point of view, among the 7 countries in which dirofilariosis of buccal mucosa has been noted, the highest number of human infections caused by these nematodes have been recorded in Sri Lanka, USA and India, where, to the best of our knowledge, amounts to as many as 173 cases [33–35], 114 cases [32,36] and 92 cases [27–29,31,37–48], respectively. Therefore, it is not surprising that the submucosal, buccal form was found in the most patients from these regions.

Similarly, the increased number of human dirofilariosis was determined on the territory of Bulgaria (49 cases) [49–51], Serbia (40 cases) [20,21], Brazil (33 cases) [52–55] and Hong Kong (8 cases) [26,56–58] where the mucosal form was also reported. In addition, studies that have examined the epidemiological profile of canine dirofilariosis in these countries have shown high infection rates in dogs in Sri Lanka (almost 60%) [35], some parts of Serbia (up to 42.6% for *D. repens* infection and up to 22.9% for *D. immitis* infection) [20], India (12% to 37% for *D. repens* infection) [39], some parts of Brazil (up to 45% for *D. immitis* infection) [3] and Hong Kong (10% for *D. immitis* infection) [56]. On the other hand, significantly lower prevalence rates have been determined in dogs in Bulgaria (4.8% for *D. repens* infection) [59] and USA (1.4% for *D. immitis* infection, with 5.5% prevalence within the state of Texas) [60,61] (Fig. 5).

Based on the literature data, this is the second described case of dirofilariosis involving buccal mucosa in Europe and the first one in the world confirmed by molecular methods. Considering rareness of this form of the disease, it is no wonder that establishing of right diagnosis is a challenge.

Infection typically begins as a buccal mass and it often takes months before swelling bring patient to a hospital. Existence of such a nodule in

oral cavity is usually misinterpreted by clinician as either benign or malignant tumor. The lesion can also be mistaken for sialolithiasis, granulomatous diseases, mucocele, lymphadenopathy, cyst or abscess, as well as salivary gland neoplasms [22,24]. In our case, due to sudden onset of diffuse swelling and with no distinctive lump, tumor was not an option but instead bacterial infection was assumed. Eosinophilia, which was also present in some other patients with buccal dirofilariosis [23,24,29,30], was in this case attributed to allergic reaction.

When the node appeared, it was movable and firm, similarly to the majority of other cases [23,25–28,30]. Other symptoms, such as itching, pain and tenderness described in some of the cases were not reported by our patient [25,26,28,30]. His medical history did not reveal any significant medical conditions and the fact that he did not leave the country suggests that this was autochthonous infection.

Performed routine imaging methods were inconclusive and without clinical suspicion, while specific imaging techniques, such as post contrast MR studies, that could have revealed parasitosis, were not requested. This long wandering during diagnostic procedures and clinical examination give rise to unnecessary, excessive use of a large number of different antibiotics, the long-term upset, fear and after diagnosis, distrust of the patient. Hence, promising in-house serological tests that detect antibodies will be of a great help in future for indirect diagnosis of human dirofilariosis [14,15].

To conclude, raising awareness of this zoonosis is needed, especially since it is expected that global warming, migration of people and animals, as well as changes in vector population ecology will influence its emerging in areas, so far considered as *Dirofilaria* free-zones. This claim is also supported by the recently published data of autochthonous *D. repens* infection in patient from Finland as an example of “northernmost” case of human dirofilariosis [62].

This is the reason why in both, endemic and non-endemic regions, dirofilariosis should be considered as possible cause of nodular lesions, including the ones that occur in oral cavity. It is particularly important to think about this parasitosis in patients not responding to routine therapy. In that way, physicians can avoid misdiagnosis, which would reduce the number of drugs used, shorten the time until diagnosis, improve patient satisfaction and also help radiologists to use appropriate imaging techniques.

Funding sources

The study was supported by the Serbian Ministry of Education and Science grant No III41007 and grant No III175034. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Declaration of Competing Interest

The authors declare no conflicts of interest.

