Strongyloides stercoralis infection in imported and local dogs in Switzerland - From clinics to molecular genetics

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Abstract

Strongyloides stercoralis is a worldwide distributed intestinal nematode affecting mainly humans and dogs. Canine strongyloidosis is generally characterized by diarrhoea, malabsorption and bronchopneumonia, and may be fatal in cases of impaired immunity. In recent years, molecular and epidemiological studies suggested that host-adapted populations of *S. stercoralis* with different zoonotic potential may exist. Clinical and subclinical cases of *S. stercoralis* infection have been increasingly diagnosed in imported (France, Belgium, Bulgaria) and locally born dogs in Switzerland, showing that this parasite is currently circulating in Europe. Three of these clinical cases will be described here. All three dogs presented severe disease, characterised by harsh diarrhoea, dehydration, vomiting, respiratory and/or neurologic signs, and needed intensive care and hospitalisation. One of these dogs was related to a Swiss breeding kennel, in which the infection was subsequently diagnosed in several other dogs. Faeces were analysed by three coproscopical methods including (i) the Baermann technique, which consistently identified the typical *S. stercoralis* first-stage larvae in both clinical and subclinical infections, (ii) the sedimentation-zinc chloride flotation and (iii) sodium acetate - acetic acid - formalin concentration (SAFC) methods, which allowed the additional identification of parasitic females and/or eggs in two of the clinical cases. Interestingly, *S. stercoralis*

isolated from all three independent clinical cases exhibited an identical genetic background on the nuclear *18S* rDNA (fragment involving hypervariable regions I and IV) and the mitochondrial cytochrome oxidase subunit I (*cox1*) loci, similar to that of zoonotic isolates from other geographical regions, and not to that of dog-adapted variants. Due to the clinical relevance and zoonotic potential of this parasite, the awareness of both diagnosticians and clinicians is strongly required.

Introduction

Strongyloides stercoralis is a worldwide distributed intestinal nematode that affects mainly humans and dogs. Higher prevalences are generally observed in tropical and subtropical regions (Thamsborg et al. 2017). *S. stercoralis* undergoes a complex life cycle involving both parasitic and free-living generations. Parasitic females are located in the small intestine mucosa and produce eggs containing first-stage rhabditoid larvae (L1) by parthenogenesis, which hatch in the intestine and are shed with the faeces. In the environment, they develop either directly through second-stage rhabditoid larvae (L2) into infective third-stage filariform larvae (L3) (homogonic development) or alternatively, through several stages and moultings into free-living female and male worms that mate and produce a generation of parasitic L3 (heterogonic development) (Thamsborg et al. 2017; Deplazes et al. 2016). Dogs get mainly infected by percutaneous penetration of L3, or through the oral mucosa. Lactogenic transmission may be possible if the bitch is infected late in gestation or during lactation, but it is considered not common (Shoop et al. 2002). After infection, L3 migrate to the small intestine via lungs, and reach maturity after two moults, developing into parthenogenetic females. However, the existence of further alternative migration routes was assumed (Schad et al. 1989).

The infection in dogs can be asymptomatic; however, life threatening disease characterized by diarrhoea, malabsorption and bronchopneumonia may occur. In case of impaired immunity (e.g. due to illness or administration of immunosuppressive drugs) autoinfection, hyperinfection and extraintestinal dissemination (e.g. trachea, nasal cavities, lungs, oesophagus, stomach, cranial cavity) of the parasite, with severe clinical signs were reported in dogs (Cervone et al. 2016; Genta 1986; Grove et al. 1983; Mansfield et al. 1996; Schad et al. 1984).

Strongyloides stercoralis was successfully transmitted from humans to dogs experimentally, and it has been largely considered a zoonotic nematode (Deplazes et al. 2016; Thamsborg et al. 2017; Jariwala

et al. 2017). However, since different genotypes have been identified during the last years, this fact has been subject of discussion. It was assumed that host-specialized populations of *S. stercoralis* may exist, and that zoonotic transmission might occur less frequently than previously thought (Ramachandran et al. 1997; Hasegawa et al. 2010; Thamsborg et al. 2017; Takano et al. 2009). Recent comparative studies mainly based on the nuclear 18S rDNA (small subunit, *SSU*) and the mitochondrial cytochrome oxidase subunit I (*cox1*) locus revealed the existence of two genetically different populations of *S. stercoralis* in dogs: one population appeared to be dog-specific, while another population was shared by dogs and humans (Jaleta et al. 2017; Nagayasu et al. 2017). Although *S. stercoralis* infections in dogs may be frequent in some tropical regions, they are considered rare in Europe. Reports on *S. stercoralis* infections in dogs during the last years (2007-2018) in Europe are summarized in Table 1.

In this study we present three clinical cases of *S. stercoralis* infection including one case from a Swiss breeding kennel involving several (imported and locally born) dogs, and two further cases from imported dogs unrelated to the first case. We also provide data on the diagnosis and treatment of this disease. Furthermore, molecular typing of the *S. stercoralis* isolates involved in the three clinical cases was performed to shed some light on the genetic background and zoonotic potential of canine *S. stercoralis* parasites circulating in Europe. For better understanding, we first present each case with its outcome and afterwards the molecular characterisation of the isolated parasites from all cases.

Materials and Methods

Cases of *Strongyloides stercoralis* infection in dogs (Summarized in Suppl. Table 1)

Case 1

A female 11-month-old Yorkshire terrier (Dog No. 1) was presented to the emergency service of the Small Animal Clinic of the Vetsuisse Faculty in Bern with acute diarrhoea, vomiting, anorexia, apathy and chronic respiratory problems. The dog had been imported from France into Switzerland eight months before and held in a familiar breeding kennel together with 36 other dogs since purchase. During the previous months before diagnosis, the dog received amoxicillin and metacam for 2.5 months (i.e. until 1.5 months before admission) due to a severe respiratory disease, clinically diagnosed as kennel cough by the referring veterinarian that affected half of the adult dogs and most puppies in the kennel during a total period of 3.5 months (as not all dogs got ill simultaneously). After a

short clinical recovery, the dog presented pruritus, and a treatment with dexamethasone (Dexadreson®) was initiated, which was prolonged over one month (until admission). Two weeks later, respiratory signs reappeared, and shortly afterwards, diarrhoea was also noted; therefore, amoxicillin and enrofloxacin administration was started few days before admission.

