

Expertise in parasite identification enables recognition and reporting of the emergence and spread of exotic parasitic diseases brought into the UK in imported pet dogs.

Background.

The Pet Travel Scheme (PETS) was introduced in 2000 and replaced some quarantine requirements in the UK. Prominent concern was raised at the time re. the serious risk posed to dogs by four vector-borne parasitic diseases, endemic in Europe but exotic to the UK: leishmaniasis, dirofilariasis (due to *Dirofilaria immitis*), ehrlichiosis and babesiosis. Although designed to enable holiday makers to travel with companion animals, PETS also facilitated commercial importation of dogs under the Balai Directive and the practice of adopting rescue dogs from abroad, largely from the Mediterranean basin and Eastern Europe. Huge year-on-year increases in movement of dogs across the UK border followed, peaking at 85,000 in 2011 under the first iteration of PETS.

Thousands of imported dogs have been tested by Liverpool Veterinary Parasitology Diagnostics (LVPD), revealing a substantial number of infections, mostly *Leishmania infantum*. While numerous publications have documented cases of exotic parasitic disease in dogs since the introduction of PETS, (including autochthonous and fatal cases of babesiosis, and reports of their vectors i.e. *Dermacentor reticulatus* and *Rhipicephalus sanguineus*), they all remained within the confines of the four diseases described above. Then, in 2016 LVPD identified *Thelazia callipaeda* recovered from the conjunctiva of one-year old dog imported from Romania, and reported the first UK case of canine ocular thelaziosis. Further exotic parasites have subsequently emerged in the UK, including *Dirofilaria repens*, *Linguatula serrata*, and *Onchocera lupi*. Here we describe in brief, cases of emerging parasitic disease processed by LVPD, all of which are of one-health concern.

1. *Thelazia callipaeda*.

Commonly referred to as the 'oriental eye worm', *Thelazia callipaeda* is a vector-borne spirurid nematode, capable of infecting the eyes and associated tissues of dogs and other mammalian host species, including humans. The competent vector is the male drosophilid fruit fly *Phortica variegata*, which is present in the UK, highlighting the potential for autochthonous transmission and establishment of endemicity. Morphological species identification by LVPD enabled the first case of canine ocular thelaziosis in the UK to be reported in 2016.

