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CASE REPORT

In vivo confocal microscopy for detection of subconjunctival *Onchocerca lupi* infection in a dog

Michele L. Edelmann,* Mason Jager,† Filipe Espinheira* and Eric C. Ledbetter*

**Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA; and †Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA*

Address communications to:
E. C. Ledbetter
Tel.: (607) 253-3060
Fax: (607) 253-3534
e-mail: ecl32@cornell.edu

Abstract

A seven-year-old male castrated mixed-breed dog was diagnosed with bilateral subconjunctival masses. *In vivo* confocal microscopy facilitated visualization of *Onchocerca lupi* adult nematodes and their characteristic cuticular morphology. Long, thin, white nematodes were extracted during excisional biopsy. Histopathologic and parasitologic evaluation confirmed the diagnosis of *O. lupi*. In addition to surgical debulking of the parasitic granulomas, the dog received systemic doxycycline, prednisone, and ivermectin therapy. *In vivo* confocal microscopy was repeated one year after initial diagnosis, and no remaining nematodes were visible. To the authors' knowledge, this is the first report of use of *in vivo* confocal microscopy as a noninvasive diagnostic and monitoring tool for canine onchocerciasis.

Key Words: canine, *in vivo* confocal microscopy, nematode, ocular, *Onchocerca lupi*, onchocerciasis

INTRODUCTION

Onchocerca lupi is an established ocular parasite of dogs with newfound zoonotic potential.[1] Reported *O. lupi* cases in dogs have been on the rise for the past 10–15 years in both the United States and Europe.[2–15] *O. lupi* is a filarial nematode that utilizes wolves, dogs, and cats as definitive hosts.[5,8,12,13] Blackflies (Simuliidae) are the usual vectors of human and animal onchocerciasis and are endemic in all geographical regions where *O. lupi* has been recorded.[5,13] A recent study identified *Simulium tribulatum* flies as the putative vector for *O. lupi* in southern California.[16] Adult nematodes live and mate in the subconjunctival/periorbital space, producing microfilariae that are ingested by blackflies and transmitted to the next definitive host through skin bites.[11] Unlike *Dirofilaria spp*, *Onchocerca* microfilariae occur in the skin, but never in the bloodstream.[17] Disease is associated with a long prepatent period of months to years. Most documented *O. lupi* cases in the United States involve animals currently or previously residing in the southwest, particularly New Mexico.[9]

In early stages, canine onchocerciasis is associated with nonspecific ocular discomfort manifested by lacrimation, photophobia, conjunctivitis, ± exophthalmos, and periorbital swelling. In chronic stages, granulomatous tissue reaction to the adult parasites creates subconjunctival nodules.[8–10] Reported treatment protocols include some or all of the following: surgical debulking of parasitic granulomas, adulticidal melarsomine, *Wolbachia* endosymbiont eradication via doxycycline, microfilaricidal ivermectin, and anti-inflammatory systemic prednisone.[8–10] While surgical debulking is generally always recommended, in the largest case series of US canine *O. lupi* to date, medical therapy alone was found to be an effective treatment strategy in some cases and is suggested to be a reasonable approach for dogs where surgical debulking is not feasible due to logistical (extensive disease), financial, or anesthetic constraints.[9]

Current diagnostic techniques include PCR,[3,7] biopsy with histopathologic identification of the adult nematodes, and skin snip testing for microfilariae.[13] A recent investigation into the utility of serology/ELISA for noninvasive diagnosis of *O. lupi* showed low sensitivity; serology only successfully diagnosed 3/6 skin snip-positive animals.[17] A separate study in two asymptomatic *O. lupi* skin snip-positive dogs found that ocular ultrasound (10–18 MHz) and CT could aid in detecting the location of subclinical disease, manifested as nonspecific mineralized foci.[3] MRI did not detect the small ocular and retro-orbital lesions found on ultrasound and CT.[3] Neither ultrasound nor CT

provided sufficient detail to visualize individual nematodes and thus were not suggested for use as a sole diagnostic technique.[3]

Histopathologic diagnosis of *O. lupi* is based on the presence of characteristic cuticular ridges of the female nematodes, the presence of two internal striae per ridge, and the size and morphology of the microfilariae within the uterus of the adult females.[9] Histopathologic diagnosis generally involves sedation or anesthesia and invasive biopsy. While excisional biopsy in itself may be therapeutic, successful treatment with exclusively medical management has been described.[9] In these cases, a noninvasive diagnostic technique could be useful to reduce time and trauma. This case report describes the use of noninvasive *in vivo* confocal microscopy as a rapid diagnostic tool for subconjunctival *O. lupi*.