At clinical examination, abdominal breathing, marked loss of weight, and alopecia with desquamation and pustules in neck, legs, and perineal regions were noticed. Thoracic radiographs showed a mixed alveolar and interstitial lung pattern, more evident in the periphery of the caudal lobes. Abdominal ultrasonography suggested the presence of hepatopathy, enteropathy and ascites. Blood analyses revealed marked hypoalbuminemia, hypocalcaemia, hypoglycemia, hypocobalaminemia, elevated C-reactive protein (CRP) levels and hypercoagulability. First, a serious protein-losing enteropathy associated with a viral, bacterial or parasitic pneumonia was suspected or, less probable a thromboembolism. Immediately, a therapy based on glucocorticoids in anti-inflammatory dose, parenteral glucose infusion (Plasmalyte®), clopidogrel (antiaggregant), cobalamin (250 µg sc, 4 applications at weekly intervals) as well as enrofloxacin (Baytril®) (5 mg/kg/day for 7 days) and fenbendazole (Panacur®) (50 mg/Kg/day 5 days, repeating after 3 days interval) was initiated.

Subsequently, further complementary diagnostic methods were performed. Commercial rapid tests for Parvovirus, *Leptospira* and *Angiostrongylus vasorum* infections (IDEXX Parvo Snap Test; Zoetis Witness Lepto and IDEXX Angio Detect Test) yielded negative results. Next, a coproscopical examination by three different techniques (i.e. SAFC (sodium acetate-acetic acid-formalin concentration); sedimentation-zinc chloride flotation (s.d. 1.35) and Baermann techniques) (Deplazes et al. 2016) was performed at the Institute of Parasitology in Bern. The analysis by the SAFC method was negative. By sedimentation-flotation, a few nematode larvae were observed, but their distinctive morphological characteristics were not clearly recognisable. The Baermann technique, however, revealed a high number of rhabditoid larvae, which were morphologically identified as L1 of *S. stercoralis* (Fig 1 a-c). After the first parasitological diagnosis, the glucocorticoid therapy was immediately stopped. A coproscopical control 3 days after beginning of fenbendazole therapy still showed viable L1 in the faeces, and diarrhoea and coughing were still present. Consequently, ivermectin (0.2 mg/kg sc) was administered (off-label) once and repeated after 2 weeks. Diarrhoea ceased 24 h after the first ivermectin administration. During the following days, the general condition of the dog improved, and it left the clinic 7 days after admission. At control 10 days later, the respiratory

signs disappeared completely, serum albumin levels almost normalized but faeces were still soft. Coproscopical analyses were negative.

Subsequently, all dogs from the kennel were coproscopically examined. *S. stercoralis* L1 were detected by the Baermann method in faeces (several pools) from 33 asymptomatic (or showing only soft faeces) Swiss Yorkshire terriers, in two 9-month-old Yorkshire terriers showing diarrhoea and respiratory problems, which had been imported from Bulgaria 4 months before, and finally in one adult female Swiss Yorkshire with cough. Additionally, *Giardia duodenalis* cysts were detected in all analysed faecal samples by SAFC, and *Isospora canis* oocysts were found in faeces from the first dog groups (asymptomatic dogs) by the sedimentation-zinc chloride flotation method. All adult dogs and puppies were treated with fenbendazole (50 mg/Kg/day 5 days twice with 3 days interval) and ivermectin in the above-mentioned doses. Bitches that were pregnant when the diagnosis was first made were initially treated with selamectin spot-on solution (Stronghold®) and after delivery also with ivermectin. A coproscopical control from all dogs (*n*=6 pooled samples according to housing groups) performed 10 days after finishing the second ivermectin treatment was negative for *S. stercoralis*, but two of the pools were still positive for *Giardia*.

As further dogs in the kennel showed diarrhoea from time to time, the ivermectin dose was increased to 0.4 mg/kg, and the duration of the treatment was prolonged by the veterinarian of the kennel. Finally, all dogs received a total of 5 ivermectin doses (once 0.2 mg/kg, and four times 0.4 mg/kg). All dogs showed a good tolerance to the medication, except one, which presented transient ataxia and trembling after the first ivermectin dose. After the fourth ivermectin dose, the digestive signs completely disappeared in the kennel.

Case 2

(One month after Case 1) A female 3-month-old Chihuahua (Dog No. 2) was transferred to the urgency service of the Small Animal Clinic in Bern by a private Veterinarian after presenting epileptiform episodes that were treated with midazolam. The dog had been imported from France two days before. At admission it was in lateral recumbency, comatose, hypothermic, showing tremors and diarrhoea. Blood analyses revealed hypoglycaemia, metabolic acidosis with low bicarbonate levels, hypoalbuminemia and hyperphosphatemia. Coproscopical analyses were performed at the Institute of Parasitology as detailed above. The SAFC method revealed the presence of *G. duodenalis* cysts and trophozoites. By the sedimentation/flotation method *I. canis* oocysts and thin-shelled larvated

nematode eggs (82.7 [76.3-86.0] x 40.2 [37.7-42.5] µm; *n*=9) were detected (Fig 2 a, b). Free rhabditoid larvae (337.5 [276-380] µm; *n*=9) (Fig 2 a) were observed by both sedimentation/flotation and Baermann methods.

The dog received parenteral glucose infusion, omeprazole, fenbendazole (50 mg/kg for 5 days) and toltrazuril (8 mg/kg/day for 5 days). Three days after beginning with fenbendazole therapy, live L1 were still present in the faeces and ivermectin was administered (0.2 mg/kg) and recommended to be repeated after 2 weeks. The dog recovered clinically after 5 days of treatment, left the Clinic and no further follow up was possible.

