

CASE REPORT

Rare Case of Orbital Granuloma Caused by Coexisting Male and Female *Dirofilaria repens* Worms: Implications for Human Host Status

ABSTRACT

Introduction:

Dirofilariasis is a ubiquitous zoonotic infection mainly involves the canine animals. Mosquitoes acts as intermediate hosts. Humans are accidental hosts and considered dead end of the parasite transmission. *D. repens* typically involve the subcutaneous region presenting as a soft tissue granuloma. Possible emergence of humans as definitive host is discussed.

Presentation of case:

This case report involves a 45year old male from Puducherry, India with orbital soft tissue granuloma caused by *Dirofilaria repens*. Histopathology of the granuloma shows presence of both male and female worms co-existing in a single lesion. The clinical, radiological, and histopathological findings are presented providing insights into this rare condition.

Discussion:

Humans are considered as unsuitable hosts for *D.repens* infestation. The larvae deposited in the subcutaneous tissue are unable to reach maturation because of the hosts defending immune system which arrests their growth leading to the development of a granuloma. Diagnosis of the condition often occurs when the dead or damaged nematodes are isolated from the patient's excised tissue.*D. repens* typically involve as single worm infestation in humans. Presence of both male and female worm co-existing in a single lesion is an unusual presentation.

Conclusion:

This case report underscores the need for awareness of rare parasitic infestations such as dirofilariasis which can mimic as soft tissue orbital granulomas, should be included in the differential diagnosis of orbital mass lesions. The unique finding of dirofilarial couple encysted in a granuloma provides evidence, humans may be emerging as a definitive host to *D. repens*.

Key words: Dirofilariasis, orbital granuloma, Histopathology, Definitive host

INTRODUCTION

Dirofilaria is a nematode from the Onchocercidae family, affecting dogs and wild carnivores. Key species include *D. repens* and *D. immitis*. Dogs serve as definitive hosts, while mosquitoes *Aedes*, *Culex*, *Anopheles* of the family Culicidae, act as intermediate hosts^[1]. When a mosquito feeds on an infected host, it ingests microfilariae, which mature into third larval stage in the mosquito's Malpighian tubules and migrate to its proboscis. These larvae are transferred to a new host during a mosquito bite. In the definitive host, such as dogs and coyotes, the larvae mature into adult worms, with *D. repens* typically causing subcutaneous nodules and *D. immitis* leading to pulmonary lesions^[2]. Adult females measure 100–170 mm by 460–650 μm, while males are smaller measuring 50–70 mm by 190–260 μm. It is believed humans are accidental hosts and not involved in further transmission^[2,3].

Globally, around 800 cases of dirofilariasis have been documented, with endemic regions including Eastern Europe, Southern Europe, Asia Minor, Central Asia, and Sri Lanka. In India, there has been a noticeable increase in dirofilariasis cases, primarily due to *Dirofilaria repens*, especially in the regions geographically closer to Sri Lanka^[4]. One major factor postulated for this rise in incidence is environmental changes, particularly the increase in temperature. Warmer temperatures prolong the feeding and breeding periods of mosquito vectors, leading to a larger pool of larvae and consequently a higher incidence of the disease^[5].

Usual presentation of *D. repens* in humans is a solitary subcutaneous nodule commonly involving the upper body (76%) and infrequently lower body (24%). Orbital and ocular involvement accounts for 31% cases which can be periocular, subconjunctival or intraocular^[6]. The subconjunctival space is the most frequently involved site (>60%) followed by the eyelids and orbit (25%). The high predisposition of *Dirofilaria repens* to cause subcutaneous nodules is attributed to its lower immunogenicity in subcutaneous regions and its ability to reduce the host's immune response. Additionally, *Dirofilaria repens* harbors an endosymbiotic bacterium that plays a crucial role in reducing the host's innate immune response, contributing to the parasite's lower immunogenicity and enhanced survival^[7].