CASE REPORT

An approximately 7-year-old, 30.9 kg, male castrated mixed-breed dog presented to the Cornell University Hospital for Animals (CUHA) Ophthalmology Service in December 2015 for a 1-month history of a red, swollen lesion on the right eye without overt signs of discomfort. The owners reported that a similar problem had been noted in the left eye several months prior but that this had resolved with neomycin–polymyxin B-dexamethasone ointment OS TID for 2 days. Otherwise, there was no known history of ocular or other health problems. The dog had been adopted from a shelter in New Mexico 3 years prior and received monthly heartworm (ivermectin, Heartgard®) and flea/tick topical (fipronil, Frontline®) preventatives.

A complete ocular examination was performed, including slit lamp biomicroscopy (Kowa SL-15; Kowa Company, Ltd., Tokyo, Japan), indirect ophthalmoscopy (Heine, USA), and applanation tonometry (Tonopen XL, Reichert, Depew, NY). The ophthalmic examination revealed a 5–6 mm superotemporal perilimbal bulbar subconjunctival erythematous mass OD. The patient was comfortable and nonblepharospastic with no ocular discharge OU. There was no evidence of corneal or adnexal trauma or injury. The palpebral reflex, menace response, pupillary light responses, and dazzle were present OU. The Schirmer tear test results were >15 mm/min OU. There was a 2-mm mid-superior lid margin mass OD. The cornea was fluorescein stain negative OU, with mild temporal corneal edema OD associated with the subconjunctival mass. The anterior chamber was clear OU. The iris was normal with mid-range pupils OU. The intraocular pressures were 14 OD and 6 OS (mmHg). The lens exhibited mild senile nuclear sclerosis OU. The vitreous, retina, and optic nerve were normal OU. Routine physical examination was unremarkable.

The dog was discharged with empiric therapy for possible nodular granulomatous episcleritis. Prednisolone acetate 1% ophthalmic suspension OD six times daily was prescribed. The owners were informed that, if there was no improvement with this medication trial, excisional biopsy would be recommended.

The dog was reexamined 1 month later. The owners reported that they had given prednisolone as directed, but that the subconjunctival mass OD was unchanged. Ocular examination OD was indeed unchanged aside from the presence of focal yellow discoloration within the hyperemic subconjunctival nodule. There had been no progression of associated corneal edema OD. However, a new 3–4 mm superior bulbar subconjunctival nodule OS was detected (Fig. 1). This nodule was nonhyperemic, unlike OD. The Schirmer tear test results were 20 mm/min OD and 15 mm/min OS. The cornea remained fluorescein stain negative OU. The intraocular pressures were 13 OU (mmHg).

The dog was admitted to the hospital for *in vivo* confocal microscopy examination and excisional biopsy. Dexmedetomidine (6 μg/kg) and butorphanol (0.2 mg/kg) were administered intravenously for sedation. *In vivo* confocal microscopy of the nodules was performed using a modified Heidelberg Retina Tomograph II and Rostock Cornea Module. This microscope emits a 670-nm-wavelength diode laser light source and has a focal depth of 1500 μm. Images obtained revealed numerous leukocytes and hyper-reflective fibrous tissue structures within the nodule wall (Fig. 2a). The center of the nodule contained adult nematodes with characteristic ridged, hyper-reflective cuticles (Fig. 2b). High-frequency ocular ultrasound (20 MHz) was also performed, and the nodules appeared hyperechoic, but it was not possible to distinguish adult nematode forms.