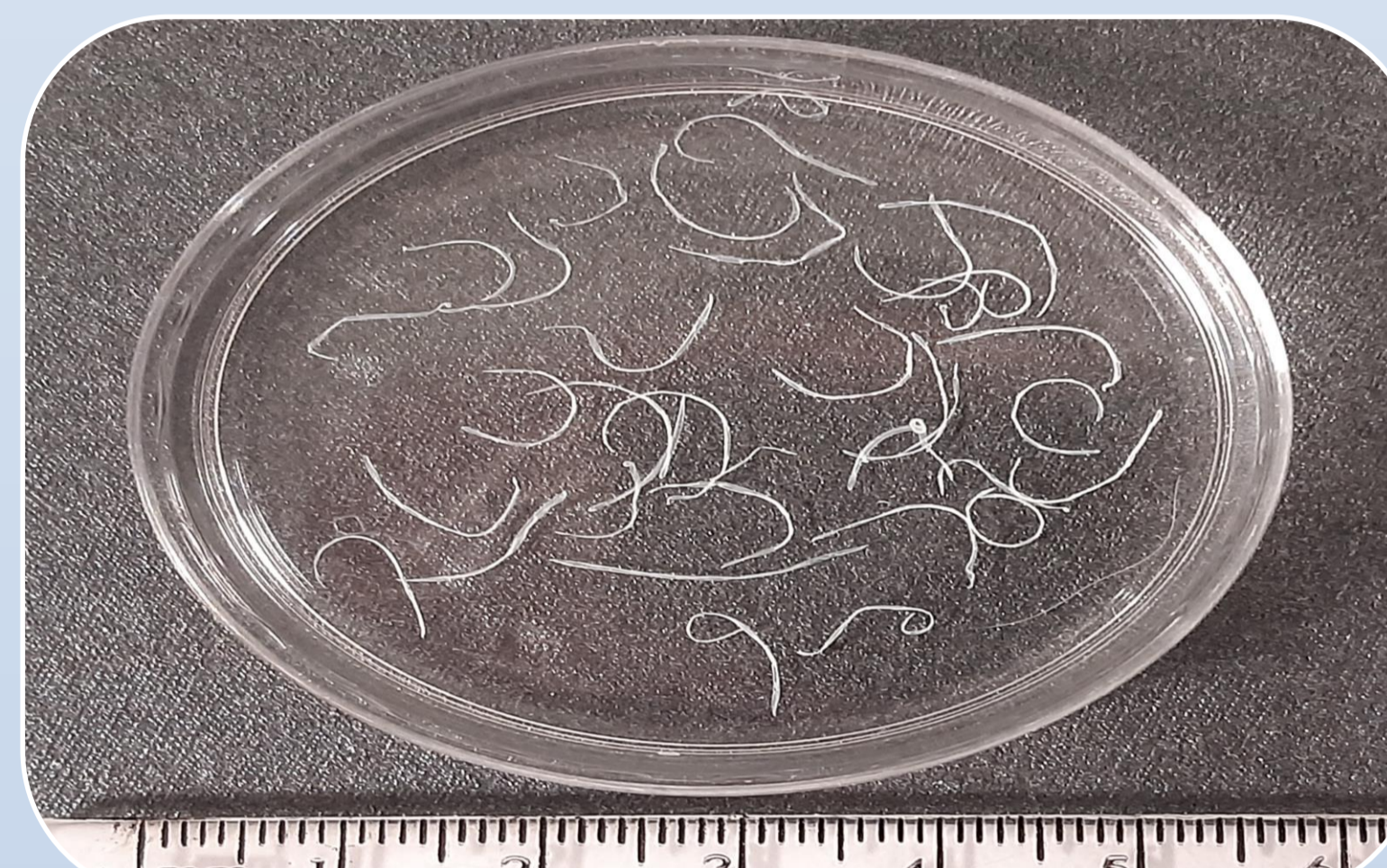


Fig 1a. Numerous *Thelazia callipaeda* recovered from the conjunctiva of affected eyes of one imported dog.

