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Parasitology Research

Filarial infection caused by *Onchocerca boehmi* (Supperer, 1953) in a horse from Italy --Manuscript Draft--

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Full Title:	Filarial infection caused by <i>Onchocerca boehmi</i> (Supperer, 1953) in a horse from Italy
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Abstract:	<p>Equids may be infected by a range of skin-dwelling filarial nematodes, including four species of the genus <i>Onchocerca</i>. Current literature on equine onchocercosis is fragmentary, and often limited to isolated case reports. The present study describes a clinical case caused by <i>Onchocerca boehmi</i> (syn. <i>Elaeophora boehmi</i>) in an 8-years old horse from northern Italy. The animal presented a firm and painless mass on the proximal third of the right metacarpal region. Ultrasound examination showed a peritendinous enlargement around the palmaro-lateral part of the tendons, characterized by an elongated hypoechoic and well-defined structure, embedding a coiled hyperechoic line. The metacarpal nodule was resected and histologically examined. Fragments of a parasitic nematode were detected, isolated and examined. Total genomic DNA was extracted from individual fragments using a commercial kit and the nematode was identified as <i>O. boehmi</i>. Comparative sequence analysis of the nematode cytochrome oxidase subunit 1 (cox1) sequence with data available in the GenBank™ database revealed high sequence similarity (i.e., 91%) with that of <i>Onchocerca lupi</i>. Thus far, <i>O. boehmi</i> has only been described in two isolated reports in Austria and Iran, and information about its life-cycle and vectors is lacking. The systematic position of this species within the genus <i>Onchocerca</i> is in concordance with our morphological and molecular results. In this article, we describe the first autochthonous case of equine onchocerciasis in Italy caused by <i>O. boehmi</i>, and discuss novel parasitological, clinical and pathological data on these neglected pathogens of horses.</p>
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Author Comments:	<p>TTo the Editor of Parasitology International Dear Editor, Please find herein enclosed the manuscript entitled "Infection by Onchocerca boehmi Supperer, 1953 in a horse in Italy" by Yasen Mutafchiev, Vincenzo Veneziano, Alessio Giannelli, Francesca Abramo, Mario Santoro, Maria Stefania Latrofa, Cinzia Cantacessi, Coralie Martin, Domenico Otranto, Andrea Bertuglia, Barbara Riccio, and myself, to be considered for publication in Parasitology International.</p> <p>As you know, horses may be infected by a range of skin-dwelling onchocercid nematodes, including Onchocerca reticulata or O. cervicalis. Unfortunately, the current literature on equine onchocerciasis is fragmentary, and often limited to isolated case reports. In addition, no information is available for other species infecting horses, as in the case of O. boehmi. In the present study, we provide the full description of a clinical case of equine onchocerciasis caused by O. boehmi in a 8-years old gelding Belgian show jumper from northern Italy. The diagnosis of the infection was performed by a comprehensive diagnostic panel, which included ultrasound and histological examination. In addition, the parasite was morphologically identified and molecularly characterized by using microscopy (either light or SEM) and cytochrome oxidase subunit 1 sequencing, respectively. Thus far, O. boehmi has only been described in two isolated reports, and information about its biology is lacking. In the present study, we hypothesize that the unusual anatomical localization of this onchocercid described in this report has resulted from the migration of the parasite from the circulatory system (i.e., arteries and veins of limbs) to the subcutaneous tissues of the metacarpal region.</p> <p>I hope you will find the manuscript of interest for the readers of your journal.</p> <p>Thank you for your kind cooperation,</p> <p>Yours faithfully, Riccardo Paolo Lia</p>
Suggested Reviewers:	<p>Guilherme Verocai gverocai@gmail.com Expert on Onchocerca species</p> <p>Shigehiko Uni uni@med.osaka-cu.ac.jp Expert on Onchocerca species</p> <p>Antti Oksanen antti.oksanen@evira.fi Expert on skin dwelling parasites of horses</p>