Steven's tenotomy scissors were used to sharply incise the conjunctiva and bluntly undermine the masses. Upon

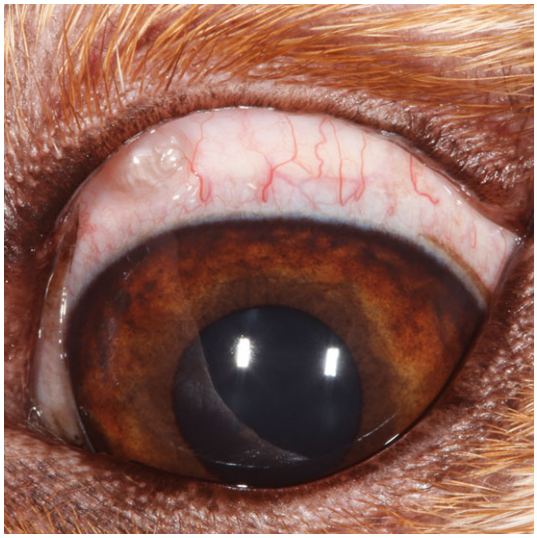

Figure 1. Left eye; nonerythematous superior bulbar subconjunctival nodule.

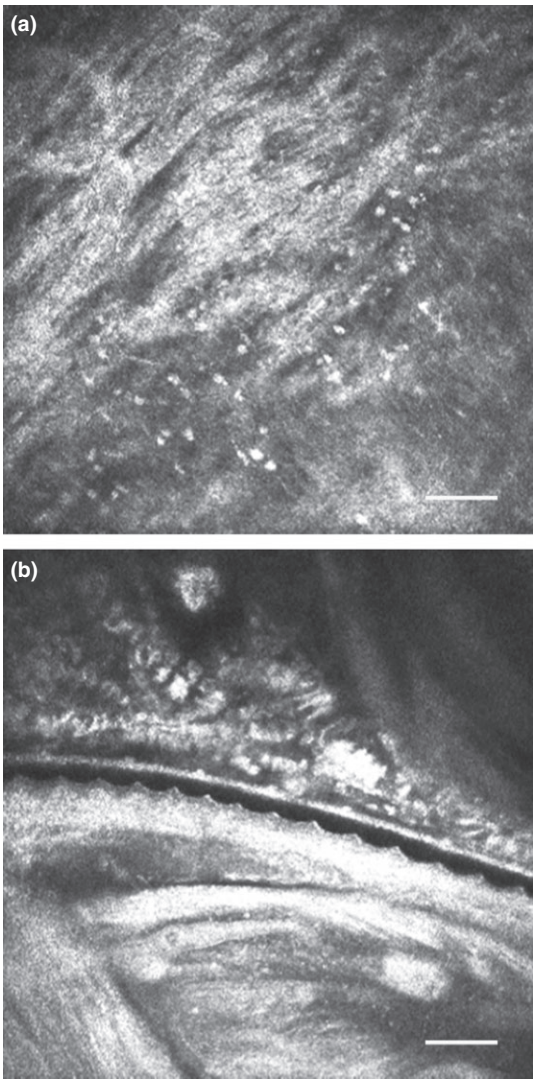

Figure 2. *In vivo* confocal microscopy images. (a) Nodule wall with numerous leukocytes and hyper-reflective fibrous tissue structures; (b) characteristic, ridged hyperechoic cuticle of an adult. Scale bars = 50 μm.

