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Occurrence, diagnosis and follow-up of canine strongyloidiosis in naturally infected shelter dogs

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Running Title: Diagnosis of canine strongyloidiosis

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Abstract

Strongyloidiosis by *Strongyloides stercoralis* is a disease of increasing interest to human and animal medicine. The scientific knowledge on canine strongyloidiosis is hindered by the poor diagnostics available. To assess the most sensitive and specific diagnostic methods, faeces and blood from 100 shelter dogs were screened for *S. stercoralis* by coprological, molecular and serological tests. Thirty-six dogs (36%) scored positive to *S. stercoralis* by coprology (22.3% to Baermann) and/or 30% to real time-PCR. According to two composite reference standards (CRS) based on all coprological methods and rt-PCR (first CRS) or in combination with serology (second CRS), the most sensitive test was IFAT (93.8%; CI 82.8-98.7), followed by rt-PCR (80.6%; 95% CI 64-91.8) and Baermann (60.6%; 95% CI 42.1-77.1). The inconsistent shedding of L1 during the 4-week follow-up in infected dogs suggests the importance of multiple faecal collections for a reliable diagnosis. A combination of serological and coprological tests is recommended for the surveillance and diagnosis of *S. stercoralis* infection in dogs.

Key words: *Strongyloides stercoralis*; dogs; diagnosis; coprology; serology; molecular biology.

Key findings

- The occurrence of strongyloidiosis was assessed in dogs by coprological, molecular and serological tests.
- The combination of rt-PCR and Baermann is an optimal approach to detect patent infection.
- The IFAT assay is highly sensitive and specific with antibody titers ≥1:320.
- The inconsistent L1 shedding suggests the importance of multiple faecal sampling for a proper diagnosis.
- Serological and coprological tests is recommended for the surveillance of *S. stercoralis* infection.

Introduction

Strongyloidiosis by *Strongyloides stercoralis* (Rhabditida: Strongyloididae) is a parasitic disease of humans and dogs, which may bear the brunt of this parasitosis (Nutman 2017; Thamsborg *et al*. 2017). The infection is typical of, but not exclusive for, tropical and subtropical areas where the environmental conditions favour the free-living phase of this nematode (Junior *et al*. 2006; Martins *et al*. 2012; Bisoffi *et al*. 2013; Schär *et al*. 2013; Thamsborg *et al*. 2017). The adult nematodes have a complex, rather unique, life cycle consisting of parasitic female worms residing in the host small intestine reproducing via parthenogenesis, and free-living male and female in the environment, reproducing sexually (Viney, 2017). In the host the new-born larvae are shed through faeces and may cause a systemic migration (autoinfective cycle), allowing the parasite to persist in healthy individuals or to disseminate in chronically infected and immunocompromised hosts (Schär *et al*. 2013; Buonfrate *et al*., 2015). Evidence for zoonotic transmission suggests that dogs may play a role as spreaders of the parasite to humans mainly in poor context, with low hygiene standards and less opportunities for socioeconomic development (Jaleta *et al*. 2017; Thamsborg *et al*. 2017).

Despite the increased attention into human strongyloidiasis in developing and less developed countries (Schär *et al*. 2014; Anselmi *et al*. 2015; Cabezas-Fernández *et al*. 2015; Buonfrate *et al*. 2016), data on the epidemiology of canine infection is restricted to tropical and subtropical geographical areas, such as Brazil and Cambodia (Gonçalves *et al*. 2007; Schär *et al*. 2014; Jaleta *et al*. 2017). In Europe, canine strongyloidiosis has been reported in Germany (Epe *et al*. 1993), Finland (Dillard *et al*. 2007), Greece (Papazahariadou *et al*. 2007), France (Cervone *et al*. 2016) and Italy (Paradies *et al*. 2017). The medical and veterinarian relevance of this parasite has probably been largely underestimated due to difficulty of diagnosis. Indeed, one of the major limitations in the assessment of this infection in dogs is represented by the diagnostic procedures for the detection of *S. stercoralis* (Buonfrate *et al*. 2017a).