References

[1] M.V. Reddy, Human dirofilariasis: An emerging zoonosis, Trop. Parasitol. 3 (2013) 2–3 http://www.tropicalparasitology.org/text.asp?2013/3/1/2/113879.
[2] D. Otranto, F. Dantas-Torres, E. Brianti, D. Traversa, D. Petrić, C. Genchi, G. Capelli, Vector-borne helminths of dogs and humans in Europe, Parasit. Vectors 6 (2013) 16, https://doi.org/10.1186/1756-3305-6-16.
[3] F. Simón, M. Siles-Lucas, R. Morchón, J. González-Miguel, I. Mellado, E. Carretón, J.A. Montoya-Alonso, Human and animal dirofilariasis: the emergence of a zoonotic mosaic, Clin. Microbiol. Rev. 25 (2012) 507–544, https://doi.org/10.1128/cmr.00012-12.
[4] N. Becker, D. Petric, M. Zgomba, C. Boase, M.B. Madon, C. Dahl, A. Kaiser, Mosquitoes and Their Control, 2nd edition, Springer, Berlin Heidelberg, 2010.
[5] H.P. Fuehrer, H. Auer, M. Leschnik, K. Silbermayr, G. Duscher, A. Joachim, *Dirofilaria* in humans, dogs, and vectors in Austria (1978-2014)-from imported pathogens to the Endemicity of *Dirofilaria repens*, PLoS Negl. Trop. Dis. 10 (2016) e0004547, , https://doi.org/10.1371/journal.pntd.0004547.t001.
[6] A.S. Damle, J.A. Iravane Bajaj, M.N. Khaparkhuntikar, G.T. Maher, R.V. Patil, Microfilaria in human subcutaneous dirofilariasis: a case report, J. Clin. Diagn. Res. 8 (2014) 113–114, https://doi.org/10.7860/JCDR/2013/6886.4123.
[7] L. Fontanelli Sulekova, S. Gabrielli, M. De Angelis, G.L. Milardi, C. Magnani, B. Di Marco, G. Taliani, G. Cancrini, *Dirofilaria repens* microfilariae from a human node fine-needle aspirate: a case report, BMC Infect. Dis. 16 (2016) 248, https://doi.org/10.1186/s12879-016-1582-3.
[8] M. Kłudkowska, Ł. Pielok, K. Frąckowiak, A. Masny, E. Gołąb, M. Paul, *Dirofilaria repens* infection as a cause of intensive peripheral microfilariemia in a Polish patient: process description and cases review, Acta Parasitol. 63 (2018) 657–663, https://doi.org/10.1515/ap-2018-0077.
[9] D. Otranto, M.L. Eberhard, Zoonotic helminths affecting the human eye, Parasite. Vector. 4 (2011) 41, https://doi.org/10.1186/1756-3305-4-41.
[10] M.I. GheorghiŢă, M.C. ForŢofoiu, C.I. Dumitrescu, D. Dumitrescu, A. Camen, C. Mărgăritescu, Intramuscular human *Dirofilaria repens* infection of the temporal region - case report and review of the literature, Romanian J. Morphol. Embryol. 58 (2017) 585–592 http://www.rjme.ro/RJME/resources/files/580217585592.pdf.
[11] C. Genchi, L.H. Kramer, F. Rivasi, Dirofilarial infections in Europe, Vector-Borne. Zoonot. 11 (2011) 1307–1317, https://doi.org/10.1089/vbz.2010.0247.