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

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Abstract

Equids may be infected by a range of skin-dwelling filarial nematodes, including four species of the genus *Onchocerca*. Current literature on equine onchocercosis is fragmentary, and often limited to isolated case reports. The present study describes a clinical case caused by *Oncocerca boehmi* (syn. *Elaeophora boehmi*) in an 8-years old horse from northern Italy. The animal presented a firm and painless mass on the proximal third of the right metacarpal region. Ultrasound examination showed a peritendinous enlargement around the palmaro-lateral part of the tendons, characterized by an elongated hypoechoic and well-defined structure, embedding a coiled hyperechoic line. The metacarpal nodule was resected and histologically examined. Fragments of a parasitic nematode were detected, isolated and examined. Total genomic DNA was extracted from individual fragments using a commercial kit and the nematode was identified as *O. boehmi*. Comparative sequence analysis of

34 the nematode cytochrome oxidase subunit 1 (*cox1*) sequence with data available in the GenBank™
35 database revealed high sequence similarity (i.e., 91%) with that of *Onchocerca lupi*. Thus far, *O.*
36 *boehmi* has only been described in two isolated reports in Austria and Iran, and information about its
37 life-cycle and vectors is lacking. The systematic position of this species within the genus *Onchocerca*
38 is in concordance with our morphological and molecular results. In this article, we describe the first
39 autochthonous case of equine onchocerciasis in Italy caused by *O. boehmi*, and discuss novel
40 parasitological, clinical and pathological data on these neglected pathogens of horses.

41 *Keywords:* equine onchocerciasis, *Onchocerca boehmi*, horse, limb nodules.

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43 **Introduction**

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The genus *Onchocerca* (Spirurida, Onchocercidae) includes more than 30 species of nodule-inducing nematodes inhabiting different anatomical regions of the subcutaneous tissues, ligaments, and aponeuroses of domestic mammals (Anderson 2000, Uni et al. 2015). The microfilariae released by the female worms migrate through the dermis of specific body areas, and they are ingested by blood feeding intermediate hosts (e.g., blackflies). In the vector, larvae moult twice, reaching the infective third larval stage (L3) in about 3-4 weeks. The L3s are then transmitted to a susceptible vertebrate host via the blood meal (Onmaz et al. 2013). The infection is patent in about 12-16 months (Taylor et al. 2007). *Onchocerca reticulata* Diesing, 1841, and *Onchocerca cervicalis* Railliet and Henry, 1910 are the best-known filarial worms of equids due to their wide geographical distribution and potential clinical relevance (Muller 1979). In particular, *O. cervicalis* infection was firstly described in Australia as “Queensland itch” (Riek 1953), and is characterised by the occurrence of an allergic dermatitis, likely induced by the skin dwelling microfilariae (Lees et al. 1983). Microfilariae may also invade the eyes, causing ocular signs (Cello 1971; Munger 1983), while *O. cervicalis* adults may cause inflammatory reactions in the nuchal ligament, which range from acute oedematous necrosis to chronic granulomatous changes. Conversely, the infection by *O. reticulata* is usually characterised by the presence of subcutaneous nodules over or within the flexor tendons and suspensory ligaments, where it is potentially associated with swelling and lameness (Scott and Miller 2003). Equids may also be infected by *Onchocerca railletii* Bain, Muller, Khamis, Guilhon & Schillhorn van Veen, 1976, a species detected in subdermal masses in the withers or penis and in the perimuscular conjunctive tissue of domestic donkeys from Africa (Bain et al. 1976). Another species of the genus, *Onchocerca boehmi* (Supperer, 1953) (syn *Elaeophora boehmi*), was described in the arteries and veins of the limbs of Austrian horses. In most cases, horses infected by *O. boehmi* are asymptomatic (Supperer, 1953).

The scientific literature on equine onchocerciasis is fragmentary and often dated. For example, *O. cervicalis* has been long considered a synonymous of *O. reticulata*, until Bain (1975) morphological differences between these two species. Similarly, epidemiological data on onchocercid species infecting horses are scarce. For instance, infection by *O. cervicalis* has been diagnosed in the United States (Stannard and Cello 1975), Canada (Marcoux et al. 1977), Australia (Riek 1954), and South America (Marques and Scrofernecker 2004). In Europe, few studies have been performed (Anderson 2000), and onchocercids have seldom been identified at species level. In this article, we describe the

76 first autochthonous case of equine onchocerciasis in Italy caused by *O. boehmi*, and discuss novel
77 parasitological, clinical and pathological data on these neglected pathogens of horses.