Proper diagnostic tools are pivotal to identify infected individuals and to evaluate the prevalence of the infection amongst host populations. Though some serological (i.e., IFAT, ELISA) and molecular tests (i.e., rt-PCR) have been employed for the diagnosis in dogs (Ferreira-Júnior *et al*. 2006; Buonfrate *et al*. 2017a; Buonfrate *et al*. 2018), the detection of the parasites by Baermann method remains the most common technique. Nonetheless, the latter tool is characterised by a low sensitivity and requires multiple testing to unequivocally rule out the presence of larvae in faecal samples (Bisoffi *et al*. 2014; Buonfrate *et al*. 2015). Again, the comparison of the accuracy of serological, molecular and coprological tests for *S. stercoralis* diagnosis in dogs has never been conducted. This study assessed the occurrence of *S. stercoralis* infection in shelter dogs by comparing the sensitivity and specificity of serological, coprological and molecular methods and the larval shedding by weekly examination of faeces in a cohort of infected dogs.

Materials and methods

Study design

From April to September 2017, faeces and blood samples were collected from 100 dogs living in a shelter in the province of Bari (41°04'47" N, 16°55'17" E, Apulia region, Italy) where cases of *S. stercoralis* infection were described (Paradies *et al*. 2017). The municipal shelter is divided in two main areas (i.e., A1 and A2), formed by 6 and 10 units, respectively. Each unit is composed by wire mesh cages (approximately 3×3 m), housing about 800 dogs according to their gender, and existing hierarchies within each group. Stool and blood from healthy animals or with gastrointestinal signs (i.e., diarrhea, weight loss, reduced appetite) were sampled directly from the rectal ampulla and from the cephalic vein, respectively. Additionally, from dogs scored positive to *S. stercoralis* at least at one coprological technique, faecal samples were weekly collected for one month and analyzed by parasitological and molecular tests. The enrolled dogs were not treated with any anthelmintic drugs at least over the month prior to sample collection. Animals were grouped according to their age in younger and elder than seven years old. Dogs with severe health condition were treated with ivermectin 200 µg/kg/sid/os for two consecutive days as reported for human strongyloidiasis (Bisoffi *et al*. 2011). The protocol of this study was approved by the Ethical Committee of the Department of Veterinary Medicine of the University of Bari, Italy (Prot. Uniba 6/17).

Faecal examination

The coprological diagnosis of *S. stercoralis* infection was performed through direct microscopy, Baermann, and culturing technique. For the Baermann examination, 5 g of faeces were analyzed and any recovered larvae were identified according to their morphology (Euzeby, 1981) and molecularly processed through partial cytochrome *c* oxidase subunit 1 (*cox*1) gene amplification and sequencing (see Hasegawa *et al*. 2010). The Koga agar plate test (Koga *et al*. 1991) was performed by plating 2 g of fresh stool on the agar plate and observed the live larvae and adult worms at the microscope after incubation at 27 ± 2°C for 2 days. In addition, faecal flotation was performed on 2 g of faeces to diagnose other helminthic infections. Presence of *Tritrichomonas foetus* was evaluated microscopically, followed by DNA isolation and amplification of marker genes to rule out other trichomonads and confirm the genotype identity as previously described (Šlapeta *et al*. 2010; 2012).

Complete blood count

The complete blood count (CBC) was performed by using the ADVIA® 2120 SIEMENS analyzer (Siemens Healthineers, Italy) and supported by blood smear evaluation. Dogs with $1.2–1.8\times10^3$ eosinophils/μl at CBC were considered affected by mild eosinophilia, while severe eosinophilia was documented by $>1.8\times10^3$ eosinophils/μl.

Molecular assay

For DNA extraction, 200 mg of faeces were suspended in 200 µl of phosphate-buffered saline containing 2% polyvinylpolypyrolidone (Sigma-Aldrich) and freezed overnight at -20°C until the extraction. After thawing, 2 µl of a 1:1000 dilution of Phocin Herpes Virus type-1 (PhHV-1) with Cq=18, was added to each sample to serve as an internal control (the virus stock was kindly provided by Dr. Pas S., ErasmusMC, Department of Virology, Rotterdam). Subsequently, the samples were boiled and extracted using MagnaPureLC.2 instrument (Roche Diagnostics), following the protocol DNA I Blood Cells High performance II, using the DNA isolation kit I (Roche Diagnostics). DNA samples were stored at -20°C until further rt-PCR analysis. The real-time assay was performed as described by Verweij *et al.* (2009). The small-subunit (SSU) rRNA gene sequence was chosen as amplification target and primers and probes synthesized by MWG Biotech S.r.l. (Ebersberg, Germany). Amplification reaction were performed in a volume of 25 µl; the PCR cycle protocol consists of 3 min at 95ºC followed by 40 cycles of 15 sec at 95ºC and 30 sec at 60ºC, and 30 sec at 72°C. Appropriate positive and negative controls were included in all the experiments. As control for PCR inhibitors and amplification quality, the PhHV-1 control DNA was amplified with the appropriate primers/probe mix (see Verweij *et al*. 2009) in the same reaction in a multiplex PCR. The reactions, detection and data analysis were performed with the CFX96 detection system (Bio-Rad Laboratories, Milano, Italy).

