

# 1 **Filarial infection caused by *Onchocerca boehmi* (Supperer, 1953) in a horse from** 2 **Italy**

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## 21 22 **Abstract**

23  
24 Equids can be infected by a range of skin-dwelling filarial nematodes, including four species of the  
25 genus *Onchocerca*. Current literature on equine onchocercosis is fragmented, and often limited to  
26 isolated case reports. The present study aimed to describe a clinical case of equine onchocercosis  
27 caused by *Oncocerca boehmi* (Supperer, 1953) (syn. *Elaeophora boehmi*) in an 8-years old gelding  
28 Belgian show jumper from northern Italy. The horse was presented with a firm and painless mass on  
29 the proximal third of the right metacarpal region. Ultrasound examination showed a peritendinous  
30 enlargement around the palmaro-lateral region of the tendons, characterized by an elongated  
31 hypoechoic and well-defined structure, embedding a coiled hyperechoic line. The metacarpal nodule  
32 was resected and histologically examined. Fragments of a parasitic nematode were detected, isolated  
33 and analysed. The morphological examination led to the identification of the nematode as *O. boehmi*.

34 Total genomic DNA was extracted from individual nematode fragments using a commercial kit and  
35 comparative analysis of the cytochrome oxidase subunit 1 (*cox1*) sequence with data available in the  
36 GenBank<sup>TM</sup> database revealed a close similarity (i.e., 91%) with the corresponding sequence from  
37 *Onchocerca lupi*. Thus far, *O. boehmi* has only been reported from Austria and Iran, and information  
38 of its life-cycle and vectors is lacking. The systematic position of this species within the genus  
39 *Onchocerca*, and not in the genus *Elaeophora* where it was originally placed, is in accordance with  
40 our morphological and molecular analyses. In this article, we describe the first autochthonous case of  
41 equine onchocercosis in Italy caused by *O. boehmi*, and discuss novel parasitological, clinical and  
42 pathological data on these equine pathogens.

43 *Keywords:* equine onchocercosis, *Onchocerca boemi*, horse, limb nodules, ultrasound, histology.

44

## 45 **Introduction**

46

47 The genus *Onchocerca* (Spirurida, Onchocercidae) includes more than 30 species of nodule-inducing  
48 nematodes inhabiting different anatomical regions of the subcutaneous tissues, ligaments, and  
49 aponeuroses of domestic mammals (Anderson 2000, Uni et al. 2015). The microfilariae released by  
50 the female nematodes migrate through the dermis of specific body areas, and they are ingested by  
51 insect intermediate hosts (e.g., black flies and biting midges) during blood feeding. In the insect  
52 vector, larvae moult twice, reaching the infective third larval stage (L3) within ~3-4 weeks. The L3s  
53 are subsequently transmitted to a susceptible vertebrate host via the blood meal (Onmaz et al. 2013).  
54 The infection becomes patent after ~12-16 months (Taylor et al. 2007). *Onchocerca reticulata*  
55 Diesing, 1841, and *Onchocerca cervicalis* Railliet and Henry, 1910 are the best-known filarial  
56 nematodes of equids due to their wide geographical distribution and high clinical relevance (Muller  
57 1979). In particular, infection by *O. cervicalis* was firstly reported from Australia as “Queensland  
58 itch” (Riek 1953); the disease is characterised by the occurrence of an allergic dermatitis, likely  
59 induced by the skin-dwelling microfilariae (Lees et al. 1983). Microfilariae may also invade the eyes,  
60 causing ocular symptoms (Cello 1971; Munger 1983), while *O. cervicalis* adults may cause  
61 inflammatory reactions of the nuchal ligament, which range from acute oedematous necrosis to  
62 chronic granulomatous changes. Conversely, infection by *O. reticulata* is usually characterised by the  
63 presence of subcutaneous nodules over or within the flexor tendons and suspensory ligaments, where  
64 it can induce swelling and lameness (Anderson 2000; Scott and Miller 2003). Equids may also be  
65 infected by *Onchocerca raillieti* Bain, Muller, Khamis, Guilhon and Schillhorn van Veen, 1976, a  
66 species mainly detected in subdermal masses in the withers or penis and in the perimuscular  
67 conjunctive tissue of domestic donkeys in Africa (Bain et al. 1976). Another species of the genus,  
68 *Onchocerca boehmi* (Supperer, 1953) (syn *Elaeophora boehmi*), was first described based on  
69 specimens collected from the arteries and veins of the limbs of horses from Austria. In most cases,  
70 horses infected by *O. boehmi* are asymptomatic (Supperer 1953).