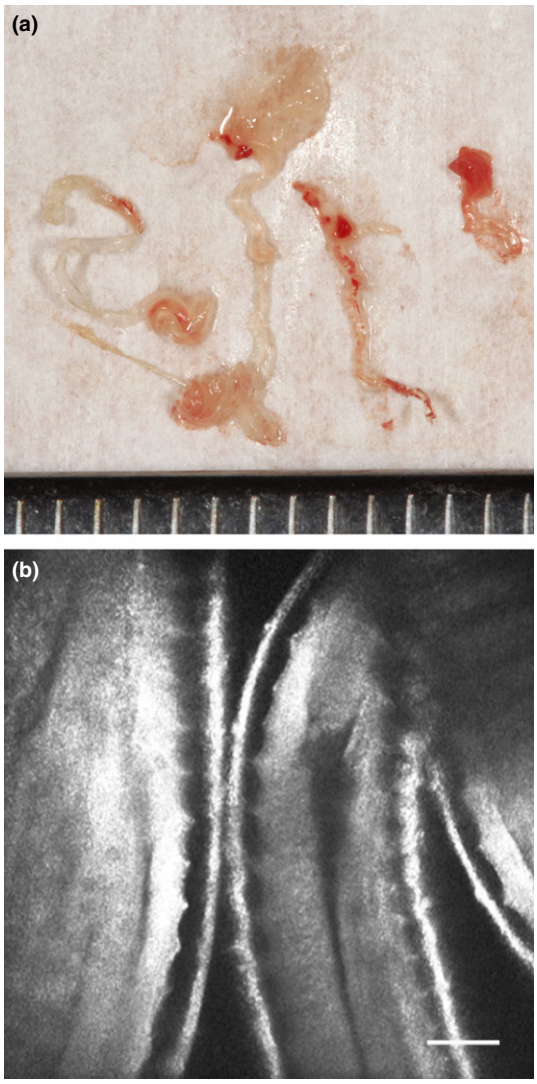

Figure 3. (a) Thin, white, adult nematodes extracted during excisional biopsy (scale in millimeters); (b) *ex vivo* confocal image of adults. Scale bar = 50 μm.

the initial incision, countless, thin, long, white, friable adult nematodes were released (Fig. 3a). Some nematodes were mobile. The parasitic masses were noted to deeply infiltrate the periocular connective tissues. The nodules were debulked to the fullest extent possible, taking care to avoid rectus muscle trauma. The conjunctival incisions were left open to heal by second intention. *Ex vivo* confocal microscopy examination of the extracted adult parasites was performed and confirmed morphology identical to that which was seen *in vivo* (Fig. 3b).

Histopathologic and gross parasitologic evaluation confirmed the nematodes to be *O. lupi* based on characteristic cuticular morphology of the females. The identification of the nematodes was confirmed by PCR assay.[8] On histopathologic evaluation, there was severe, chronic, multifocal granulomatous episcleritis with intralesional adult filarial nematodes (Fig. 4a). The adult nematodes measured 275 μm in diameter had an outer layer of evenly spaced annular cuticular ridges with an inner layer composed of striae, polymyarian and coelomyarian musculature, and a single-cell-thick gastrointestinal tract (Fig. 4b). Two inner striae were present per single external cuticular ridge (Fig. 4c). Some sections contained reproductive tracts with ova. Several parasite sections were surrounded by well-formed granulomas characterized by large numbers of epithelioid macrophages, plasma cells, neutrophils, and fibrosis (Fig. 4a).

Complete blood count results were within normal limits. Serum biochemistry was also unremarkable; all values were within normal limits aside from clinically insignificant mild hypokalemia 4.0 mEq/L (rr 4.1–5.6) and mild hypophosphatemia 2.6 mg/dL (rr 2.9–5.2). As adulticidal melarsomine therapy was planned, the patient was evaluated for