Humans are generally regarded as unsuitable for completing the life cycle of *Dirofilaria repens* and are considered as accidental hosts. In canines the parasite has been studied to have a

lengthy pre-patent period of 170 to 238 days, with microfilariae typically appearing in the bloodstream around day 164 post-infection^[8]. Ernakova et al. found sexually mature dirofilarial parasites in 10.4% of nodules, suggesting that humans might act as dual facultative hosts. Long pre-patent period, low immunogenicity of *D. repens*, and detection of microfilariae in the bloodstream have led some researchers to believe that humans could potentially be definitive hosts^[9,10]. However this assumption can only be confirmed if both adult male and female *Dirofilaria* worms demonstrated within a solitary nodule. In this report we provide histopathological evidence of the presence of adult male and female dirofilarial worms encapsulated within a single subcutaneous granuloma providing evidence for humans to be considered as definitive hosts in the life cycle of *D. Repens*.

PRESENTATION OF CASE:

A 45-year-old male from Puducherry, India presented with painless swelling of the right eye lower lid for the past 3 months. Patient gave history of mosquito bite 3 months back following which he developed localized intense itching and mild pain which subsided after taking a course of antihistamines. However swelling continued to persist with gradual increase in size. There was no recent travel history and no contact with pets. On examination an ill-defined, firm, mobile, and non-tender mass, approximately 2x2 cm in size, not attached to the bone or the overlying skin was present at the inferior orbital margin below the lower lid [Figure 1]. Extraocular movements were full. Anterior segment and posterior segment examination was within normal limits. Systemic examination was normal.



Figure 1: Preoperative picture showing ill-defined lesion below the right lower lid

Investigations Hb, total WBC, ESR, Peripheral smear, Absolute eosinophil count, chest Xray and Mantoux were normal. CT scan showed infraorbital soft tissue lesion seen in anteroinferior aspect of the right orbit bordering the globe and the inferior rectus muscle insertion [Fig. 2-a and b]. Differential diagnosis of lipoma, dermoid cyst, tubercular/ sarcoid/ foreign body granuloma was considered.

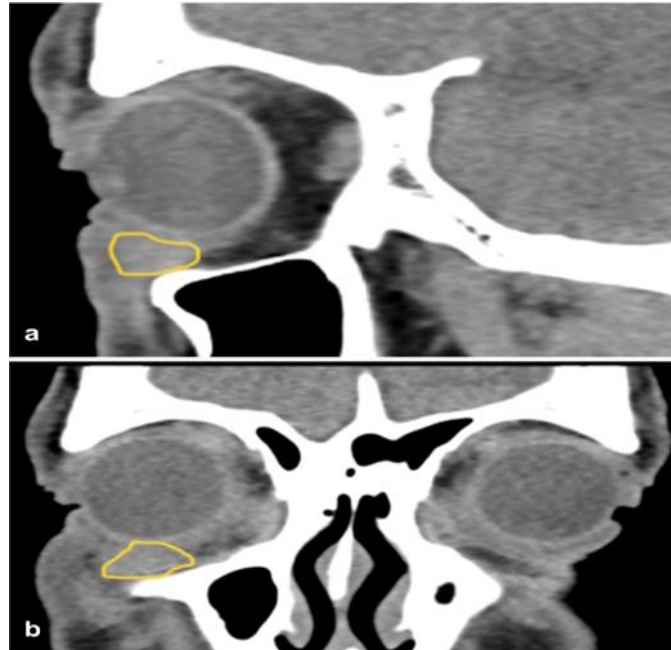


Figure 2: CT Orbit sagittal section[a]/coronol section(b) showing infraorbital soft tissue lesion in anteroinferior aspect of the right orbit bordering the globe and the inferior rectus muscle insertion.No calcification/ fat noted. Globe and intraconal space were normal

Excisional biopsy was performed and sent for histopathological analysis. Gross examination revealed a single, grey-brown, homogenous soft tissue fragment measuring 1.8 x 1.3 x 0.7 cm. Histopathological examination showed loose, hyperemic fibro-collagenous tissue with granuloma formation and a dense inflammatory cell infiltrate surrounding multiple lobules of dead worms belonging to the *Dirofilaria* group [Fig. 3a].