Serological assays

The ELISA is a commercial kit (Bordier, Affinity Products, Switzerland) based on somatic antigens from *Strongyloides ratti* larvae. The results were expressed in optical density (OD) considering as positive an OD of the tested sample higher than that of the positive control. The OD of the positive control could change among runs, hence a result expressed as a normalized ratio was preferable for study purpose, so that the results of the tests conducted in different runs were comparable. Positive tests were defined by OD values ≥1 (normalized ratio). The IFAT is an in-house technique based on somatic antigens from *S. stercoralis* larvae (obtained by agar plate culture) as described (Boscolo *et al*. 2007). Samples were scored as positive when an antibody titer ≥1:80 was detected.

Statistical analysis

The accuracy of the diagnostic methods was assessed against a panel of true positives defined as the samples positive at any coprological techniques and/or rt–PCR. As coprological techniques lack sensitivity, a composite reference standard (CRS) was also used, as a statistical method suggested in absence of a diagnostic gold standard (Rutjes *et al*. 2007). According to the CRS, true positives were defined as samples positive either to coprological methods (i.e., Baermann, direct smear and culture) and/or rt-PCR (first CRS) and to any fecal method and/or positive to both serological tests (second CRS).

The agreement between the different diagnostic tests alone or in combination was measured through Cohen's kappa. Test sensitivity was calculated as the proportion of positive results over all positive samples, for both reference methods. Uncertainty was quantified with the 95% confidence intervals. The corresponding ROC curves were plotted for IFAT serology. Prevalence of the infection in the study population was calculated as the proportion of dogs diagnosed with *S. stercoralis* infection over all dogs screened. All analyses were done in R, version 3.4.0 (R Core Team, 2017) and Stata SE 14.0.

The association between *S. stercoralis* infection and dog data (i.e., location, gender, age, clinical signs and eosinophilia) was investigated using a logistic regression model. The statistical significance of the model was assessed by chi-square statistic and p-value. The odds ratio of *S. stercoralis* infection, as defined by CRS, was calculated for each level of the categorical variables. The association between infection by *S. stercoralis* and other intestinal parasites was evaluated by chi-square test. The percentage of co-infection with other intestinal parasites compared to dogs positive and negative to *S. stercoralis* (as defined by CRS) was evaluated by Fisher exact test with permutational adjustment of p-values. The same analysis was performed to compare the occurrence of symptomatic dogs. Logistic regression was performed with MedCalc Statistical Software version 18, adjustment of p-values was performed with the PROC MULTTEST of the software SAS V9.4 for personal computer.

Results

Of 100 dogs sampled (i.e., 51 females and 49 males) 69% were younger and 31% elder than 7 years old. Thirty-six dogs (36%) scored positive to *S. stercoralis* at coprological methods and/or rt-PCR, with 22.3% (19 of 85) animals positive to Baermann and 30% by rt-PCR (n = 100). Faeces of 15 animals were not examined by Baermann because of the small amount (<5g). The agreement between the two tests was assessed by Cohen's kappa, and resulted fair: 0.40 (95% CI: 0.19-0.62). The morphological identification of *S. stercoralis* collected by Baermann was confirmed by the

molecular analysis by the sequencing of partial *cox*1 gene, which showed 100% nucleotide identity with free-living adult of *S. stercoralis* (GenBank accession n. AJ558163).

Sensitivity and specificity of each diagnostic method is reported in Table 1. Comparing the first CRS, the sensitivity of rt-PCR resulted 80.6% (95% CI: 64-91.8), whereas that of Baermann was lower (60.6%, 95% CI: 42.1-77.1). IFAT was the test with the highest sensitivity (91.2-93.8%, according to the CRS used), while the sensitivity of ELISA was closer to that of Baermann (44.1-60.4%). When the second CRS was considered (hence the results of serology was added into the analysis), 11 positive cases were further identified, and the overall prevalence of infected dogs raised to 56%. All but one case resulting positive to ELISA (n=30) were also positive to IFAT, which identified further 36 positive samples. The two CRSs showed substantial agreement: 0.72 (95% CI: 0.59-0.85). The ROC curves were plotted for IFAT, and they showed an upward trend with specificity for increasing titers, reaching values of around 98% for titers ≥1:320, when the calculation was assessed against either the first CRS or the second CRS (Fig 1 a,b).