71

72 Current scientific literature on equine onchocercosis is fragmented and often dated. For example, *O.*  
73 *cervicalis* has been long considered a synonym of *O. reticulata*, until Bain (1975) highlighted  
74 important morphological differences between these two species. Similarly, epidemiological data on  
75 onchocercid species infecting horses are scarce. Infection by *O. cervicalis* has been diagnosed in the  
76 United States (Stannard and Cello 1975), Canada (Marcoux et al. 1977), Australia (Riek 1954), and  
77 Brazil (Marques and Scrofernecker 2004). In Europe, only a few studies have been performed  
78 (Anderson 2000), and onchocercids have seldom been identified at species level. In this article, we

79 describe the first autochthonous case of equine onchocercosis in Italy caused by *O. boehmi*, and  
80 discuss novel parasitological, clinical and pathological data on these pathogens of horses.

81

## 82 **Materials and Methods**

83

### 84 *Case presentation*

85 An 8-years-old 570 kg gelding Belgian horse, used in show-jump competitions, housed in northern  
86 Italy (Genoa, Liguria region, Italy), was presented in July 2013 at the Veterinary Teaching Hospital  
87 of the University of Turin (Piedmont, Italy) with an evident lump in correspondence of the right  
88 metacarpal region. This lesion had appeared six months prior to presentation as a diffuse swelling,  
89 during the spring season, that had progressively increased in size. The owner sought the advice of  
90 veterinary clinicians in order to investigate the occurrence of tendinitis in correspondence of the mid-  
91 metacarpal region. During the clinical examination, the horse was presented with a firm and painless  
92 mass located palmaro-laterally on the proximal third of the right metacarpal region and a mild  
93 swelling in correspondence of the medial aspect of the left metacarpal region (**Figure 1**). Several  
94 firm and small subcutaneous nodules were observed on the back of the animal, along the epiaxial  
95 muscles. The horse was mildly lame only at the start of the clinical examination. Palpation did not  
96 allow defining the relationship between the mass and the superficial digital flexor tendon (SDFT).  
97 Previous treatments included DMSO (dimethyl sulfoxide) Gel 99.9% as a topical application, twice  
98 daily over 3 weeks, to reduce the swelling. An oral administration of ivermectin paste was previously  
99 recommended by the practitioner, at double label dose (400 µg/kg body weight), on the basis of  
100 previous experience with similar subcutaneous nodules of suspected parasitic aetiology.

101

### 102 *Ultrasonographic examination*

103 An ultrasonographic examination was conducted using a mobile Logiq E Vet Ultrasound machine  
104 (General Electric Company Fairfield, CT, USA) with a linear multifrequency transducer (8-12 MHz).  
105 The examination was carried out on site, with the horse in standing position. No sedatives were  
106 administered. Prior to the ultrasound examination, both palmar metacarpal regions were prepared  
107 using standard procedures. Images were obtained using a standoff pad coupled to the transducer. The  
108 examination showed the presence of a peritendinous enlargement around the palmaro-lateral aspect  
109 of the SDFT, on the right forelimb, exerting a mass-effect on the whole soft tissues. The abnormal  
110 peritendinous mass was characterized by an elongated hypoechoic and well-defined structure,  
111 including a coiled hyperechoic line. On the left forelimb, the ultrasound examination revealed the  
112 same ultrasonographic pattern on the medial aspect of the mid metacarpal region, but with a more

113 echogenic structure and lacking the hyperechoic linear structure. Ultrasonographic findings of both  
114 structures were consistent with a peritendinous localization of a verminous nodule (**Figure 2**).