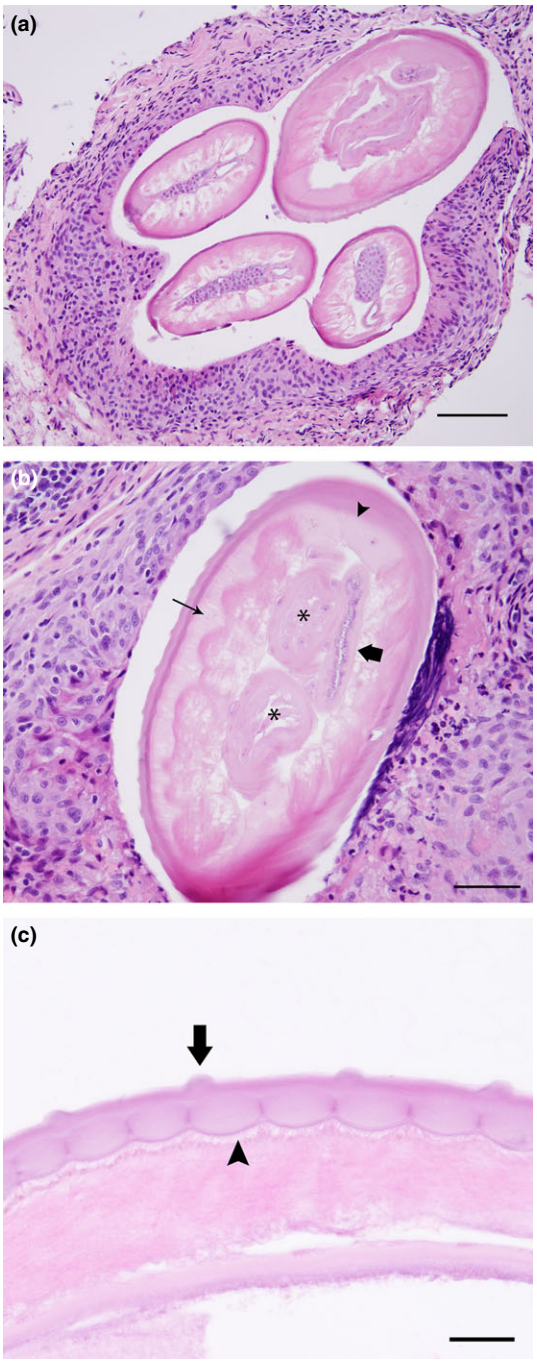

Figure 4. (a) Multiple cross sections of an adult nematode surrounded by granulomatous inflammation with epithelioid macrophages, lymphocytes, plasma cells, neutrophils, and fibrosis. Scale bar = 100 μm; (b) the nematode is approximately 275 μm in diameter with an outer layer of evenly spaced external cuticular ridges, coelomyarian/polymyarian musculature (narrow arrow), lateral cords (arrowhead), a single-cell-thick gastrointestinal tract (broad arrow), and paired uteri (asterisks). Scale bar = 50 μm; (c) higher magnification of a longitudinal section reveals the presence of two inner striae (arrowhead) per single external cuticular ridge (arrow). Scale bar = 20 μm.

subclinical *Dirofilaria immitis* infection. *Dirofilaria*, *Borrelia*, *Anaplasma*, and *Ehrlichia* (SNAP 4DX) testing was negative. Three-view thoracic radiographs were unremarkable. Given plans to initiate microfilaricidal ivermectin therapy and the dog's unknown genetic background, MDR-1 mutation testing was performed and results were negative.

The dog was initiated on neomycin–polymyxin–bacitracin OU QID × 10 days to prevent bacterial infection during conjunctival second-intention healing, tramadol 3 mg/kg PO TID × 5 days for pain associated with surgery, doxycycline 5 mg/kg PO BID × 2 weeks for endosymbiotic bacterial eradication, and prednisone 0.5 mg/kg PO BID × 2 weeks to reduce inflammation associated with parasite death, which was then tapered over the following 4 weeks (0.3 mg/kg PO BID × 2 weeks, then 0.3 mg/kg PO SID × 2 weeks). Monthly preventative therapy was transitioned from ivermectin (Heartgard®) to moxidectin (Advantage Multi®) per the recommendation of an experienced parasitologist (Dwight Bowman, MS, PhD) to reduce the likelihood of microfilariae transmission to fly vectors. Adulticidal melarsomine therapy was recommended but declined by the dog's owners due to concern for possible side effects. Microfilaricidal ivermectin 50 μg/kg was administered subcutaneously and was repeated monthly for 6 months thereafter. Instructions were given to return every 6 months thereafter for ivermectin injections for 2 more years.

The dog was followed over the following year at each recommended ivermectin injection time point. A new, superonasal subconjunctival mass OD was detected 1-week postdebulking surgery. Medical treatment was continued and ocular examination was unchanged until examination 6 months later, when the mass was not visible. Schirmer tear test values and ocular pressures remained within normal limits throughout the period of follow-up. The owners reported no ocular discomfort at any point aside from postexamination transient irritation manifested by squinting and redness. The owners attributed this to proparacaine used for applanation tonometry. At subsequent examinations, rebound tonometry was performed to avoid use of additional proparacaine. The last follow-up was performed one year after surgical debulking. The dog remained visual and comfortable with a persistent superonasal subconjunctival nodule OD and a new temporal bulbar conjunctival nodule OS (Fig. 5a). A comprehensive *in vivo* confocal microscopic examination was performed on both eyes, including the two visible nodules. There was a noticeable reduction in leukocyte presence within the nodule walls and unchanged hyper-reflective fibrous tissue structures (Fig. 5b). No remaining adult nematodes were visible within the nodules (Fig. 5b) by confocal microscopy. As such, continued medical treatment with ivermectin was elected rather than repeat invasive surgical excision of the nodules.