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80 **Materials and Methods**

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83 *Case presentation*

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

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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

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

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113 *Surgical removal of the nodule*

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

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126 *Histopathological analysis*

127 Histopathological examination of the excised metacarpal nodule was performed; the tissue was fixed
128 in a 10% formalin solution (pH 7.4) and processed using standard procedures (Mutafchiev et al.
129 2013).

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131 *Parasitological and molecular analyses*

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

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

150

151 **Results**

152

153 *Histopathological analysis*

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

165

166 *Morphological and molecular identification*

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168 Nematode fragments (n=83) recovered from the nodule varied in length from 0.25 to 8.83 mm,
169 amounting to 18.6 mm total length and a diameter ranging from 127 to 320 µm. The fragments
170 contained only empty ovaries and were considered as belonging to unknown number of unfertilized
171 female worms (**Figure 4A, 4B**). The anterior and posterior extremities were not detected. The cuticle
172 was 16–25 µm thick with three distinct layers: an external layer 3–4 µm thick with transverse
173 striations 7–12 µm apart interrupted along the medial lateral linings (**Figure 4C, 4D, 5A**) and ornate
174 with fine irregularly anastomosing crests (**Figure 4D, 4E, 5B**); a median layer 10–18 µm thick, with
175 annular striae with length corresponding to the distance between the external transverse striations
176 (**Figure 4E**), and an internal hyaline layer 3–5 µm thick.

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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

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

185 A fragment of 950 bp of the *cox1* gene was amplified. BLAST analysis of this sequence revealed the
186 highest nucleotide similarity (i.e., 91%) with that of *Onchocerca lupi* Rodonaja, 1967 available from
187 GenBank™ (Accession Number EF521410).

188 189 **Discussion**

190
191 The present study describes a case of *O. boehmi* infection in a horse from Italy, where equine
192 onchocerciasis had never been reported and it is therefore unknown to veterinary practitioners. In
193 equine practice, the appearance of skin nodules is often asymptomatic, and it goes therefore
194 unnoticed by owners (B. Riccio, personal communication). However, in the present report, the
195 clinical presentation was accompanied by an impaired function of the suspensory ligament and
196 occurrence of mild lameness. Interestingly, prior to this case, no clinical symptoms associated to
197 infestation by *O. boehmi* had been described. Given the anatomical localisation of the worms and the
198 nodules, we therefore hypothesize that the nematode might have undertaken an erratic migration
199 from the in circulatory system (i.e., the arteries and veins of limbs) to the subcutaneous tissues of the
200 metacarpal region. *Onchocerca boehmi* had only been diagnosed in two isolated reports, and
201 information about its biology is lacking. According to the original report by Supperer (1953), adults
202 were detected in the medial or external layer of tissues within the artery wall 6.7% of Austrian
203 horses, while a second survey from Iran indicated that 8.69% of horses examined had microfilariae in
204 the blood (Mirzayans and Maghsoodloo 1977).

205 The presence of numerous parasites in the nodule allowed to assess the histopathological lesions
206 caused by the nematode. Eosinophils were the main inflammatory cells found in the nodule as
207 already reported (Scott and Miller 2003); indeed these cells are typically detected in parasitic
208 cutaneous nodules, and they play a direct role in the defence against the parasites via their main basic
209 protein. Remarkably, eosinophils also play pivotal roles in hypersensitivity disorders (Scott and
210 Miller 2003), and they are seen in eosinophilic granuloma of horses, which are characterised by the
211 presence of cutaneous nodules and by the histopathological occurrence of collagen flame figures

212 (Scott and Miller 2003). Flame figures, albeit rare, were observed in the case herein described.
213 Onchocerciasis in horses is characterised by both parasitic encystment (adult parasites) and
214 hypersensitivity, the latter usually caused by microfilariae; nevertheless, dead or dying microfilariae
215 were not observed in the tissue examined and the lesions were not pruritic.