[12] F. Dantas-Torres, D. Otranto, Dirofilariosis in the Americas: a more virulent *Dirofilaria immitis*? Parasite. Vector. 6 (2013) 288, https://doi.org/10.1186/1756-3305-6-288.
[13] E.D. Cabrera, E. Carretón, R. Morchón, Y. Falcón-Cordón, S. Falcón-Cordón, F. Simón, J.A. Montoya-Alonso, The Canary Islands as a model of risk of pulmonary dirofilariasis in a hyperendemic area, Parasitol. Res. 117 (2018) 933–936, https://doi.org/10.1007/s00436-018-5774-1.
[14] S.A. Tasić-Otašević, S.V. Gabrielli, A.V. Tasić, N.L. MiladinovićTasić, J.T. Kostić, A.M. Ignjatović, L.D. Popović Dragonjić, Z.G. Milošević, V.S. Arsić-Arsenijević, G.A. Cancrini, Seroreactivity to *Dirofilaria* antigens in people from different areas of Serbia, BMC Infect. Dis. 14 (2014) 68, https://doi.org/10.1186/1471-2334-14-68.
[15] L. Ciuca, F. Simòn, L. Rinaldi, L. Kramer, M. Genchi, G. Cringoli, D. Acatrinei, L. Miron, R. Morchon, Seroepidemiological survey of human exposure to *Dirofilaria* spp. in Romania and Moldova, Acta. Trop. 187 (2018) 169–174, https://doi.org/10.1016/j.actatropica.2018.07.012.
[16] S. Otašević, S. Momčilović, S. Savić, B. Gajić, S. Gabrielli, Survey of canine and human cases of dirofilariosis in parts of Serbia, Proceedings of 6th European *Dirofilaria* and *Angiostrongylus* Days, Parasite. Vector., Belgrade, Serbia, 2018, p. 623, , https://doi.org/10.1186/s13071-018-3183-z.
[17] S. Savić, S. Gabrielli, M. Žekić, D. Marčić, B. Gajić, S. Momčilović, A. Potkonjak, L. Ćurčin, S. Otašević, Prevalence and diagnostic methods for dirofilariosis in dog population from azylum and shelters in Vojvodina, Serbia, Proceedings of 6th European *Dirofilaria* and *Angiostrongylus* Days, Parasite. Vector., Belgrade, Serbia, 2018, p. 623, , https://doi.org/10.1186/s13071-018-3183-z.
[18] M. Casiraghi, O. Bain, R. Guerrero, C. Martin, V. Pocacqua, S.L. Gardner, A. Franceschi, C. Bandi, Mapping the presence of *Wolbachia pipientis* on the phylogeny of filarial nematodes: evidence for symbiont loss during evolution, Int. J. Parasitol. 34 (2004) 191–203, https://doi.org/10.1016/j.ijpara.2003.10.004.
[19] A.M. Džamić, T. Kalezić, I. Čolović Čalovski, D. Rašić, M. Cvetković, S. Mitrović, Superficial *Dirofilaria repens* infection: a case series in Serbia, Fifth European *Dirofilaria* and *Angiostrongylus* Days (FiEDAD) 2016, Parasite. Vector., Vienna, Austria, 5 2016, p. A21, , https://doi.org/10.1186/s13071-016-1902-x.
[20] S.A. Tasić-Otašević, M.S. Trenkić Božinović, S.V. Gabrielli, C. Genchi, Canine and human *Dirofilaria* infections in the Balkan Peninsula, Vet. Parasitol. 209 (2015) 151–156, https://doi.org/10.1016/j.vetpar.2015.02.016.