115

#### 116 *Surgical removal of the nodule*

117 Surgical removal of the peritendinous mass was performed, with the horse standing and sedated  
118 using a constant infusion rate. In particular, the infusion rate was prepared by adding 2 mg of  
119 medetomidine to a 0.5 litre bag of saline (4 µg/mL) and this volume was administered at a rate of 1  
120 drop/sec (10 drops/mL infusion set drip rate), which provides approximately 80 min of infusion. A  
121 local analgesia was administered using a high metacarpal nerve block, with a 2% solution of  
122 mepivacaine. The nodule was resected from the SDFT peritendon and the deep metacarpal fascia.  
123 Haemorrhage was controlled using an Esmark bandage, applied proximally to the carpal region. The  
124 skin was closed using routine procedures and a half-limb bandage was applied post-operatively. Post-  
125 surgery standard anti-inflammatory and antibiotic therapies were administered over 3 days following  
126 the procedure, and the horse was not trained for two weeks post-surgery. The horse made a full  
127 recovery.

128

#### 129 *Histopathological analysis*

130 Histopathological examination of the excised metacarpal nodule was performed; the tissue was fixed  
131 in a 10% formalin solution (pH 7.4) and processed using standard procedures (Mutafchiev et al.  
132 2013).

133

#### 134 *Parasitological and molecular analyses*

135 A sub-section of the nodule was fixed and preserved in 70% ethanol, and dissected under a  
136 stereomicroscope. For light-microscopy, nematode fragments were cleared and examined as  
137 temporary mounts in lactophenol, while those used for scanning electron microscopy observations  
138 were prepared and studied, as described elsewhere (Mutafchiev et al. 2013). A female of *O. boehmi*  
139 (one slide) from the Supperer collection deposited in the University of Veterinary Medicine Vienna  
140 (UVMV) was used as comparative material. In addition, total genomic DNA was extracted from  
141 parasite fragments recovered from an individual specimen using a commercial kit (DNeasy Blood &  
142 Tissue Kit, Qiagen, GmbH, Hilden, Germany) in accordance with the manufacturer's instructions; a  
143 partial region of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*; ~689 bp) was  
144 amplified as previously described (Otranto et al. 2011). The amplicon obtained was purified using  
145 Ultrafree-DA columns (Amicon, Millipore; Bedford, USA) and sequenced directly using the Taq  
146 Dye Deoxy Terminator Cycle Sequencing Kit (v.2, Applied Biosystems) in an automated sequencer

147 (ABI-PRISM 377). Sequences were determined from both strands (using the same primers  
148 individually as for the PCR) and the electropherograms were verified by eye. The nucleotide  
149 sequence of the *cox1* fragment was conceptually translated into an amino acid sequence using the  
150 invertebrate mitochondrial code by MEGA 6.0 software (Tamura et al. 2013). Finally, the nucleotide  
151 sequence was compared with those available in the GenBank<sup>TM</sup> database by BLAST analysis.

152

## 153 **Results**

154

### 155 *Histopathological analysis*

156 Both haematoxylin and eosin and trichromic stains revealed a number of multifocal coalescing  
157 parasitic and necrotic granulomas. Each granuloma was characterised by a central cavity containing  
158 one or more parasitic sections (possibly due to coiled bodies); the cavity was lined by necrotic  
159 material and eosinophilic products of degranulation, surrounded by macrophages and by an external  
160 layer of dense collagen. Parasitic granulomas were separated by a dense interstitial eosinophilic and  
161 macrophage infiltrate on a background of fibroplasia. Rare collagenolytic granulomas were scattered  
162 around the nodule. A visible body wall with an outer cuticle with subcuticular striations and an  
163 inner hypodermal layer could be observed for some of the parasites. Small intestine and empty uteri  
164 were also observed. Based on their morphological features, the parasites were identified as  
165 nematodes (**Figure 3**).