Cross section of two worms revealed distinct features. Both worms displayed prominent multilayered cuticle, indicating both worms belonged to *Dirofilaria repens* genus. Cross section of one of the worms showed noticeable lateral cords, a curved body, two uterine tracts, and a digestive tract, suggesting it is a female *D. repens*. Cross section of another worm exhibited parts of the intestine, granular testes, and characteristic spicules, indicative of a male *D. repens*. Both the worms were encapsulated within a single granulomatous tissue comprising of inflammatory response lymphocytes, eosinophils, plasma cells, and epithelioid cells.

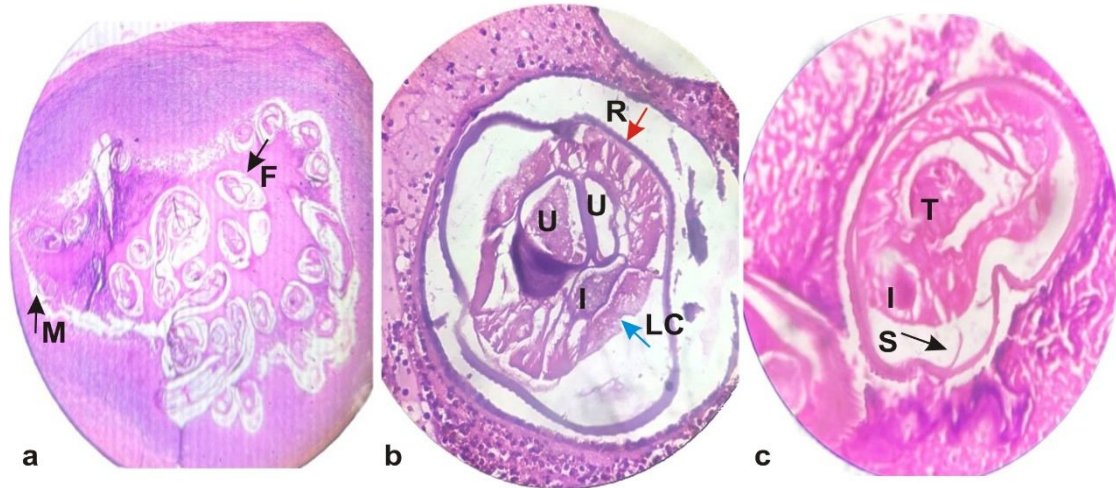


Figure 3: Histopathological section (Hematoxylin and eosin) of the granuloma showing [a] multilobulate cut section of the dead *Dirofilaria* worms with surrounding dense inflammatory cells, M- cross section of male worm and F- cross section of female worm (under low magnification-10x). [b] Cross sectional view of the female worm showing the typical external cuticular ridges(R) (red arrow), lateral chords (blue arrow) and two uterine tracts(U) and intestine(I)(40x). [c] Cross sectional view of the male worm having granular testis(T), intestine(I) and characteristic spicules(S) (black arrow). (R-cuticular ridges, U-uterine tract, I-intestine, LC-lateral chords, T-testis, S-spicules)

Post-operatively patient did not have any complications and recovered completely. He was referred to General medicine department and treated with oral Ivermectin. Patient was followed up for 3 months and did not have any complication or recurrence.

DISCUSSION

This case highlights the rare occurrence of orbital dirofilariasis granuloma. Human dirofilariasis can be divided to two types pulmonary and extrapulmonary. Pulmonary dirofilariasis is caused by *D. immitis* species which is mostly asymptomatic but patients may present with cough, fever, chest pain and pleural effusion. Extrapulmonary dirofilariasis mainly caused by *D. repens* species can be subcutaneous, visceral and ophthalmic. *D. repens*, *D. ursi*, *D. tenuis*, and *D. striata* exhibit

longitudinal cuticular ridges and are usually found in subcutaneous tissues whereas the *D. immitis* will have a smooth cuticle^[11].