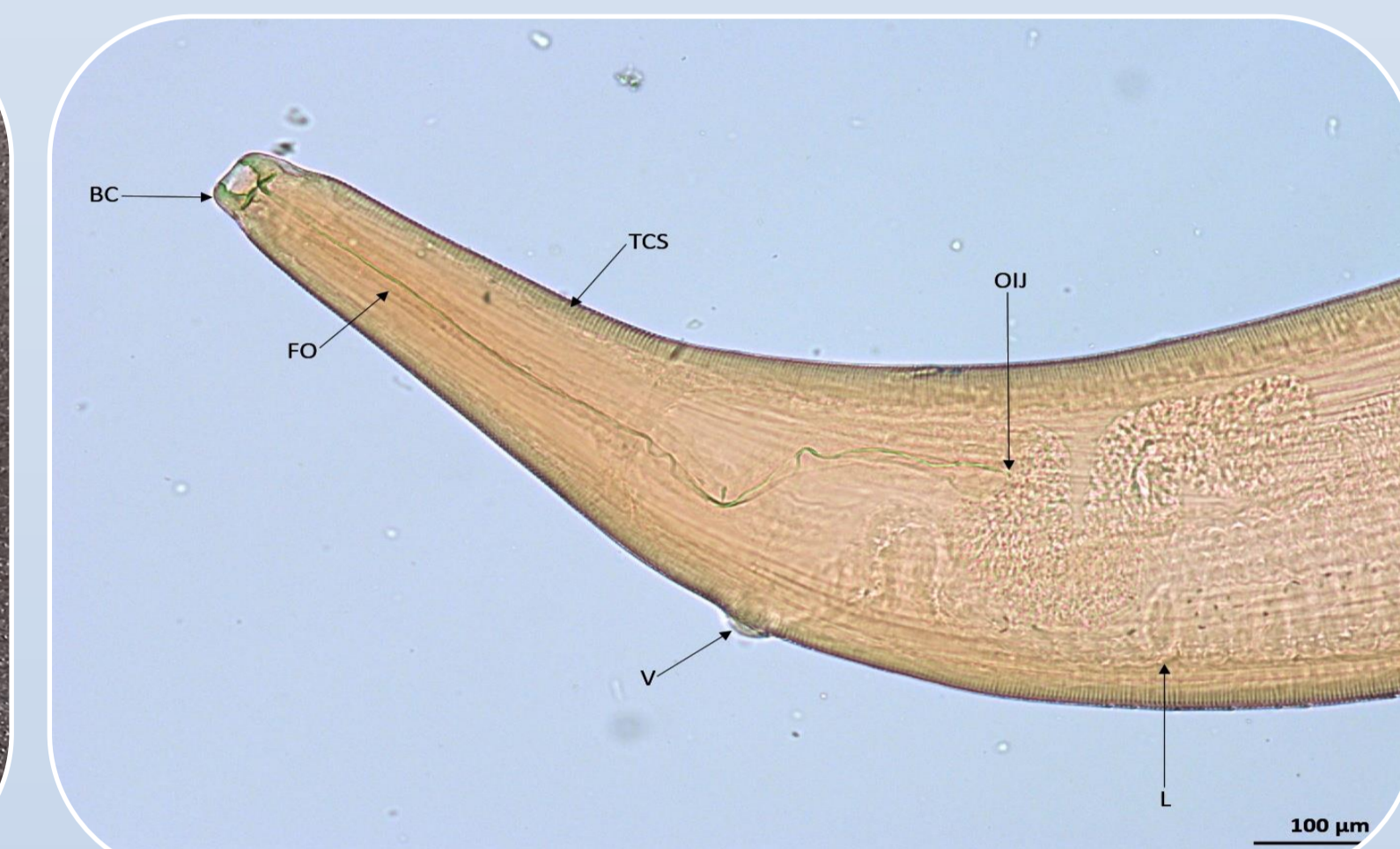


Fig 1b. Light micrograph of a gravid female *Thelazia callipaeda*, anterior. Buccal capsule (BC), filariform oesophagus (FO), transverse cuticular striations (TCS), vulva (V), oesophagus-intestinal junction (OIJ) and L1 larvae (L) (x10).

2. *Dirofilaria repens*.

Although less pathogenic in dogs than *Dirofilaria immitis*, this filarial nematode is an important zoonotic parasite, causing chronic nodular disease. Endemic foci have been reported in most European countries. Despite presence of the culicine mosquito vector in the UK and record summer temperatures in recent years, ambient temperature remains thus far an effective barrier to autochthonous transmission.

Species identification of circulating blood microfilariae is possible via Knott's test, with clinical signs, gross morphology, antigen testing and molecular analysis all being useful diagnostic aids if required. In a case submitted to LVPD in 2022, a particularly dexterous vet surgeon managed to extract an entire, fully-intact specimen from a skin nodule of a dog. This was most unusual, given how *Dirofilaria repens* is embedded and the tightly coiled within host tissue.



Fig 2a. *Dirofilaria repens* recovered from a flank skin nodule of an imported dog.