216 The morphology of the cuticle of the nematode fragments collected resembled to that of the voucher
217 material from Austria; this allowed to consider both samples conspecific. In particular, while *O.*
218 *boehmi* is characterized by cuticle without external ridges and three distinct layers with specific
219 morphology, other *Onchocerca* parasitizing equids, i.e. *O. cervicalis* and *O. reticulata* have a cuticle
220 with well-distinct external annular ridges (Bain 1981). Conversely, the cuticle of *O. raillieti*, which is
221 smooth and does not bear any external ridges, is thicker than that of *O. boehmi* (up to 50–55 µm vs
222 22–25 µm) and has longer striae (up to 16–20 µm vs 6–12 µm) (Bain et al. 1976; present study). Data
223 reported in the present study indicate that the systematic position of *O. boehmi* should be ranked
224 within the genus *Onchocerca*, as suggested by Bain et al. (1967), and not in the genus *Elaeophora*, as
225 corroborated by morphological and molecular results here produced.

226 Equine onchocerciasis has been reported worldwide, but most epidemiological information date back
227 from the 70s'. For instance, *Onchocerca* sp. has been diagnosed in horses from the United States,
228 where Stannard and Cello (1975) reported a mean 48% prevalence, whereas Lloyd and Soulsby
229 (1978) recovered microfilariae in 61% of examined animals from the eastern part of the country.
230 Schmidt and colleagues (1982) examined the nuchal ligament of 83 horses from Midwestern U.S.,
231 and 37% of were positive for adult parasites. Klei et al. (1984) detected microfilariae in 76% (out of
232 84) of ponies from the Gulf Coast area and in 82.4% of horses (out of 51) from the Louisiana State.
233 Of 664 horses from southeaster and midwestern US, 341 (51.4%) were positive for cutaneous
234 microfilariasis by *O. cervicalis* (Cummings and James, 1985). Monahan et al. (1985) diagnosed *O.*
235 *cervicalis* infection in 30.5% (25/82) ponies in USA. Finally, Lyons and colleagues (2000) reported
236 *O. cervicalis* in 24% of horses (out of 157) examined for several species of internal parasites at
237 necropsy in Kentucky. Infection by *O. cervicalis* infection has reported also in Canada (Marcoux et
238 al. 1977; Lees et al. 1983). Indeed, during a survey on 383 slaughtered horses from the western
239 Canadian provinces, *O. cervicalis* microfilariae were detected in 11.8% of umbilical samples (Polley
240 1984). Riek (1954) examined the nuchal ligaments of 282 Australian horses from Queensland and
241 found that 79.8% were infected with *Onchocerca* (erroneously reported as *O. reticulata*), whereas
242 Ottley et al. (1983) sampled a small group of horses and ponies from Queensland and the Northern
243 Territory, and diagnosed *O. cervicalis*, *O. gutturosa* and *O. reticulata* in those animals. In South
244 America, Mancebo et al. (1997) found *O. cervicalis* microfilariae in 24% of the 257 adult work

245 horses examined in Argentina. A similar result was reported in Brazil by Marques and Scrofernecker
246 (2004), who described *O. cervicalis* microfilariae in the midventral skin samples of 17.9%
247 (215/1,200) horses examined, while adult worms were recovered from the nuchal ligaments of 200
248 (16.6%) animals. In Europe, a few studies have been performed thus far. In England, Mellor (1973)
249 detected adult *Onchocerca* sp. in nuchal ligaments of 15.8% (33/209) British horses; in France,
250 Moignoux (1954) reported that 6% of horses living in Camargue were infected by subcutaneous
251 *Onchocerca* microfilariae. However, Collobert et al. (1995) found that only 1% (out of 368 horses)
252 were positive for *Onchocerca* at post mortem examinations in Normandy. In other European
253 countries, out of 160 horse skin biopsies examined on horses showed a very low prevalence 3.7% of
254 *Onchocerca* microfilariae from Spain and Poland (Franck et al. 2006). Finally, in Finland, skin
255 biopsies from 42 horses were all negative for microfilariae (Solismaa et al. 2008). These data indicate
256 that equine onchocerciasis is common in horse populations; however, as a consequence of the non-
257 specific clinical presentation and challenging diagnosis, the infection prevalence is most likely
258 underestimated. Additional large-scale studies are required to ascertain the presence and diffusion of
259 *O. boehmi* and other onchocercid species in Italian and European horse populations.