In humans, considered intermediate host, the infection process starts when a mosquito bite deposits microfilarial larvae onto the skin. These larvae then enter the subcutaneous tissue, where they may either migrate to other areas or begin to mature, forming a subcutaneous nodule. The host's immune system typically responds to the *Dirofilaria repens* antigens by killing the larvae before its maturation and surrounded by dense connective tissue capsule. This immune response halts the development of the nematodes, leading to their destruction. Diagnosis often occurs when the dead, damaged, or live nematodes are isolated from the patient's tissue, as the larvae undergo some developmental stages in the abnormal host before being identified and eliminated by the immune system^[12].

Humans are considered atypical hosts for these parasites, as the host's immune response typically eradicates the infectious larvae. However, contrary to common belief, Claudio et al. suggested that human's exhibit weak immune response to the parasite because of subcutaneous localization of the parasite, which helps in evading hosts defenses, allowing for potential maturation. In our case, the patient presented with swelling of the lower eyelid without other inflammatory symptoms^[7]. We hypothesize that the reduced inflammation observed could be due to the factors mentioned, and that a diminished immune response may have allowed the worms to reach sexual maturity, ultimately leading to granuloma formation.

Epidemiological data from India indicates that *Dirofilaria* infections typically involve a single worm encysted within a granuloma. Among 73 reported cases, only two patients were documented to have multiple worm infestations. Sekhar et al. (2000) and Sangit et al. (2012) described instances of paired adult female dirofilarial worms in a granuloma^[3,13,14]. Notably, none of the reports mention the coexistence of adult male and female *Dirofilaria*. This case represents the first documented occurrence of a matured adult male and female *Dirofilaria* found encysted in a subcutaneous nodule.

Ermakova et al., in a study involving 266 cases of human dirofilarial infestation, concludes humans serve as a dual facultative hosts and even possibly biological dead end^[9]. However, a report by Ana Bakrac presents contrasting findings. Bakrac observed microfilariae in the bloodstream of a patient with a subcutaneous nodule caused by an adult female *Dirofilaria*. Ana

Bakrac suggests the presence of microfilariae indicates likely presence of an adult male, even if not specifically detected, challenging the notion that humans are dead-end hosts. Bakrac concludes that if the simultaneous presence of sexually matured adult male and female dirofilarial worms can be demonstrated, then humans are likely definitive hosts^[10]. In our patient, sexually matured adult dirofilarial worms, males with testis and spicules, females with uterine cavities, were found coexisting in a soft tissue granuloma. This simultaneous presence of adult male and female worms within a single granulomatous nodule has not been histopathologically demonstrated previously, lending support to Ana Bakrac's proposition that humans may not be dead-end hosts but rather definitive hosts for this helminth.

Limitation in this report is the identification of the parasite by morphological means. In India, many studies rely on morphological features for parasite identification. While DNA analysis through polymerase chain reaction (PCR) offers more precise identification, it is not always feasible in clinical laboratories due to the need for numerous specific probes for accurate diagnosis^[15].

This case report underscores the need for awareness of rare parasitic infestations such as dirofilariasis which can mimic as soft tissue orbital granulomas. Molecular test should be performed to achieve a more accurate etiological diagnosis. Specific serological tests need to be improved in future research work, as well as molecular markers to detect possible existence of cryptic species or subpopulations within *D. repens*.

CONCLUSION

This case report underscores the need for awareness of rare parasitic infestations such as dirofilariasis which can mimic as soft tissue orbital granulomas. Previous studies have reported adult worms of same sex coexisting. We report a first case of histopathologically confirmed adult male and female worms encysted in orbital soft tissue granuloma. This unique finding of dirofilarial couple encysted in a granuloma provides evidence, suggesting, humans may be emerging as a definitive host to *D. repens*. We advocate further research into the epidemiology, pathogenesis and reproductive cycle of human dirofilarial infestations.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1. Chat GPT used for grammar check and proof reading.