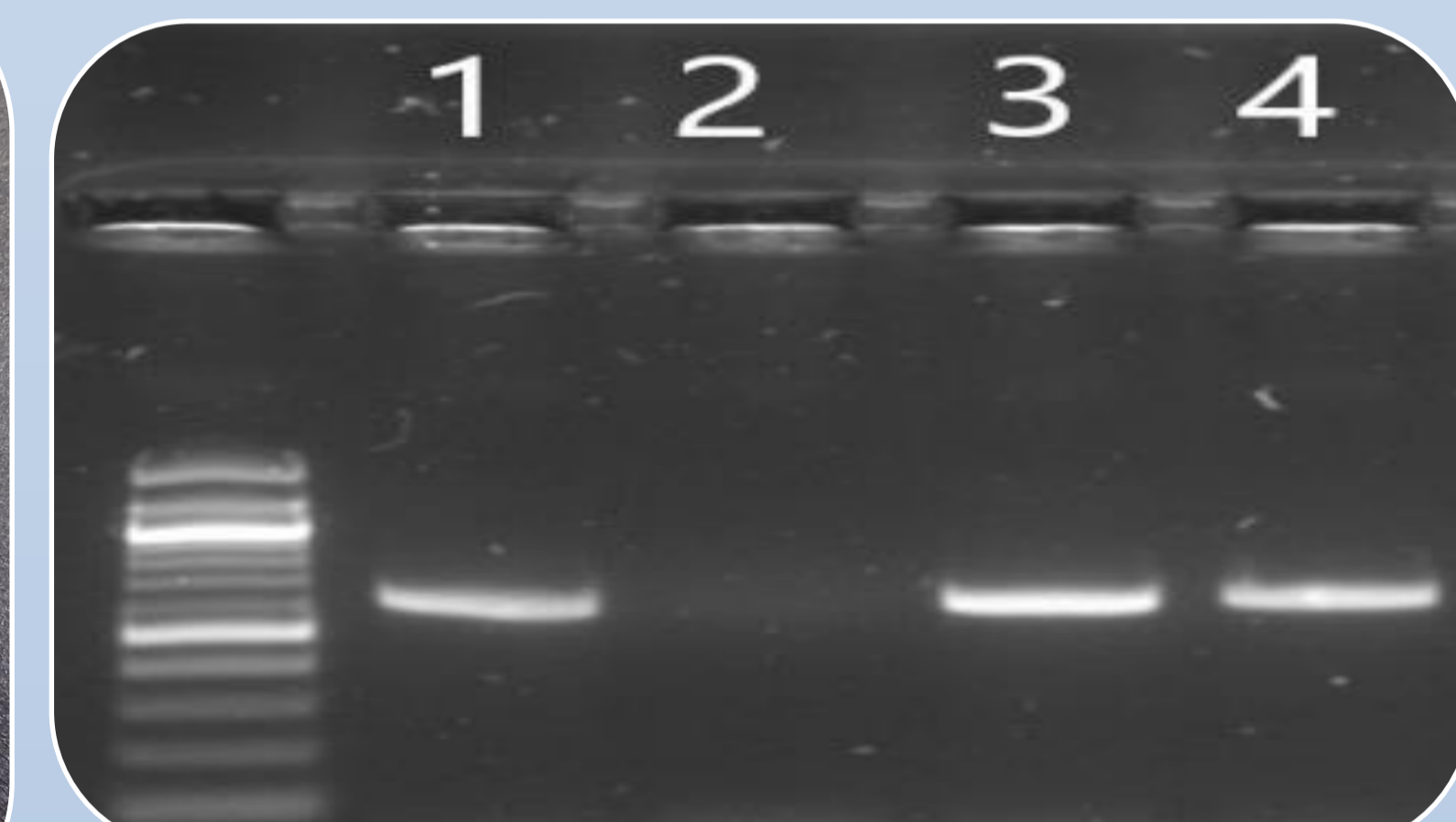


Fig 2b. Amplicons of the filarial and spirurid mitochondrial *cox1* gene produce a single band of 689bp. Lane 1 – Positive control. Lane 2 – Negative control. Lane 3 – *Thelazia callipaeda*. Lane 4 – *Dirofilaria repens*.

3. *Linguatula serrata*.

Known as 'tongue worm', this pentastomid parasite is found in the nasal cavities or sinuses of dogs, and has zoonotic potential. Strict hygiene should be observed in cases where infection is confirmed or suspected to minimise the risk of contact with infective eggs. Gross and microscopic morphological features are diagnostic. Chromium 10X sequencing data has also been generated from an LVPD case for species confirmation, and taxonomic work.



Fig 3a. *Linguatula serrata* recovered from an imported dog, after being expelled by sneezed during consultation.