166

### 167 *Morphological and molecular identification*

168 Nematode fragments (n=83) recovered from the nodule varied in length from 0.25 to 8.83 mm,  
169 amounting to 186 mm total length and a diameter ranging from 127 to 320  $\mu$ m. The fragments  
170 contained only empty ovaries and were considered as belonging to an uncertain number of  
171 unfertilized female nematodes (**Figure 4A, 4B**). The anterior and posterior extremities could not be  
172 seen. The cuticle was 16–25  $\mu$ m thick with three distinct layers: an external layer 3–4  $\mu$ m thick with  
173 transverse striations 7–12  $\mu$ m apart interrupted along the medial lateral linings (**Figure 4C, 4D, 5A**)  
174 and ornate with fine irregularly anastomosing crests (**Figure 4D, 4E, 5B**); a median layer 10–18  $\mu$ m  
175 thick, with annular striae with length corresponding to the distance between the external transverse  
176 striations (**Figure 4E**), and an internal hyaline layer 3–5  $\mu$ m thick. The somatic musculature was  
177 coelomyarian.

178 The morphological identification was confirmed by comparing samples with the voucher material  
179 collected by Supperer, which consisted of a single developing young and unfertilised female  
180 measuring 54.5 mm in length, without a posterior extremity. The specimen had a maximum body

181 width of 170  $\mu\text{m}$  at about mid-body and a width, measured at the level of vulva and oesophago-  
182 intestinal junction, of 104  $\mu\text{m}$ ; the oesophagus was 1,259  $\mu\text{m}$  long and the vulva was situated at 575  
183  $\mu\text{m}$  from the cephalic extremity. The cuticle at mid-body was 15–22  $\mu\text{m}$  thick (thicker on lateral  
184 sides) with three distinct layers: an external layer 2  $\mu\text{m}$  thick with fine transverse striations 3–5  $\mu\text{m}$   
185 apart, median layers 10–15 thick with annular striae with length coinciding with distance between  
186 external transverse striations, and internal layer a without specific structure with a regular thickness  
187 of 4–5  $\mu\text{m}$  (**Figure 6**).

188 A fragment of 689 base pairs of the *cox1* gene was amplified. BLAST analysis of this sequence  
189 revealed the highest nucleotide similarity (i.e., 91%) to that of *Onchocerca lupi* Rodonaja, 1967  
190 available from GenBank<sup>TM</sup> (Accession Number EF521410).

191

## 192 **Discussion**

193

194 The present study describes a case of *O. boehmi* infection from a horse in Italy, where equine  
195 onchocercosis had never been reported and it is therefore unknown to veterinary practitioners. In  
196 equine practice, the appearance of skin nodules is often asymptomatic, and it often goes unnoticed by  
197 owners (B. Riccio, personal communication). However, in the present report, the clinical presentation  
198 was accompanied by an impaired function of the suspensory ligament and occurrence of mild  
199 lameness. Interestingly, prior to this case, no clinical symptoms associated to infestation by *O.*  
200 *boehmi* had been described. Given the anatomical localisation of the nodules, we hypothesize that the  
201 nematode had undertaken an erratic migration from the circulatory system (i.e., the arteries and veins  
202 of limbs) to the subcutaneous tissues of the metacarpal region. Previously, *O. boehmi* had only been  
203 diagnosed in two isolated reports, and information about its biology is lacking. According to the  
204 original report by Supperer (1953), adults were detected in the medial or external layer of tissues  
205 within the artery wall in Austrian horses, while a second survey from Iran indicated that 14 out 161  
206 horses examined (8.69%) had microfilariae in the blood (Mirzayans and Maghsoodloo 1977).