REFERENCES

- 1) Parasmitha Das Choudhury, Dina Raja, Vaishali Sarma: Human subcutaneous dirofilariasis: A diagnostic dilemma. *Trop Parasitol.* 2023 Jul-Dec; 13(2): 118–121.
- 2) Matthew Camacho, Antonietti, Yoseph Sayegh, Jordan D. Colson, Anne, Kevin D. Clauss, Hannah Muniz-Castro, Wendy W. Lee, Sonia H. Yoo, Thomas E. Johnson, and Sander R. Dubovy Ocular Dirofilariasis: A Clinicopathologic Case Series and Literature Review, *Ocul Oncol Pathol.* 2024 Apr; 10(1): 43–52.
- 3) Reshma G Kini, J B Leena, Prathvi Shetty, Raphael Hart Lyngdoh, D Sumanth, Lovely George: Human dirofilariasis: an emerging zoonosis in India. *J Parasit Dis.* 2015 Jun;39(2):349-54.
- 4) S Pampiglione, F Rivasi, G Angeli, R Boldorini, R M Incensati, M Pastormerlo, M Pavesi, A Ramponi. Dirofilariasis due to *Dirofilaria repens* in Italy, an emergent zoonosis: report of 60 new cases. *Histopathology.* 2001 Dec 9; 38, 344-354.
- 5) Genchi C, Rinaldi L, Mortarino M, Genchi M, Cringoli G. Climate and dirofilaria infection in Europe. *Vet Parasitol.* 2009;163(4):286–92

- 6) M Smitha, VR Rajendran, E Devarajan, PM Anitha: Case report: Orbital dirofilariasis. Indian J Radiol Imaging. 2008 Feb; 18(1): 60–62
- 7) Claudio Genchi, Laura Kramer: Subcutaneous dirofilariosis: an infection spreading throughout the old world. Parasit Vectors. 2017 Nov 9;10(Suppl 2):517.
- 8) L. Ciuca, A. Vismarra, W. Lebon , F. Beugnet, R. Morchon, L. Rinaldi , G. Cringoli L. Kramer c, M. Genchi. New insights into the biology, diagnosis and immune response to *Dirofilaria repens* in the canine host. Veterinary Parasitology: X 4 (2020) 100029.
- 9) Larisa Ermakova, Sergey Nagorny, Natalia Pshenichnaya, Yury Ambalov and Kerim Boltachievc: Clinical and laboratory features of human dirofilariasis in Russia. IDCases. 2017; 9: 112–115.
- 10) Ana Pupić-Bakrač, Jure Pupić-Bakrač, Ana Beck, Daria Jurković, Adam Polkinghorne, and Relja Beckd :*Dirofilaria repens* microfilaremia in humans: Case description and literature review. One Health. 2021 Dec; 13: 100306.
- 11) Nobuaki Akao. Human Dirofilariasis in Japan. Trop Med Health. 2011 Mar; 39(1 Suppl 2): 65–71.
- 12) Beata Rymgayłło-Jankowska, Marta Ziaja-Sołtys, Beata Flis, Anna Bogucka-Kocka and Tomasz Zarnowski : Subcutaneous Dirofilariosis of the Eyelid Brought to Poland from the Endemic Territory of Ukraine. Pathogens. 2023 Jan 28;12(2):196.
- 13) Sekhar HS, Srinivasa H, Batru BR, Mathai E, Shariff S, Macaden RS (2000) Human ocular dirofilariasis in Kerala, Southern India. Indian J PatholMicrobiol. 2000; 43:77–79
- 14) Sangit VA, Haldipurkar SS (2012) Subconjunctival dirofilariasis mimicking scleritis: first report from Western India. Indian J Ophthalmol. 2012; 60:76–77

15) Simón F, Morchón R, González-Miguel J, Marcos-Atxutegi C, Siles-Lucas M. What is new about animal and human dirofilariosis? Trends Parasitol. 2009;25:404–9.

UNDER PEER REVIEW