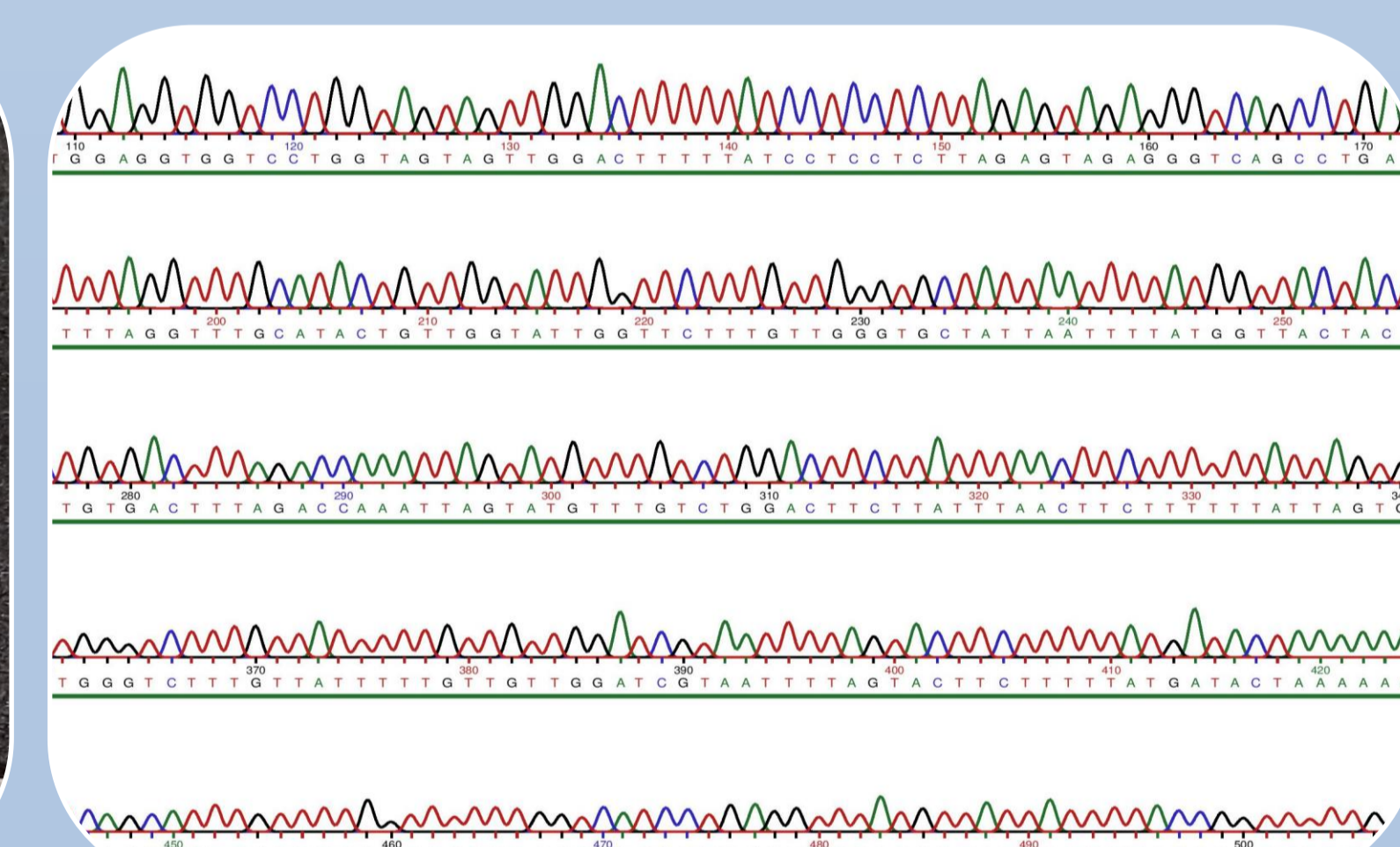


Fig 2c. Sanger sequencing chromatogram of filarial mitochondrial *cox1* amplicons.

4. *Onchocera lupi*.

Another vector-borne, zoonotic parasite, this filarial nematode mostly affects dogs and usually resides in episcleral tissue nodules, causing potentially severe ocular disease which can require enucleation. The competent vector is thought to be the blackfly, *Simulium* spp, which is widespread in the UK.

In a recent case reported by LVPD in Jan 2023, formalin-fixed, paraffin-embedded (FFPE) sub-conjunctival tissue was submitted for analysis. *Onchocera lupi* was identified in histological sections based on morphological characteristics. Further tissue sections were then used to extract DNA. The filarial mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene was amplified by PCR with NTF and NTR primers, and amplicons were analysed through Sanger sequencing. Sequence data showed 100% homology with published sequences of *Onchocera lupi* in the NCBI BLAST database, confirming our morphological findings.