260 Based on these observations, we therefore argue that veterinary practitioners should be aware of the
261 presence and of the clinical issues associated with infection by these equine parasites, whose
262 occurrence is very often underestimated whose identification may require the application of non-
263 conventional diagnostic methods. Finally, the life cycle of *O. boehmi* remain unknown and further
264 studies should be performed to elucidate the biology of this poorly-knowm onchocercid.

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269 voucher material.

270 271 272 **Conflict of interest statement**

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

274 275 **References**

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- 277 Anderson RC (2000) The Superfamily Filarioidea. In *Nematode Parasites of Vertebrates; Their*
 278 *Development and Transmission*. Second edition. New York: CABI Publishing; 517-523
- 279 Bain O (1975) Redescription de cinq espèces d'onchocercques. *Ann Parasitol Hum Comp* 50:763-788
- 280 Bain O (1981) Le genre *Onchocerca*: hypothèses sur son évolution et clé dichotomique des espèces.
 281 *Ann Parasitol Hum Comp*, 56:503-526
- 282 Bain O, Muller RL, Khamis Y, Guilhon J, Schillhorn van Veen T (1976) *Onchocerca raillieti* sp. n.
 283 (Filarioidea) chez l'Ane domestique en Afrique. *J Helminthol* 50:287-293
- 284 Cello RM (1971) Ocular onchocerciasis in the horse. *Equine Vet J* 3:148-154
- 285 Collobert C, Bernard N, Lamidey C (1995) Prevalence of *Onchocerca* species and *Thelazia*
 286 *lacrimalis* in horses examined post mortem in Normandy. *Vet Rec* 136:463-465
- 287 Cummings E, James ER (1985) Prevalence of equine onchocerciasis in southeastern and midwestern
 288 United States. *J Am Vet Med Assoc* 186:1202-1203
- 289 Franck MT, Colombet J, Hugnet C, Ducos de Lahitte J, Desmaizières LM, Delverdier M, Franc M
 290 (2006) Research of skin microfilariae on 160 horses from Poland, France and Spain. *Revue Méd*
 291 *Vét* 157:323-325
- 292 Gardiner CH, Poynton SL (1999) An atlas of metazoan parasites in animal tissues. Armed Forces
 293 Institute of Pathology, Washington, DC pp. 1-39
- 294 Klei TR, Torbert B, Chapman MR, Foil L (1984) Prevalence of *Onchocerca cervicalis* in equids in
 295 the Gulf Coast region. *Am J Vet Res* 45:1646-1647
- 296 Lees MJ, Kleider N, Tuddenham TJ (1983) Cutaneous onchocerciasis in the horse: five cases in
 297 Southwestern British Columbia. *Can Vet J* 24:3-5
- 298 Lloyd S, Soulsby EJJ (1978) Survey for infection with *Onchocerca cervicalis* in horses in eastern
 299 United States. *Am J Vet Res* 39:1962-1963
- 300 Lyons ET, Swerczek TW, Tolliver SC, Bair HD, Drudge JH, Ennis LE (2000) Prevalence of selected
 301 species of internal parasites in equids at necropsy in central Kentucky (1995-1999). *Vet Parasitol*
 302 92:51-62
- 303 Mancebo OA, Verdi JH, Bulman GM (1997) Comparative efficacy of moxidectin 2% equine oral gel
 304 and ivermectin 2% equine oral paste against *Onchocerca cervicalis* (Railliet and Henry, 1910)
 305 microfilariae in horses with naturally acquired infections in Formosa (Argentina). *Vet Parasitol*,
 306 73:243-248
- 307 Marcoux M, Frechette JL, Morin M (1977) *Onchocerca cervicalis* infection in Quebec: clinical signs
 308 and diagnostic methods. *Can Vet J* 18:108-110
- 309 Marques SMT, Scrofernecker ML (2004) *Onchocerca cervicalis* in horses from Southern Brasil.
 310 *Trop Anim Health Prod* 36:633-636