Case 3

(Five months after Case 1) A female 5-month-old French bulldog (Dog No. 3) imported from Belgium into Switzerland 2 months earlier was presented to the Small Animal Clinic in Bern with bloody diarrhoea and vomiting. The dog had diarrhoea since it was bought, and since f approximately one week before admittance, also blood was observed in the faeces. A coprological analysis performed at a private veterinary clinic one week before (no method was specified) gave negative results. Thoracic radiologic examination showed no abnormalities. After being hospitalized at our veterinary hospital, coprological analyses were performed at the Institute of Parasitology. Numerous *S. stercoralis* eggs (72.4 [62.1-81.5] x 37.2 [32.3-39.3] µm; *n*=7) containing larvae in different evolution stages (Fig 3 a, b), free rhabditoid L1 (Fig 3 a, b) and parasitic females (Fig 3 c, d) were detected by the sedimentation/flotation and SAFC methods; by Baermann, abundant living L1 were seen. The dog was treated with ivermectin 0.2 mg/kg sc and fenbendazole 50 mg/kg for 5 days. The dog clinically recovered and left the hospital 6 days after admission. The coproscopical control one week after initiated the treatment was negative. Cases 1 to 3 are summarized in Suppl. Table 1.

Molecular characterisation of *S. stercoralis* isolates

To confirm the microscopical diagnosis and to obtain information on the genetic background and zoonotic potential of *S. stercoralis* parasites involved in these clinical cases, a molecular characterisation based in the amplification and sequencing of fragments of the mitochondrial *cox1* gene and of the nuclear 18S rDNA, including the hypervariable regions (HVR) I and IV was performed

(Table 2). The amplified small subunit (*SSU*) fragment indicating the localization of the HVR I and HVR IV and of the primers used in this study is presented in fig 2 in Jaleta et al. (2017).

Briefly, *S. stercoralis* L1 were isolated from all three dogs (Dogs No. 1 to 3) by the Baermann method, washed in PBS by centrifugation and conserved at -20°C until processing. DNA from the larvae was extracted with a commercial kit (DNeasy Blood & Tissue Kit, QIAGEN) as indicated by the manufacturer. PCR reactions were performed in a total volume of 50 µl (25 µl QIAGEN Multiplex Master Mix 2X, 19 µl nuclease free water, 0.5 µl of each primer according to Table 2 and 5 µl template) using a GeneAmp® PCR System 9700 (Applied Biosystems) instrument and following thermocycling programs: *cox1*: 94°C/15', 40 x (94°C/45'', 52°C/45'', 72°C/90''), 72°C/10', 4°C/∞; *SSU* HVR I: 94°C/15', 35 x (94°C/30'', 52°C/15'', 72°C/90''), 72°C/10', 4°C/∞; *SSU* HVR IV: 94°C/15', 35 x (94°C/30'', 57°C/15'', 72°C/90''), 72°C/10', 4°C/∞. The obtained PCR products were visualized after electrophoresis in 2% agarose gels stained with ethidium bromide, subsequently purified with a commercial kit (DNA Clean & Concentrator-5 Zymo Research, USA) and sequenced in both directions by a commercial company (Microsynth, Balgach, Switzerland) using primers indicated in Table 2. The obtained sequences were compared with those available in GenBank using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

PCR products with the expected sizes were obtained in all cases. All amplified products showed 99-100% identity with published *S. stercoralis* sequences, confirming the morphological diagnosis. The obtained DNA sequences were deposited in GenBank, after trimming of the primers binding region (accession numbers MH932095-MH932103). Interestingly, all three isolates showed the same genetic background with an identical molecular pattern in all three analysed markers. The obtained *cox1* sequences (accession No. MH932101-MH932103) showed 100% identity (649/649 bp) with sequences of *S. stercoralis* (AJ558163.1, LC050212.1) from parasites derived from an isolate originally obtained from a dog in the USA (UPD strain) (Schad et al. 1984; Stoltzfus et al. 2012). They also showed 100% identity (606/606 pb) with *S. stercoralis* isolates from dogs from Japan (accession No. LC179456-58; LC179244-53) and from a human patient from Thailand (LC179281), but with only 93% query cover, as only partial *cox1* sequences were available in GenBank (Nagayasu et al., 2017). The polymorphism in the HVR-I observed among isolates of *S. stercoralis* is a single base insertion/deletion (indel) that results in a stretch of either four or five thymidine nucleotides within this

region (4T or 5T sequence) (Nagayasu et al., 2017). In this study, the amplified *SSU* fragment including HVR I (accession No.MH932098-MH932100) displayed an HRV I haplotype with a 4T sequence: 5´- TTTT-ATATT…A -3´. Additionally, a polymorphism A was observed in the polymorphic site 18S rDNA 458 T/A, identified at position 458 of the reference AF279916 (Online Resource 1). Following the nomenclature of Jaleta et al., (2017) we named this haplotype as HVR I haplotype VI (Online Resource 1). This haplotype VI was observed previously in *S. stercoralis* isolates from a dog (accession No. AB453316.1) and a chimpanzee (accession No. AB453314.1) in Japan (Hasegawa et al. 2009).

Parasites isolated from Dogs No. 1, 2 and 3 displayed HRV IV haplotype A (5´- ATTTTGTTTATTTTA-ATAT-3´). This haplotype has been observed in parasites isolated from dogs, humans and non-human primates (Hasegawa et al. 2009) and was assumed to characterise the zoonotic population of *S. stercoralis* (Jaleta et al. 2017). Moreover, the whole amplified sequence surrounding HVR IV of the parasites in this study (Accession No. MH932095-MH932097) showed a 100% identity (680/680 pb) with GenBank sequences of *S. stercoralis* isolated from the faeces of a human patient in Myanmar (accession No. AB923888.1) (Hino et al. 2014).

Discussion

S. stercoralis infections in dogs are not considered common in Europe, however some cases have been recorded during the last years in England (Wright et al. 2016); Finland (Dillard et al. 2007); France (Cervone et al. 2016); Greece (Papazahariadou et al. 2007; Kostopoulou et al. 2017); Iceland (Eydal und Skírnisson 2016); Italy (Zanzani et al. 2014; Riggio et al. 2013; Paradies et al. 2017; Sauda et al. 2018; Iatta et al. 2018); Norway (Hamnes IS, Davidson R & Øines Ø 2009); Republic of Macedonia (Cvetkovikj et al. 2018); Slovakia (Štrkolcová et al. 2017) and Romania (Mircean et al. 2012) (summarized in Table 1). There are no recent reports from Central Europe. In Austria, *S. stercoralis* infection was first recorded in a dog in 1974 at the Institute of Parasitology in Vienna, and in two Austrian breeder kennels in the 80s (Prosl 1985). In Germany, the parasite was detected in 0.3% of 3,329 dogs analysed between 1984 and 1991 at the Institute of Parasitology, University of Veterinary Medicine Hannover (Epe et al. 1993). However, it was not observed in further 4,012 dogs analysed by the sedimentation/ zinc sulphate (s.g 1.3) flotation and Baermann methods between 1998 and 2012 at the same Institute (Epe et al. 2004; Raue et al. 2017). In the present study, the diagnosis