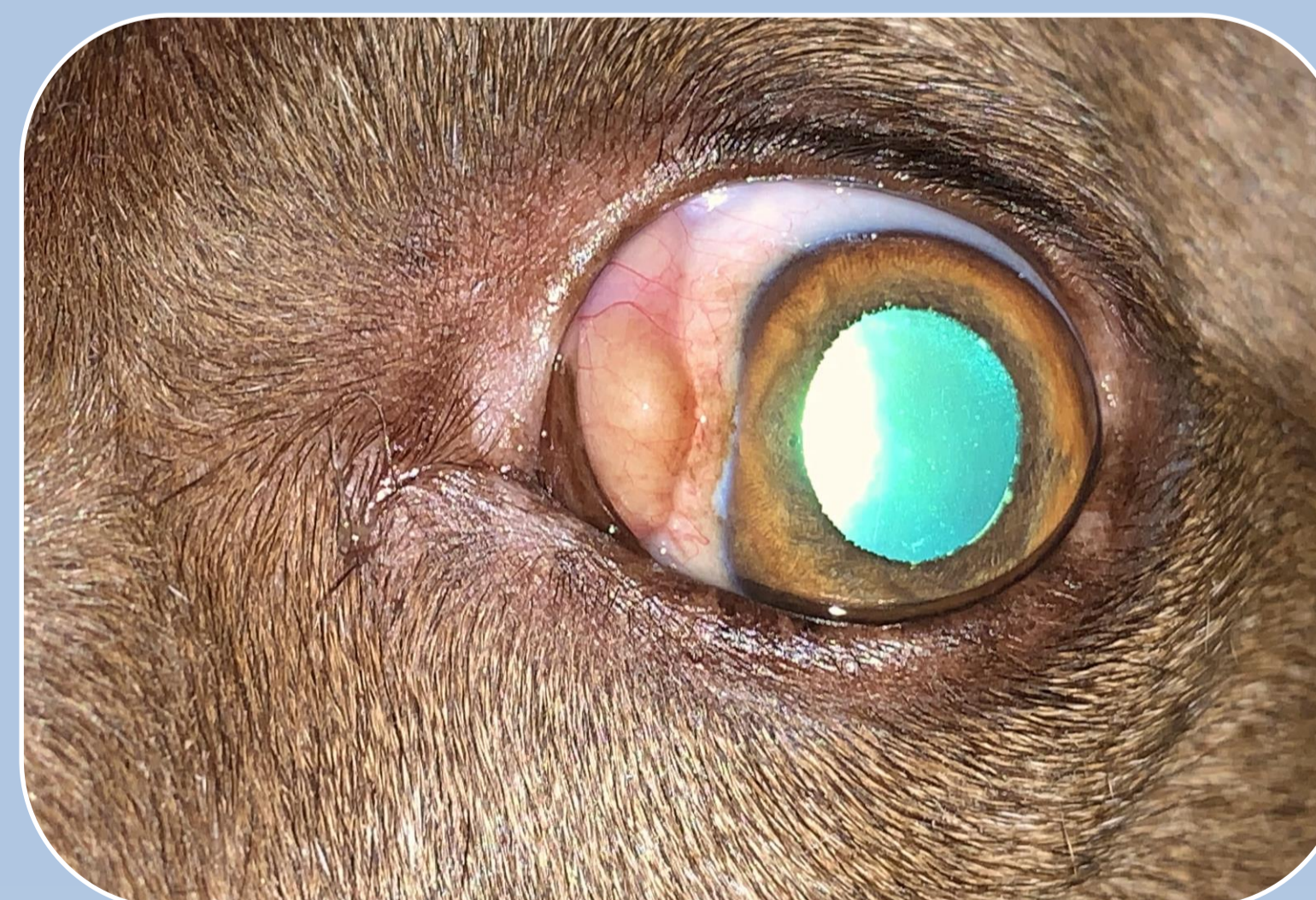


Fig 4a. Sub-conjunctival mass of an imported dog infected with *Onchocera lupi*.