Of the 20 dogs positive to *S. stercoralis* at least at one faecal examination test (19 by Baermann and 1 by direct microscopy) at the baseline (T0), a subset of 17 dogs (Fig 2) were studied over a four-week period (T1-T4) by coprological (i.e., Baermann, direct microscopy, culture) and rt-PCR to evaluate intermittency of larval shedding (Table 2). Eleven of the 17 dogs were positive at rt-PCR, whereas of the 6 negative, *S. stercoralis* larvae were isolated by Baermann and molecularly identified by conventional PCR and sequencing. Ten (58.8%) out of the 17 dogs scored negative at the Baermann at either T3 and/or T4, though rt-PCR confirmed the parasitological results in only five animals (3 of which were already negative at T0) (Table 2). Only two dogs of the above ten animals, were treated with ivermectin per os soon after the diagnosis because of their severe health conditions. Out of the 17 Baermann positive dogs at T0, 12 were retested four times (T1-T4) showing repeated positive result by Baermann and/or rt-PCR in 50-75% (6-9 dogs), whereas 6 dogs had at least one Baermann test negative before returning positive again (Table 2). Ten dogs rt-PCR positive at T0 were retested four times (T1-T4) with repeated positive result in 60-80% (6-8 dogs) and 5 dogs had at least one test negative before returning positive result again (Table 2).

The risk of *S. stercoralis* infection in dogs was not significantly associated to any of the two areas of the shelter (A1 vs A2: OR=0.61, 95% CI 0.26-1.4, p=0.2491). The units of each areas of the shelter had no any role in determining risk of *S. stercoralis* infection neither according to A1 (chi-square=3.701, df=5, p=0.5931) nor to A2 (chi-square=6.116, df=9, p=0.7283). Dogs elder than 7 years were not related to higher risk of *S. stercoralis* infection than the younger (OR=1.27, 95% CI: 0.52-3.13, p=0.6017). Same result was observed for gender, in particular the risk of female respect to male dogs was OR=0.44 (95% CI 0.19-1.02) and was not statistically significant (p=0.052).

Eosinophilia, analyzed as continuous variable, was not related to higher risk of *S. stercoralis* infection (OR=0.99, 95% CI 0.99-1, p=0.6529).

Of the 36 dogs scored positive to *S. stercoralis* at coprological methods and/or rt-PCR, 44.4% (16/36) were symptomatic at least at one clinical sign showing no statistical significant difference with the asymptomatic dogs (chi-square=0.982, df=1, p=0.0986). In dogs infected by *S. stercoralis*, cachexia and/or weight loss are the most common clinical signs (47.2%, 17/36), followed by diarrhea (13.9%, 5/36). Diarrhea and weight loss were present in 8.3% dogs (3/36), while diarrhea and colitis in only one dog (2.7%). In *S. stercoralis* negative dogs, diarrhea was observed in 10.9% dogs (7/64), cachexia and weight loss in 14.1% (9/64). The differences between the two groups are not statistically significant for any symptom.

Of the 59 samples available for flotation examination, 42 (71.2%) dogs scored positive for at least one species of intestinal parasite. Among dogs infected by *S. stercoralis*, 65.4% (17/26) were co-infected with other intestinal parasites, showing no statistical significance compared to *S. stercoralis* negative dogs (72.7%, 24/33) (chi-square=0.364, df=1, p=0.5465).

Twenty-eight animals (47.4%) were infected by only one species (27.1% *Toxocara canis* and 18.6% *Trichuris vulpis*), while 14 (23.7%) by two species (Table 3). In dogs infected by *S. stercoralis*, 26.9% (7/26) dogs were co-infected with *T. canis*, followed by *T. vulpis* in 15.4% (4/26). The coinfection of *T. canis* and *T. vulpis* was observed in 19.2% (5/26) of dogs positive to *S. stercoralis*. The differences between *S. stercoralis* infected and non-infected animals are not statistically significant at the Fisher exact test, adjusted for multiplicity. Intestinal parasites were diagnosed in 71.8% and 70% dogs younger and elder than 7 years old, respectively (chi-square=0.0204, p=0.8864) (Table 3). Among gastrointestinal parasites, *T. canis* (49%) and *T. vulpis* (39%) were most frequently identified. *T. canis* was the most common nematode in animals up to seven years of age (53.8%; p=0.946) while *T. vulpis* in dogs elder than 7 years (35.9%; p=0.814). At the direct microscopy, one dog resulted positive to *Tritrichomonas foetus*, typed as 'feline genotype' because of ITS rDNA region and eight protein coding genes matching 100% the 'feline genotype' *T. foetus* (accession number will be provided in the revised version of the manuscript).