207 The occurrence of the parasite in the nodule allowed the assessment of the histopathological lesions  
208 caused by *O. boehmi*. Eosinophils were the main inflammatory cells observed in the nodule, as  
209 reported for the skin lesions caused by other *Onchocerca* species (Scott and Miller 2003). Apart from  
210 their protective roles against parasites, eosinophils are known to be involved in hypersensitivity  
211 disorders. In addition, these cells can also be detected in eosinophilic granulomas of horses, which  
212 are clinically characterized by the presence of cutaneous nodules and the occurrence of collagen  
213 flame figures visible at the histopathological examination (Scott and Miller 2003). Flame figures,  
214 albeit rare, were observed in the case herein described. Onchocercosis in horses can be characterised

215 by both encystment of (adult) parasites and hypersensitivity, the latter usually caused by  
216 microfilariae; nevertheless, dead or dying microfilariae were not observed in the tissue examined and  
217 the lesions were not pruritic.

218 The morphology of the cuticle of the nematode fragments collected resembled that of the voucher  
219 material of *O. boehmi* from Austria; therefore we consider both samples conspecific. In particular,  
220 while *O. boehmi* is surrounded by a cuticle without external ridges and three distinct layers with a  
221 specific morphology, the cuticle of other *Onchocerca* parasitizing equids, (i.e. *O. cervicalis* and *O.*  
222 *reticulata*) is characterised by well-distinct external annular ridges (Bain 1981). Conversely, the  
223 cuticle of *O. raillieti*, which is smooth and does not bear any external ridges, is thicker than of *O.*  
224 *boehmi* (up to 50–55  $\mu\text{m}$  vs 22–25  $\mu\text{m}$ ) and has longer striae (up to 16–20  $\mu\text{m}$  vs 6–12  $\mu\text{m}$ ) (Bain et  
225 al. 1976; present study). The systematic position of this species within the genus *Onchocerca*, as  
226 suggested by Bain et al. (1967), and not within the genus *Elaeophora*, is in accordance with the  
227 results of our morphological and molecular analyses.

228 Equine onchocercosis has been reported worldwide, but most epidemiological information date back  
229 to the 70s'. For instance, *Onchocerca* sp. has been diagnosed in horses from the United States, where  
230 Stannard and Cello (1975) reported a mean prevalence of 48%, whereas Lloyd and Soulsby (1978)  
231 recovered microfilariae in 61% of examined animals from the eastern part of the country. Schmidt et  
232 al. (1982) examined the nuchal ligament of 83 horses from Midwestern US, and 37% of them were  
233 positive for adult parasites. Klei et al. (1984) detected microfilariae in 76% (out of 84) of ponies from  
234 the Gulf Coast area and in 82.4% of horses (out of 51) from the Louisiana State. Of 664 horses from  
235 Southeastern and Midwestern USA, 341 (51.4%) were positive for cutaneous microfilariae of *O.*  
236 *cervicalis* (Cummings and James, 1985). Monahan et al. (1985) diagnosed *O. cervicalis* infection in  
237 30.5% (out of 82) of ponies in USA. Finally, Lyons and colleagues (2000) reported *O. cervicalis* in  
238 24% of horses (out of 157) examined for several species of internal parasites at necropsy in  
239 Kentucky. Infection by *O. cervicalis* was reported also in Canada (Marcoux et al. 1977; Lees et al.  
240 1983). Indeed, during a survey of 383 slaughtered horses from the western Canadian provinces, *O.*  
241 *cervicalis* microfilariae were detected in 11.8% of umbilical samples (Polley 1984). Riek (1954)  
242 examined the nuchal ligaments of 282 Australian horses from Queensland and found that 79.8% of  
243 these were infected with *Onchocerca* (erroneously reported as *O. reticulata*), whereas Ottley et al.  
244 (1983) sampled a small group of horses and ponies from Queensland and the Northern Territory, and  
245 diagnosed *O. cervicalis*, *O. gutturosa* and *O. reticulata* in these animals. In South America, Mancebo  
246 et al. (1997) detected *O. cervicalis* microfilariae in 24% of the 257 adult working horses examined in  
247 Argentina. A similar result was obtained in Brazil by Marques and Scrofernecker (2004), who  
248 described *O. cervicalis* microfilariae in the midventral skin samples of 17.9% (out of 1,200) horses