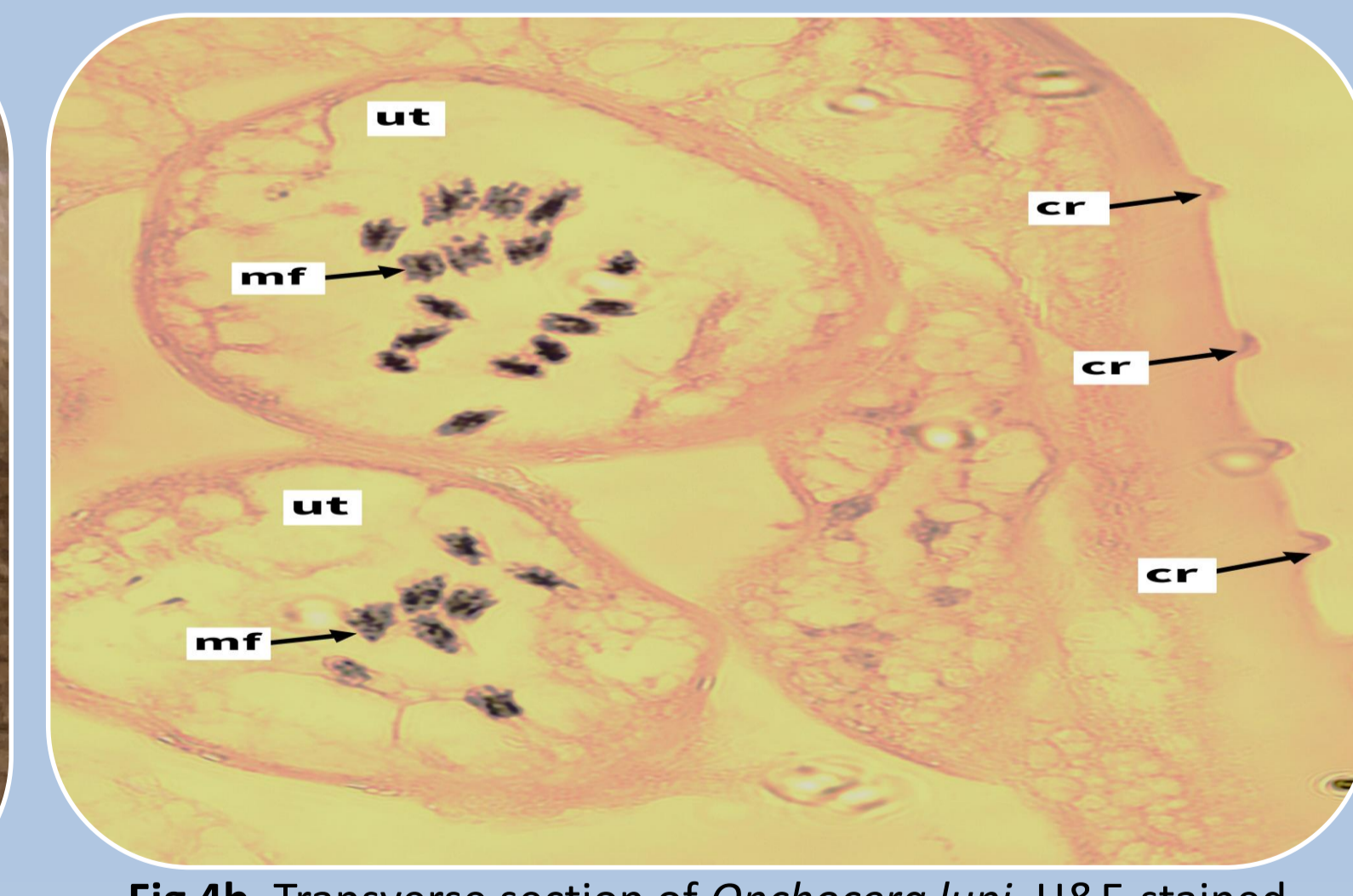


Fig 4b. Transverse section of *Onchocera lupi*. H&E-stained gravid female, with prominent cuticular ridges (cr), and twin uteri (ut) containing numerous mature microfilariae (mf).

5. Significance.

EU harmonisation of UK travel rules in 2012 led to relaxation of controls under PETS. In the following year alone, the number of dogs entering the UK under PETS increased by 61% to 140,000, driven in large part by commercial importation of puppies. These numbers increased dramatically in subsequent years, coinciding with a well-publicised rise in illegal puppy importations, and reached a pre-Covid peak of over 300,000. The UK government is currently consulting on proposed changes to rules re. commercial and non-commercial movement of pets. However, the reduced state of UK biosecurity pertaining to the current rules, seems likely to remain for some time to come.

Expertise in parasite identification at LVPD enables continued recognition and reporting of the emergence and spread of exotic parasitic diseases brought into the UK in imported pet dogs. These outputs underpin clinical treatment decisions, and help improve patient outcomes. They also alert practicing vets to zoonotic considerations when in consultations and enable them to provide relevant advice to pet owners. Furthermore, they ensure the University maintains a prominent voice in the national discourse of the veterinary profession amongst our academic and clinical peers. Lastly, case materials are used for teaching our BVSc students, making them aware of what they may encounter once they enter into practice.