- 311 Mellor PS (1973) Studies on *Onchocerca cervicalis* Raillet and Henry 1910: I. *Onchocerca cervicalis*
312 in British horses. J Helminthol 47:97-110
- 313 Mirzayans A, Maghsoodloo H (1977) Filarial infection of Equidae in the Tehran area of Iran. Trop
314 Anim Health Prod 9:19-20
- 315 Moignoux JB (1954) Enquête épidémiologique sur l'onchocercose cutanée des chevaux en
316 Camargue. Rev Path Gen Comp 54:569-573
- 317 Monahan CM, Chapman MR, French DD, Klei TR (1995) Efficacy of moxidectin oral gel against
318 *Onchocerca cervicalis* microfilariae. J Parasitol 81:117-118
- 319 Muller R (1979) Identification of *Onchocerca*. Symposia of the British Society for Parasitology, Vol.
320 17. In: Problems in the Identification of Parasites and their Vectors, ed. Taylor and Muller.
321 Blackwell Scientific, London, pp. 175-206
- 322 Munger RJ (1983) Equine onchocercal keratoconjunctivitis. Equine Vet J 15:65-70
- 323 Mutafchiev Y, Dantas-Torres F, Giannelli A, Abramo F, Papadopoulos E, Cardoso L, Cortes H,
324 Otranto D (2013) Redescription of *Onchocerca lupi* (Spirurida: Onchocercidae) with
325 histopathological observations. Parasit Vectors 6:309
- 326 Onmaz AC, Beutel RG, Schneeberg K, Pavaloiu AN, Komarek A, van den Hoven R (2013) Vector
327 and vector-borne diseases of horses. Vet Res Commun 37:65-81
- 328 Otranto D, Sakru N, Testini G, Gürlü VP, Yakar K, Lia RP, Dantas-Torres F, Bain O (2011) Case
329 report: first evidence of human zoonotic infection by *Onchocerca lupi* (Spirurida, Onchocercidae).
330 Am J Trop Med Hyg 84:55-58
- 331 Ottley ML, Dallemagne C, Moorhouse DE (1983) Equine onchocerciasis in Queensland and the
332 Northern Territory of Australia. Aust Vet J 60:200-203
- 333 Polley L (1984) *Onchocerca* in horses from Western Canada and the Northwestern United States: An
334 abattoir survey of the prevalence of infection. Can Vet J 25:128-129
- 335 Riek RF (1953) Studies on allergic dermatitis (Queensland itch) of the horse. I. Description,
336 distribution, symptoms and pathology. Aust Vet J 7:177-184
- 337 Riek RF (1954) Studies on allergic dermatitis (Queensland itch) of the horse: the aetiology of the
338 disease. Aust J Agricul Res 5:109-129
- 339 Schmidt GM, Krehbiel JD, Coley SC, Leid RW (1982) Equine onchocerciasis: lesion in the nuchal
340 ligament of Midwestern US horses. Vet Pathol 19:16-22
- 341 Scott DW, Miller WH (2003) Equine dermatology. Saunders, Elsevier Science, St. Louis, Missouri
342 pp 242-245
- 343 Solismaa M, Laaksonen S, Nylund M, Pitkänen E, Airakorpi R, Oksanen A (2008) Filarioid
344 nematodes in cattle, sheep and horses in Finland. Acta Vet Scand 50:20

345 Stannard AA, Cello RM (1975) *Onchocerca cervicalis* infection in horses from the western United
346 States. Am J Vet Res 36:1029-1031
347 Supperer R (1953) Filarosen der Pferde in Österreich. Wiener Tierärztliche Monatsschrift 40:193-220
348 Uni S, Fukuda M, Agatsuma T, Bain O, Otsuka Y, Nakatani J, Matsubayashi M, Harada M, Omar H,
349 Ramli R, Hashim R, Azirun MS, Hashim R (2015) *Onchocerca takaokai* n. sp. (Nematoda:
350 Filarioidea) in Japanese wild boars (*Sus scrofa leucomystax*): Description and molecular
351 identification of intradermal females. Parasitol Int 64:493-502.
352 Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary
353 Genetics Analysis version 6.0. Mol Bio Evol 30: 2725-2729
354 Taylor MA, Coop RL, Wall RL (2007) Parasites of horses. In *Veterinary Parasitology* 3rd edition.
355 Blackwell Publishing; 303-304

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357 **Figure captions**

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

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

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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 details 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).

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

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

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

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