of all cases was performed between December 2017 and May 2018 at the Institute of Parasitology in Bern, and there were no previous records of this parasite in dogs for the last 10 years at the Institute. Although *S. stercoralis* is generally considered as a tropical parasite, and it seems not well adapted to survive low environmental temperatures, it can nevertheless successfully fulfil its life cycle and disseminate within commercial kennels and dog shelters (Table 1) even in cold climates (Eydal und Skírnisson 2016; Dillard et al. 2007; Hamnes IS, Davidson R & Øines Ø 2009). This fact, added to the increased national and international transport of dogs (e.g. dog trade, relocation of dogs by animal welfare associations, illegal import, tourism) observed during the last years may favour a wider spreading of this parasite in Europe, thus requiring an appropriate awareness of diagnostic labs, practicing veterinarians, as well as the availability of adequate control measures.

In the present study, *S. stercoralis* infection was detected in imported but also in dogs born in Switzerland. In the Swiss breeding kennel (Case 1), the parasite might have been introduced by the dog imported from France (Dog No. 1), but also by one or both dogs imported from Bulgaria (that had diarrhoea since their arrival), or it might have been already present in the kennel before these dogs arrived, remaining undiscovered so far. Since the imported dogs had been in the kennel for several months before the diagnosis was made, the origin of the infection could not be clearly elucidated. On the other hand, Dog No. 2 must be certainly considered as an imported case of strongyloidosis, as the Chihuahua was shedding *S. stercoralis* larvae when it was brought to the Clinic after being been bought in France only two days before (prepatent period of *S. stercoralis*: at least 5 to 21 days). Dog No. 3, originating from a breeding kennel in Belgium, was showing digestive signs since its arrival. The infection might have occurred in the kennel; however, since the animal had been in Switzerland for two months before diagnosis (and this exceeds the prepatent period of *S. stercoralis*) its imported origin can be only suspected but not proven.

In the present study, all three infected and hospitalised dogs showed severe disease needing intensive care. However, milder disease and subclinical infection were also detected in further dogs from the Swiss kennel (Case 1). Digestive signs were present in all three hospitalised cases, respiratory signs were additionally observed in Dog No. 1, and neurologic signs only in Dog No. 2, indicating a rather diversity of clinical presentations of this disease (Suppl. Table 1). Although *S. stercoralis* is considered a primary pathogen, concomitant viral, bacterial and parasitic infections can influence the clinical outcome. In the Swiss kennel, underlying respiratory agents as well as *G. duodenalis* and *I. canis* infections and prolonged medication, especially with corticosteroids, may have

277 contributed to some extent to the clinical severity observed, and to the dissemination of *S. stercoralis*
278 within the kennel. Recrudescence of infection, autoinfection and hyperinfection with dissemination of
279 the parasite to extraintestinal organs were observed in infected dogs after administration of high
280 and/or prolonged doses of corticosteroids (Genta 1986; Mansfield et al. 1996; Schad et al. 1984;
281 Schad et al. 1997; Genta 1989). In the case of Dog No. 2, coinfections with the intestinal parasites *G.*
282 *duodenalis* and *I. canis* were diagnosed, which could have also intensified the clinical manifestations.
283 Regarding coproscopical analyses, eggs in different maturity stages (Dogs No. 2 and 3) and parasitic
284 females (Dog No. 3) were additionally observed besides the typical L1. It is worth to note that the size
285 of *S. stercoralis* eggs seems to be quite variable. The eggs observed in this study (62-86 x 32-42 µm)
286 were larger than those reported for *S. stercoralis* (e.g. 50-58 x 30-34 µm) by Thamsborg et al. (2017).
287 However, our observations are in agreement with previous reports, in which also a larger size for *S.*
288 *stercoralis* eggs after oviposition was observed (Tanaka 1966). Shimura (1919) described that the
289 eggs of *S. stercoralis* in dogs had a size of 56-64 x 22-30 µm in the uterus and of 60 x 40 µm after
290 oviposition and Ito (1932) reported sizes of 66-83 x 36-45 µm in the uterus and of 75-88 x 40-60 µm
291 after oviposition (as cited in Tanaka 1966). Therefore, factors that may influence the size of the eggs
292 such as maturity, possible degradation inside dead females shed with the faeces, or mechanical
293 effects during performance of the coproscopical analyses should be considered during the diagnosis.
294 Due to their similar size, S*. stercoralis* eggs could have been misdiagnosed with hookworm or free-
295 living nematode eggs (product of intestinal passage through coprophagia); however, this possibility
296 can be disregarded in the present cases as several of the observed eggs in fresh faeces contained
297 already a larva (hookworm eggs are morulated when shed), and coprophagia was not possible as the
298 dogs were housed in isolated cages in the intensive care station of the Clinic.
299 To the authors knowledge, no products for the treatment of *S. stercoralis* infections in dogs are
300 registered so far, and reported treatments were mostly attempted in only one or a few dogs. Some
301 good clinical results and/or clearance of L1 from the faeces seem to have been obtained after off-label
302 administration of ivermectin (0.2-0.8 mg/Kg BW one or several doses with different intervals)
303 (Mansfield und Schad 1992; Dillard et al. 2007; Cervone et al. 2016; Thamsborg et al. 2017; Nolan
304 2001; Umur et al. 2017; Iatta et al. 2018); however, this drug appears not to be effective to remove
305 migrating L3 from extraintestinal sites (Mansfield und Schad 1992). Administration of fenbendazole as
306 mono-drug (50 mg/kg BW/day for 5-7 days) (Cervone et al. 2016; Paradies et al. 2017; Eydal und

Skírnisson 2016; Itoh et al. 2009) or combined with Moxydectin-imidacloprid (Advocate®) (Paradies et al. 2017) was only partially effective or not effective. There is also one report on the use of febantel (31.5 mg/kg BW) + pyrantel + praziquantel (Drontal Plus®) for 1 day, repeated after 12 days for 3 days with good clinical results (Cvetkovikj et al. 2018). Unfortunately, in most cases no long-term follow-up was possible. In this study, the combination of fenbendazole and ivermectin lead to clinical improvement and to the cease of larvae shedding in Dogs No.1 to 3. In Dog No. 1 also a long-term follow up could be carried up. In Dogs No. 1 and 2 it could be observed that after three days of fenbendazole treatment viable *S. stercoralis* larvae were still being shed, thus it seems that this drug is not or not highly effective against this parasite, at least as sole treatment. Clinical improvement and cease of larvae shedding were observed only after additional administration of ivermectin.