DISCUSSION

In vivo confocal microscopy has been recently proposed for the detection and/or monitoring of a variety of ocular conditions in veterinary patients, including but not limited

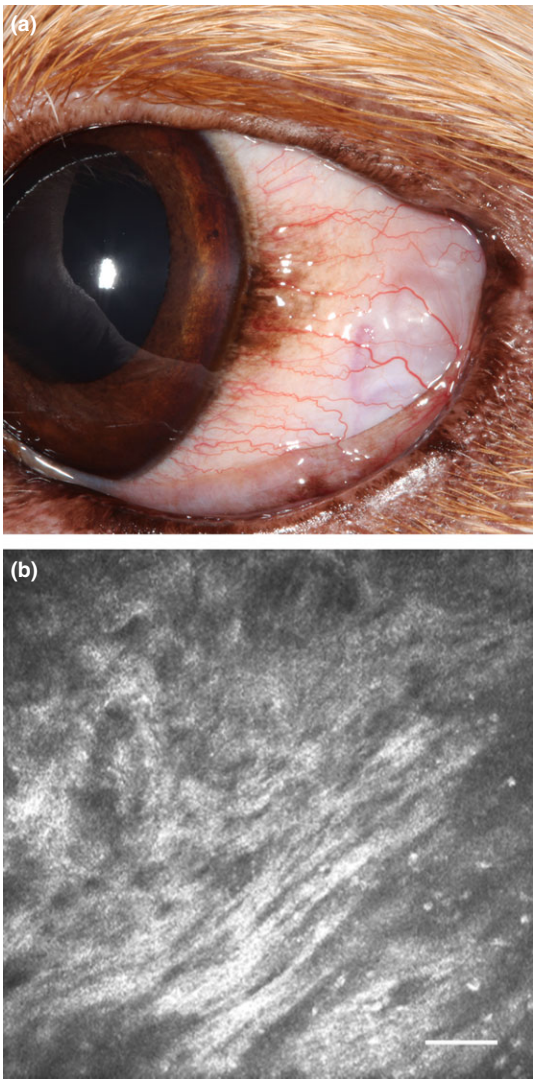

Figure 5. Last clinical follow-up, left eye. (a) Temporal bulbar conjunctival nodule; (b) *in vivo* confocal microscopic image of nodule. There was a noticeable reduction in leukocytes and unchanged hyper-reflective fibrous tissue structures. No adult nematodes were seen. Scale bar = 50 μm.

to fungal keratitis, corneal foreign bodies, and pigmentary keratitis.[18–20] The system utilizes an illumination and observation system with a common focal point, suppressing reflected light originating from outside the field of observation, so that only light reflected from the focal plane contributes to image formation.[21] This allows for excellent axial and lateral resolution and allows for the use of higher magnification than that achievable with conventional light microscopy.[22] Unlike light microscopy, *in vivo* confocal microscopy is not subject to fixation and staining artifacts, and sequential noninvasive imaging of the same structure can be performed.[23,24] Traditional diagnostic methods for *O. lupi* in dogs generally require sedation and/or tissue incision (skin snip biopsy or episcleral nodule biopsy).[9,11,13,15,17] Confocal microscopy does not require sedation or surgical biopsy to achieve a diagnosis of *O. lupi* in dogs. In addition, *in vivo* confocal microscopy provides the operator real-time images and the potential for an immediate diagnosis.

This case demonstrated recurrence of a parasitic granuloma despite initial surgical debulking. Surgical therapy was not repeated, medical therapy was continued, and while two nodules were present after 1 year, repeat confocal microscopic examination showed no remaining nematodes. In addition to complete parasite eradication, it is also possible that ocular nematodes were still present, but at different anatomic locations or in numbers too low for detection by confocal microscopy. The dog was not assessed for the presence of microfilariae; therefore, microfilariae could have been present and persistent. Ideally, the dog would have been skin snip tested before and after treatment, and the recurrent nodules excised and submitted for biopsy to confirm the absence of nematodes. In this clinical case, a desire to minimize invasive procedures was expressed by the owner given the excellent comfort of the dog. The dog remained comfortable and visual throughout treatment and follow-up.

This case lends support to the idea that medical therapy alone may be effective in some dogs[9] and may be particularly useful in dogs where surgical debulking is not feasible due to logistical (extensive disease), financial, or anesthetic constraints. These cases would benefit from a noninvasive diagnostic method as well. This report demonstrates the unique ability of *in vivo* confocal microscopy to provide a rapid, noninvasive method for the diagnosis of canine onchocerciasis and for the monitoring of treatment response.

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