249 examined, while adult nematodes were recovered from the nuchal ligaments of 200 (16.6%) animals.  
250 In Europe, a few studies have been performed thus far. In England, Mellor (1973) detected adult  
251 *Onchocerca* sp. in the nuchal ligaments of 15.8% (out of 209) British horses. Moignoux (1954)  
252 reported that 6% of horses living in Camargue (France) were infected by subcutaneous *Onchocerca*  
253 *microfilariae*. However, Collobert et al. (1995) found that only 1% of 368 horses were positive for  
254 *Onchocerca* at post mortem examinations in Normandy. In other European countries, out of 160  
255 horse skin biopsies examined in Spain and Poland, only 3.7% had detectable *Onchocerca*  
256 *microfilariae* (Franck et al. 2006). Finally, skin biopsies from 42 horses were all negative for  
257 *microfilariae* in Finland (Solismaa et al. 2008). These data indicate that equine onchocercosis is  
258 common in horse populations; however, as a consequence of the non-specific clinical presentation  
259 and diagnostic challenges, its prevalence is most likely underestimated. Additional large-scale studies  
260 are required to better investigate the presence and diffusion of *O. boehmi* and other onchocercid  
261 species in Italian and European horse populations.

262 Based on our observations, we suggest that parasitic granuloma should be included in the differential  
263 diagnosis of peritendinous swelling in horses; an accurate ultrasound examination allows to easily  
264 differentiate this condition from acute tendonitis or haematoma. The prevalence of parasitic  
265 granuloma associated with *O. boehmi* in equine populations is currently unknown, and the life cycle  
266 of this parasite is presently unclear. Further studies are needed to elucidate the biology of this poorly  
267 known onchocercid nematode and the impact of infection on equine species.

268

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274

### 275 **Conflict of interest statement**

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

277

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360 **Figure captions**

361

362 **Figure 1.** Right forelimb of the horse, showing a subcutaneous firm nodule in the palmaro-lateral  
363 aspect of the right metacarpal region. Palmar (A) and lateral (B) view of the limb.

364

365 **Figure 2.** Transversal (A, B) and longitudinal (C, D) ultrasound scans of both mid metacarpal  
366 regions, showing a verminous nodule on the palmaro-lateral aspect of the right forelimb. The parasite  
367 appears as a coiled hyperechoic line within a hypoechoic nodule, surrounding the superficial digital  
368 flexor tendon (B: red arrows). Longitudinal scan (D) shows the localization at the level of the deep  
369 metacarpal fascia.

370

371 **Figure 3.** Histopathology of the nodule. A) Granulomatous reaction around a parasite: the cavity is  
372 lined by necrotic material with products of eosinophil degranulation (\*), macrophages and giant cells  
373 (>), collagen bundles, eosinophils and lymphocytes (trichrom stain); B) Morphological features of a  
374 coiled parasite within a granuloma: small intestine, uteri (>) and lateral chord (\*) (HE stain); C)  
375 Subcuticular striations (HE stain); D) Collagenolytic granuloma at the periphery of the nodule (HE  
376 stain).

377

378 **Figure 4.** *Onchocerca boehmi*, light microscopy, horse from Italy. A) Body fragment with intestine  
379 (arrow) and two uteri (arrowheads); B) Transverse section through body, note two uteri  
380 (arrowheads); C) Surface of cuticle, note the interrupted external transverse striations along median  
381 lateral line (C2); D) Surface of cuticle exhibited when studied without coverslip, note internal striae  
382 (arrowheads), transverse striations (arrows) and ornamentation of fine irregularly anastomosing  
383 crests; E) Detail of cuticle of two body fragments, note fine external crests on the surface  
384 (arrowheads) and internal annual striae of the median layer (arrows).

385

386 **Figure 5.** *Onchocerca boehmi* scanning electron microscopy, horse from Italy. A) Transverse  
387 striations (arrows) of cuticle surface; B) Cuticle ornamentation, note transverse striations (arrows)  
388 and fine external crests (arrowheads).

389

390 **Figure 6.** *Onchocerca boehmi*, cuticle of young female, horse from Austria. Note the fine external  
391 crests (arrowheads) and the internal striae (arrows).