In the last years, the zoonotic potential of *S. stercoralis* and the role of dogs as reservoirs for humans has been subject of debate. Due to epidemiological data and to the observed genetic diversity within *S. stercoralis*, the existence of different host-adapted variants, subspecies or even species has been suggested (Thamsborg et al. 2017; Jaleta et al. 2017; Ramachandran et al. 1997; Hasegawa et al. 2009; Hasegawa et al. 2010). Recent molecular studies based on the polymorphism of the mitochondrial *cox1* gene and nuclear *18S* (hypervariable regions I and IV) rDNA sequences of *S. stercoralis* revealed that two genetically different populations occurred in dogs living in an endemic region for human and canine strongyloidosis in Cambodia. One population appeared to be restricted to dogs ("dog-adapted") while the other population was present both in dogs and humans ("dog-human shared") of the same region, arguing for its zoonotic character (Jaleta et al. 2017). These results would support the existence of a zoonotic species (*S. stercoralis*) and a dog-adapted species (*S. canis*) (Jaleta et al. 2017). Also Nagayasu et al. (2017) reported recently the existence of two genetically different lineages of *S. stercoralis* (Type A and Type B parasites), mainly characterized by the *cox1* sequence (*cox1* clades I and II haplotypes define *S. stercoralis* Type A and B respectively). While *S. stercoralis* type A were isolated from both humans and dogs from different countries (i.e humans from Japan, Myanmar, Thailand, Laos, Uganda, Central Africa and dogs from Japan and Myanmar), *S. stercoralis* type B were isolated exclusively from dogs from Myanmar. The authors suggested that Type B parasites could represent an ancestral canid-adapted *S. stercoralis* line, from which type A parasites evolved, expanding its host-spectrum to infect humans (Nagayasu et al., 2017). However, it is not known if these observations do also apply to parasites from other geographical regions. As the genetic structure of *S. stercoralis* strains circulating in Europe is largely unknown, we

performed a molecular characterisation of the isolates involved in the clinical cases in this study to aid clarifying this issue. Interestingly, in our study, all three European isolates obtained from unrelated dogs had the same genetic background for the considered markers, independent of their origin. This fact suggests that *S. stercoralis* isolates circulating among kennels/breeders in Europe may be very closely related. A full-genome sequencing approach could figure out if all three isolates are completely identical. This is noteworthy because the *cox1* sequence of *S. stercoralis* was shown to have a great variability, with at least 100 different haplotypes described in isolates from dogs and humans so far (Jaleta et al. 2017; Hasegawa et al. 2010; Laymanivong et al. 2016; Schad et al. 1984; Nagayasu et al. 2017). The *cox1* sequence from all three dogs in our study showed a 100% identity and query cover with GenBank entries (AJ558163.1, LC050212.1) derived from a same *S. stercoralis* isolate (UPD strain), originally obtained from a dog in the USA (Schad et al. 1984; Stoltzfus et al., 2012), but with none of the 17 haplotypes identified by Jaleta et al, (2017). It had also a 100% identity (but only 93% query cover) with *S. stercoralis* isolates from dogs from Japan (accession No. LC179456-58; LC179244-53) and from a human patient from Thailand (LC179281); however only partial *cox1* sequences (covering 93% of our sequences) were available from these isolates (Nagayasu et al., 2017). Therefore, we assume that the isolates obtained in our study would be phylogenetically related with parasites within the *cox1* Clade I in the study of Nagayasu et al (2017), in which both human and dog *S. stercoralis* isolates clustered, suggesting their zoonotic potential.

The HVR I haplotype detected in the present study (5' TTTT-ATATT…A 3') does not belong to any of the haplotypes (I to V) observed in parasites isolated from dogs and humans by Jaleta et al., (2017); however, this haplotype was observed previously in isolates from both dogs and non-human primates before (accession No. AB453316.1 and AB453314.1). Although the HVR I is highly conserved within many nematode species, it appears to be more variable in *S. stercoralis* and its usefulness to distinguish dog and human-adapted strains should be further clarified (Nagayasu et al., 2017). Among the three selected markers, the *SSU* HVR IV is the most conserved region, and so far, only 2 variants: haplotypes A and B have been described in *S. stercoralis* (Jaleta et al. 2017). The two haplotypes differed at three positions (two indels, one base substitution). Haplotype A seems to be the most frequent haplotype and was originally described as the HVR IV sequence of *S. stercoralis* (Hasegawa et al. 2009). Parasites showing this haplotype were isolated from humans and dogs from different geographical regions and also from chimpanzees (Hasegawa et al. 2009). In the study from Jaleta et al., (2017), the HVR IV haplotype A was the only haplotype present in *S. stercoralis* isolated from

humans and it was also found in 22.5% of the worms obtained from dogs, while the haplotype B was exclusively found in dog-derived worms and represented the most frequent haplotype in this species. In the same study, a total of 17 *cox1* different haplotypes were observed, 7 of them were associated with HVR IV haplotype A and 10 with HVR IV haplotype B. No *cox1* haplotype was shared by both HVR IV haplotypes, accounting for the existence of two different phylogenetic groups. On the other side, same HVR I haplotypes were shared by both groups, suggesting that the HVR I other than the HVR IV, does not indicate genetically separated populations. The authors stated that the *SSU* HVR IV haplotype can be adequate to identify both *S. stercoralis* populations in dogs and that haplotype A would be a marker for the zoonotic population. Parasites from all dogs in our study presented the HVR IV haplotype A variant accounting for their zoonotic potential, if this assumption proves to be valid. To our knowledge, only the owner of the dogs in Case 1 was examined coprologically after our diagnosis in the dogs and tested negative. Nevertheless, transmission from infected dogs to humans was already reported and dogs have been experimentally infected with parasites isolated from humans (Georgi und Sprinkle 1974; Thamsborg et al. 2017; Genta 1989; Grove und Northern 1988). It should be considered, that not only the presence of infected dogs in the household, but also factors from the host (e.g. level of exposure, immune status, hygiene measures), environment (e.g. humidity, temperature, sanitary conditions, level of contamination) and from the parasite (e.g. parasite burden, genetic background) are needed for the infection to be successfully established.