[21] M. Krstić, S. Gabrielli, M. Ignjatović, S. Savić, G. Cancrini, G. Ranđelović, S. Momčilović, S. Stojnev, S. Otašević, An appraisal of canine and human cases reveals an endemic status of dirofilariosis in parts of Serbia, Mol. Cell. Probes 31 (2017) 37–41, https://doi.org/10.1016/j.mcp.2016.08.005.
[22] K. Chaudhry, S. Khatana, N. Dutt, Y. Mittal, S. Sharma, P. Elhence, Systematic review of lesser known Parasitoses: maxillofacial dirofilariasis, J. Maxillofac. Oral. Surg. 18 (2019) 180–189, https://doi.org/10.1007/s12663-018-1139-7.
[23] V. Velev, M. Dinkova, A. Mirtschew, Oral live *Dirofilaria repens* infection, QJM. 111 (2018) 815–816, https://doi.org/10.1007/s12663-018-1139-7.
[24] A. Vélez-Pérez, L. Liang, E. Syklawer, V. Chavez, S. Zhang, A. Wanger, Dirofilariasis presenting as an infiltrative mass in the right buccal space, Int. J. Surg. Pathol. 24 (2016) 660–662, https://doi.org/10.1177/1066896916653212.
[25] L.L. Pereira, R.D. Coletta, L.C. Monteiro, V.Y. Ferreira, J.E. Leon, P.R. Bonan, Dirofilariasis involving the oral cavity: report of the first case from South America, Rev. Soc. Bras. Med. Trop. 48 (2015) 361–363, https://doi.org/10.1590/0037-8682-0025-2015.
[26] E.W. To, W.M. Tsang, K.F. Chan, Human dirofilariasis of the buccal mucosa: a case report, Int. J. Oral Maxillofac. Surg. 32 (2003) 104–106, https://doi.org/10.1054/ijom.2001.0217.
[27] R.S. Desai, N. Pai, A.P. Nehete, J.S. Singh, Oral dirofilariosis, Indian J. Med. Microbiol. 33 (2015) 593–594, https://doi.org/10.4103/0255-0857.167342.
[28] S. Kurup, R. Veeraraghavan, R. Jose, U. Puthalath, Filariasis of the buccal mucosa: A diagnostic dilemma, Contemp. Clin. Dent. 4 (2013) 254–257, https://doi.org/10.4103/0976-237X.114883.
[29] S.M. Balaji, Live *Dirofilaria* in buccal mucosa, Indian J. Dent. Res. 25 (2014) 546–547, https://doi.org/10.4103/0970-9290.142581.
[30] W.M. Tilakaratne, T.N. Pitakotuwage, Intra-oral *Dirofilaria repens* infection: report of seven cases, J. Oral. Pathol. Med. 32 (2003) 502–505, https://doi.org/10.1034/j.1600-0714.2003.00183.x.
[31] M. Janardhanan, S. Rakesh, V. Savithri, Oral dirofilariosis, Indian J. Dent. Res. 25 (2014) 236–239, https://doi.org/10.4103/0970-9290.135932.
[32] B.M. Collins, A.C. Jones, F. Jimenez, *Dirofilaria tenuis* infection of the oral mucosa and cheek, J. Oral Maxillofac. Surg. 51 (1993) 1037–1040, https://doi.org/10.1016/S0278-2391(10)80052-7.
[33] N. Gunathilaka, S. Siriwardana, L. Wijesooriya, G. Gunaratne, N. Perera,