Conclusions

S. stercoralis infections in dogs may occur and should be considered during the diagnosis of enteritis and respiratory disease. Breeding kennels and animal trade seem to play an important epidemiological role in the dissemination of *S. stercoralis*. Since routine faecal flotation methods have low sensitivity for detection of L1, and shedding of eggs is considered uncommon, the prevalence, as well as the clinical significance of *S. stercoralis* in dogs might be underestimated; therefore, the awareness of diagnosticians and practicing veterinarians is required. Moreover, due to its zoonotic potential, a correct diagnosis is pivotal also from the public health point of view. The genetic background of the parasites detected in this study correlates with that of the zoonotic isolates and not with the dog-adapted variants. In the present case, *S. stercoralis* infection was detected in several dogs in Switzerland, either locally born or imported from other European countries, showing that the parasite is

currently circulating in Europe. The combination of fenbendazole and ivermectin (off-label) proved to be effective as treatment. However, no necropsies to ensure a complete absence of adult parasites or migrating larvae after treatment were performed and a long-term follow-up was not possible in all cases.

Notes

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Compliance with Ethical Standards

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Data availability

The DNA sequences obtained from this study are available from GenBank (https://www.ncbi.nlm.nih.gov/genbank) (accession numbers MH932095-MH932103).

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565

566 **Figure captions**

567 **Fig 1** First-stage (L1) rhabditoid larvae of *Strongyloides stercoralis* isolated from the faeces of Dog
568 No.1 by the Baermann technique. A: whole L1; B and C Detail of the anterior and posterior ends of
569 one L1. gp: prominent genital primordium; bc: small buccal capsule; ro: rhabditoid oesophagus; st:
570 straight tail
571

572 **Fig 2** a and b: First-stage larvae (arrows) and larvated eggs (arrow heads) of *Strongyloides stercoralis*
573 isolated from the faeces of Dog No. 2 by the sedimentation-zinc chloride flotation technique. Note in b
574 a string of larvated eggs.
575

576 **Fig 3** *Strongyloides stercoralis* stages isolated from faeces of Dog No. 3. a: first -stage larva (arrow)
577 and immature egg (arrow head); b: immature egg (arrow head); c: Detail of posterior end of the
578 parasitic female. d: Parasitic female, co: long, cylindric oesophagus, v: vulva behind the middle of the
579 body.
580

581 **Online Resources**

582 **Suppl. Table 1**: Cases of *Strongyloides stercoralis* infection in dogs in Switzerland. F: female; M: male;
583 d: days; m: months; Y: yes; N: no; L1: first-stage larvae; A: adult; SAFC: sodium acetate-acetic acid-
584 formalin concentration technique
585
586 **Suppl. Fig 1:** Nucleotide arrangements in hypervariable regions (HVR) I and IV (in boxes) in 18S
587 rDNA of *Strongyloides stercoralis* as defined by Hasegawa et al. (2009) and haplotypes according to
588 Jaleta et al. (2017); dash indicates absence of nucleotide; H: human; D: dog. Red: variable
589 nucleotides *: polymorphic site 18S rDNA 458T/A, identified at position 458 of the reference AF279916
590
591

Table 1: Reports on *S. stercoralis* infections in dogs during 2007-2018 in Europe detected by coproscopical methods

Country	Region	*n* tested	*n* pos	Prevalence (%)	Type of dogs	Breed, sex, age (w, m, y) in positive cases	Origin	Clinical signs in positive cases	Coproscopic method used to obtain the reported prevalence of *S. stercoralis*	Reference
England	Lancashire	171	3	1.7	household	>6 m	ns	ns	FLOTAC	(Wright et al. 2016)
Finland		1	1		household	Yorkshire terrier,10 w	born in Finnish Kennel	yes	intestinal scrapings, histopathology	(Dillard et al. 2007)
		46	3		kennel (*n*=1)	adults	Netherlands (*n*=1) imported 3 years before; born in Finish kennel (*n*=2)	ns	Baermann	
France	Paris	1	1		household	Chihuahua, male, 10 m	animal shop in Paris > 6 m before	yes	Flotation (solution s.g. 1.2; direct smear; Baermann	(Cervone et al. 2016)

Greece	Crete	879	1	0.1	shelters, household, shepherd	ns	ns	ns	sedimentation (acid/ether); flotation (sugar-salt s.g. 1.28)	(Kostopoulou et al. 2017)
	Serres Prefecture	117	1	0.9	shepherd	<6 m	ns	ns	Telemann's sedimentation	(Papazahariadou et al. 2007)
		164	4	2.4	hunting dogs	<6 m (*n*=1); adults (*n*=3)	ns	ns		
Iceland		3,208	20	0.6	imported dogs in quarantine 1989-2016	12 breeds; age: <1.5 years (*n*=17), 2–7 years (*n*=3)	Sweden (*n*=7); Hungary (*n*=4); Belgium (*n*=2); Finland (*n*=1); Poland (*n*=1); Latvia (*n*=1); Russia (*n*=1); UK (*n*=1); USA (*n*=2).	ns	formalin-ethyl acetate sedimentation technique (FEAST); Baermann	(Eydal und Skírnisson 2016)
		17	8		kennel (*n*=1) (faecal pools)	ns	Icelandic kennel (imported and locally born dogs)	ns	Baermann	

		96	11		household	8 breeds; 2.5-7 m (*n*=9); 5 y (*n*=2)	Icelandic kennel (*n*=9); in contact with dogs from the kennel (*n*=2)	yes (*n*=6); ns (*n*=5)	FEAST; Baermann	
Italy	Latium, Tuscany	639	1	0.2	kennels, shelters	ns	ns	ns	Baermann	(Sauda et al. 2018)
	Pisa, Tuscany	239	2	0.8	household	ns	ns	no	flotation (NaCl s.g. 1.2); Baermann	(Riggio et al. 2013)
	Milan, Lombardy	463	4	0.9	faeces from public soil	ns	ns	ns	flotation (sucrose and $NaNO_3$ s.g. 1.36)	(Zanzani et al. 2014)
	Apulia	210	1	0.5	household	adult	shelter (adopted 1 year before)	no	Baermann	(Paradies et al. 2017)
		62	5	8.1	shelter (*n*=1)	adults	ns	yes		
	Apulia	85	19	22.3	shelter (*n*=1)		ns	yes (*n*=2)	Baermann	(Iatta et al. 2018)
Norway		3	3		household	3 puppies	Sweden (*n*=1); born in Norwegian kennel (*n*=2)	yes		(Hamnes IS, Davidson R & Øines Ø 2009)

Slovakia	Medzev (Roma settlement)	30	4	13.3	household	mixed-breeds	ns	bad sanitary conditions	Koga agar plate culture	(Štrkolcová et al. 2017)
	Haniska	20	2	10	shelter (*n*=1)	mixed-breeds; <7 m (*n*=1); >7 m (*n*=1)	ns			
Republic of Macedonia	Skopje	1	1		household	Pomerania, male, 6 m	Russia (imported 1 w before)	yes	Direct faecal smear; flotation ($ZnSO_4$); Baermann	(Cvetkovikj et al. 2018)
Romania		52	2	3.8	kennels, shelters, shepherd, household	ns	ns	no	flotation (NaCl s.g. 1.2)	(Mircean et al. 2012)

ns: not specified; w: weeks; m: months; y: years; pos: positive; s.g. specific gravity

Table 2. Primers used for molecular typing of *Strongyloides stercoralis* isolates and PCR product size

Target	Primer	Name	Sequence	Product size (pb)	Ref
cox1	Fwd	COI int F	5‘-TGATTGGTGGTTTTGGTAA-3‘	688 bp	(Casiraghi M. et al. 2001)
	Rev	COI int R	5‘-ATAAGTACGAGTATCAATATC-3‘		
18S rDNA HVR I	Fwd	SSU 18A	5'AAAGATTAAGCCATGCATG-3'	863 bp	(Dorris et al. 2002)
	Rev	SSU 26R	5'-CATTCTTGGCAAATGCTTTCG-3'		
18S rDNA HVR IV	Fwd	18S P4F	5'-GCGAAAGCATTTGCCAA-3'	712 bp	(Hasegawa et al. 2009)
	Rev	18S PCR	5'-ACGGGCGGTGTGTRC-3'		

cox1: mitochondrial cytochrome c oxidase subunit I locus; HVR: hypervariable region of the 18S rDNA;