Subcutaneous dirofilariasis caused by *Dirofilaria* (*Nochtiella*) *repens* in Sri Lanka: a potential risk of transmitting human dirofilariasis, SAGE open med case rep. 5, 2050313X17701373 (2017), https://doi.org/10.1177/2050313X17701373.
[34] C.H. Mallawarachchi, N.T.G.A. Chandrasena, S. Wickramasinghe, R. Premaratna, N.Y.I.S. Gunawardane, N.S.M.S.M. Mallawarachchi, N.R. de Silva, A preliminary survey of filarial parasites in dogs and cats in Sri Lanka, PLoS One 13 (2018) e0206633, , https://doi.org/10.1371/journal.pone.0206633.
[35] M.P. Senanayake, M.L. Infaq, S.G. Adikaram, P.V. Udagama, Ocular and subcutaneous dirofilariasis in a Sri Lankan infant: an environmental hazard caused by dogs and mosquitoes, Paediatr. Int. Child. Health. 33 (2013) 111–112, https://doi.org/10.1179/2046905512Y.0000000024.
[36] C. Genchi, L. Kramer, Subcutaneous dirofilariosis (*Dirofilaria repens*): an infection spreading throughout the old world, Parasite. Vector. 10 (2017) 517, https://doi.org/10.1186/s13071-017-2434-8.
[37] R.G. Kini, J.B. Leena, P. Shetty, R.H. Lyngdoh, D. Sumanth, L. George, Human dirofilariasis: an emerging zoonosis in India, J. Parasit. Dis. 39 (2013) 349–354, https://doi.org/10.1007/s12639-013-0348-8.
[38] P.J. Yaranal, M.M. Priyadarshini, B. Purushotham, Human subcutaneous dirofilariasis of forearm an unusual presentation, Indian. J. Dermatol. 60 (2015) 103, https://doi.org/10.4103/0019-5154.147849.
[39] A.S. Krishna, N. Bilahari, Savithry R.P. Ambooken, Subcutaneous infraorbital dirofilariasis, Indian, J. Dermatol. 60 (2015) 420, https://doi.org/10.4103/0019-5154.160513.
[40] S.P. Kombade, R.S. Mantri, N.A. Ambhore, R.P. Karyakarte, Subcutaneous human dirofilariasis in Vidarbha, Indian, J. Pathol. Microbiol. 58 (2015) 387–388, https://doi.org/10.4103/0377-4929.162923.
[41] S. Sukumarakurup, B.M. Payyanadan, R. Mariyath, M. Nagesh, A.P. Moorkoth, D. Ellezhuthil, Subcutaneous human dirofilariosis, Indian, J. Dermatol. Venereol. Leprol. 81 (2015) 59–61, https://doi.org/10.4103/0378-6323.148576.
[42] M. Sreekumar, J. Goethe, R.H. Chekkattu, Dirofilariasis mimicking an osteoma, Neurol. India 66 (2018) 255–256, https://doi.org/10.4103/0028-3886.222868.
[43] K.M. Seema, A case of pediatric ocular dirofilariosis, Kerala, J. Ophthalmol. 29 (2017) 136–138, https://doi.org/10.4103/kjo.kjo_62_17.
[44] S. Shivani, K. Avani, R. Mehta Preeti, L. Gatne Mukulesh, Subconjunctival dirofilariosis, JCR 5 (2015) 340–342, https://doi.org/10.17659/01.2015.0087.
[45] U. Kim, M. Gupta, N. Vidhya Shanti, Ocular *Dirofilaria repens*: not rare, DJO 24 (2013) 34–36, https://doi.org/10.7869/djo.2013.6.
[46] A. Joy, M. Narayanan, T.V. Ravi, P.S. Binu, Dirofilariasis: A Report on Emerging Zoonosis in South India, JCR 7 (2017) 306–308, https://doi.org/10.17659/01.2017.0082.
[47] K.S. Bhat, S. Kotigadde, K. Vishwanatha Bhat, P. Pare, Subconjunctival dirofilariasis: case studies with review of literature, Trop. Parasitol. 4 (2014) 119–121, https://doi.org/10.4103/2229-5070.138541.
[48] D. Das, K. Das, S. Islam, K. Bhattacharjee, H. Bhattacharjee, S.M. Das, A. Deka, A rare case of anterior chamber dirofilariosis, Oman. J. Ophthalmol. 8 (2015) 50–53, https://doi.org/10.4103/0974-620X.149868.
[49] N.S. Stoyanova, A case of subconjunctival dirofilariasis in Bulgaria, Folia. Med. (Plovdiv). 60 (2018) 323–327, https://doi.org/10.1515/folmed-2017-0097.
[50] R.N. Harizanov, D.P. Jordanova, I.S. Bikov, Some aspects of the epidemiology, clinical manifestations, and diagnosis of human dirofilariasis caused by *Dirofilaria repens*, Parasitol. Res. 113 (2014) 1571–1579, https://doi.org/10.1007/s00436-014-3802-3.
[51] I.T. Kaftandjiev, R.N. Harizanov, Rare case of epididymal dirofilariosis, QJM. 109 (2016) 351–352, https://doi.org/10.1093/qjmed/hcw034.
[52] J.R. Milanez de Campos, C.S. Barbas, L.T. Filomeno, A. Fernandez, H. Minamoto, J.V. Filho, F.B. Jatene, Human pulmonary dirofilariasis: analysis of 24 cases from São Paulo, Brazil, Chest. 112 (1997) 729–733, https://doi.org/10.1378/chest.112.3.729.
[53] D. Otranto, D.G. Diniz, F. Dantas-Torres, M. Casiraghi, I.N. de Almeida, L.N. de Almeida, J.N. dos Santos, A.P. Furtado, E.F. de Almeida Sobrinho, O. Bain, Human intraocular filariasis caused by *Dirofilaria* sp. nematode, Brazil, Emerg. Infect. Dis 17 (2011) 863–866, https://doi.org/10.3201/eid1705.100916.
[54] R.S. Cavallazzi, A.C. Cavallazzi, I.V. Souza, J.J. Deus Cardoso, Dirofilariose pulmonar humana: relato de sete casos, J. Pneumologia. 28 (2002) 100–102, https://doi.org/10.1590/S0102-35862002000200007.
[55] R. Rodrigues-Silva, R.J. Guerra, F.B. de Almeida, J.R. Machado-Silva, D.D. de Paiva, Human pulmonary dirofilariasis at Rio de Janeiro, Brazil: a case report, Rev. Soc. Bras. Med. Trop. 37 (2004) 56–59, https://doi.org/10.1590/S0037-86822004000100015.
[56] To, K.K, S.S. Wong, R.W. Poon, N.J. Trendell-Smith, A.H. Ngan, J.W. Lam, T.H. Tang, A.K. AhChong, J.C. Kan, K.H. Chan, K.Y. Yuen, A novel *Dirofilaria* species causing human and canine infections in Hong Kong, J. Clin. Microbiol. 50 (2012) 3534Y41, https://doi.org/10.1128/JCM.01590-12.
[57] W.K. Ng, T.H. Siu, B. Fung, J.H. Kong, Dirofilariasis of breast: report of two cases diagnosed by fine-needle aspiration biopsy, Diagn. Cytopathol. 26 (2002) 22–25, https://doi.org/10.1002/dc.10009.
[58] R.P. Kwok, P.P. Chow, J.K. Lam, A.C. Fok, V. Jhanji, V.W. Wong, A.L. Young, Human ocular dirofilariasis in Hong Kong, Optom. Vis. Sci. 93 (2016) 545–548, https://doi.org/10.1097/OPX.0000000000000770.
[59] R. Morchón, E. Carretón, J. González-Miguel, I. Mellado-Hernández, Heartworm disease (*Dirofilaria immitis*) and their vectors in Europe - new distribution trends, Front. Physiol. 3 (2012) 196, https://doi.org/10.3389/fphys.2012.00196.
[60] D. Bowman, S.E. Little, L. Lorentzen, J. Shields, M.P. Sullivan, E.P. Carlin, Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey, Vet. Parasitol. 160 (2009) 138–148, https://doi.org/10.1016/j.vetpar.2008.10.093.
[61] J. Drake, S. Wiseman, Increasing incidence of *Dirofilaria immitis* in dogs in USA with focus on the southeast region 2013-2016, Parasite. Vector. 11 (2018) 39, https://doi.org/10.1186/s13071-018-2631-0.
[62] R. Pietikäinen, S. Nordling, S. Jokiranta, S. Saari, P. Heikkinen, C. Gardiner, A.M. Kerttula, T. Kantanen, A. Nikanorova, S. Laaksonen, A. Lavikainen, A. Oksanen, *Dirofilaria repens* transmission in southeastern Finland, Parasite. Vector. 10 (2017) 561, https://doi.org/10.1186/s13071-017-2499-4.