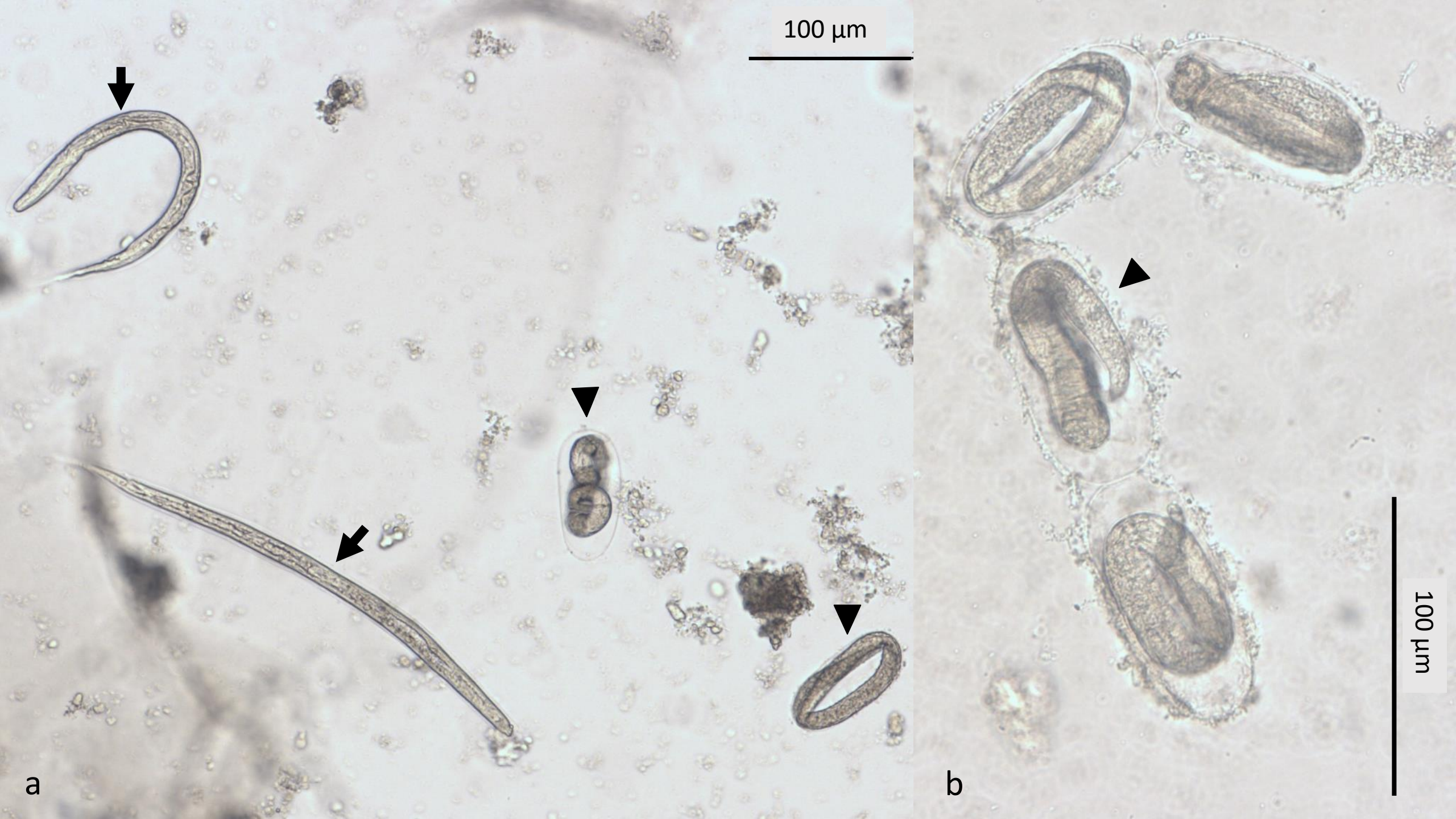
bp: base pairs

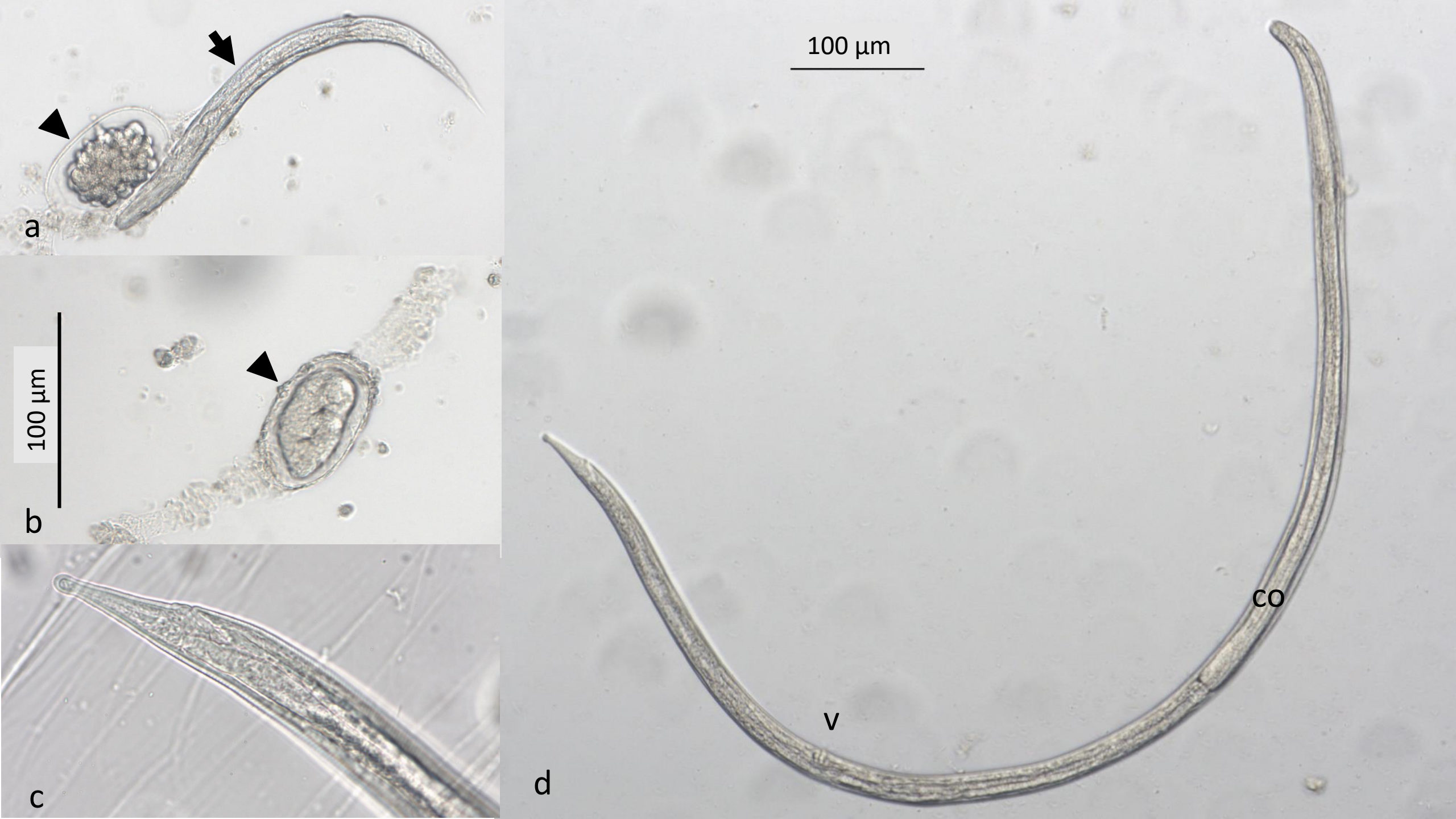
Suppl. Table 1: Cases of *Strongyloides stercoralis* infection in dogs in Switzerland

Case No	Breed	n	Origin (time since import into Switzerland)	Sex	Age	Clinical signs							Coproscopical results (1st diagnosis)			Genotyping performed
						Apathy	Anorexia	Respiratory	Digestive	Dermatological	Neurological	Asymptomatic or soft faeces	Sedimentation-Flotation (zinc chloride s.d. 1.35)	SAFC	Baermann technique	
1 (Swiss Kennel)	Yorkshire terrier	1	France (8 m)	F	11 m	Y	Y	Y	Y	Y	N	N	*S. stercoralis* L1	negative	*S. stercoralis* L1	Y (Dog No. 1)
	Yorkshire terrier	33	Switzerland	F, M	adults and puppies	N	N	N	N	N	N	Y	*I. canis*	*G. duodenalis*	*S. stercoralis* L1	N
	Yorkshire terrier	2	Bulgaria (4 m)	F	9 m	N	N	Y	Y	N	N	N	negative	*G. duodenalis*	*S. stercoralis* L1	N
	Yorkshire terrier	1	Switzerland	F	adult	N	N	Y	N	N	N	N	negative	*G. duodenalis*	*S. stercoralis* L1	N
2	Chihuahua	1	France (2 d)	F	3 m	Y	Y	N	Y	N	Y	N	*I. canis* *S. stercoralis* L1 and eggs	*G.duodenalis*	*S. stercoralis* L1	Y (Dog No. 2)
3	French bulldog	1	Belgium (2 m)	F	5 m	Y	Y	N	Y	N	N	N	*S. stercoralis* L1, eggs and adult females	*S. stercoralis* L1, eggs and adult females	*S. stercoralis* L1	Y (Dog No. 3)

F: female; M: male; d: days; m: months; Y: yes; N: no; L1: first-stage larvae; A: adult; SAFC: sodium acetate-acetic acid-formalin concentration technique


gp
100 µm
a
bc
ro
b
gp
st
c


100 µm
a
b
100 µm


a
b
100 µm
c
100 µm
v
co
d