Discussion

The infection by *S. stercoralis* has been evaluated in a population of shelter dogs by comparing coprological, serological and molecular tests in order to assess their efficiency in diagnosis and monitoring. The occurrence and the assessment of prevalence for canine strongyloidiosis still represents a *conundrum*, because of i) the lack of a gold standard diagnostic technique, ii) the low parasite burden and iii) the intermittent larval shedding (Buonfrate *et al*. 2017a). According to the

approach employed, the prevalence of strongyloidiosis detected in dog population ranged from 22.3% by coprology (including Baermann, direct microscopy and culture) to 30% by rt-PCR. This data indicate that the infection is endemic in the shelter as it is further confirmed by the higher prevalence recorded by serology (56%) which indicate that more than half of animal population was exposed to the parasite. Prevalence data are higher than those registered by Baermann in owned (i.e., 0.2-0.9%) (Junior *et al*. 2006; Gates and Nolan, 2009; Riggio *et al*. 2013; Paradies *et al*. 2017) and shelter dogs from Italy, Brazil and the United States (0.6-8.1%) (Goncalves *et al*. 2007; Paradies *et al*. 2017). Such a higher prevalence could be due to the peculiarity of the studied population since such a large number of dogs (i.e., 800) living in a confined environment and with wicked health conditions, are at high risk of infection. Indeed, in studies carried out in different epidemiological contexts the prevalence of strongyloidiosis is similar to that recorded in dogs living in villages from Cambodia and Brazil (e.g., 14.9-26.3%) (Schär *et al*. 2014; Martins *et al*. 2012). Current data demonstrate that coupling of rt-PCR with Baermann is an optimal approach to detect patent infection, and therefore reservoir dogs, although missing diagnoses could occur. Our data show that rt-PCR should be performed at least twice a week apart, while Baermann should be repeated three times to improve the identification of intermittent larval shedding.

In studies where more than one diagnostic method has been used for the diagnosis of *S. stercoralis* the prevalence of the infection recorded by serology was higher in both owned and shelter dogs (20.9-24.3%) than that by Baermann (0.6-0.9%) (Ferreira-Júnior *et al*. 2006; Gonçalves *et al*. 2007). Amongst the parasitological techniques, the Baermann detected the highest number of *S. stercoralis* infected animals (22.3%), followed by culture (6.9%), and direct microscopy (4%), suggesting that the former is the most suitable tool for the diagnosis of strongyloidiosis. Nonetheless, rt-PCR successfully diagnosed further positive samples leading to an overall prevalence of 30%. The good performance of rt-PCR in the diagnosis of human infection was already recorded in a retrospective study on 223 human patients (Buonfrate *et al*. 2017b). Therefore, although faecal examination presents inherent limitations due to the collection of fresh faeces, the combination of parasitological and molecular tests improves the diagnosis of dogs infected by *S. stercoralis*. The accuracy of serology, molecular and coprological tests for *S. stercoralis* is detected against a primary CRS (using fecal-based positivity), and a secondary CRS (which includes serological positivity). Trough Baermann examination, the risk of any false positive results was excluded considering both the morphological identification of larvae and their confirmatory sequencing. Nonetheless, the lower performance of Baermann than rt-PCR suggests that a single stool examination by Baermann may fail to detect *S. stercoralis* larvae in infected hosts, also due to the low viable worm burden. Conversely, rt-PCR may detect also unviable larvae, when present.

Even considering the IFAT limitations for the diagnosis of strongyloidiosis in dogs (i.e., difficulty in setting up the test, in result interpretation and lack of validation in dogs), the test was the most sensitive according to the CRS2 analysis (Table 1), as demonstrated for the diagnosis in humans (Requena-Méndez *et al*. 2013; Bisoffi *et al*. 2014; Buonfrate *et al*. 2017b). Although the usefulness of IFAT for both diagnosis and prevalence studies has been scantly investigated in dogs (Junior *et al*. 2006; Gonçalves *et al*. 2007), a higher prevalence of infected animals was recorded by serology than by coprology. In spite of the low specificity of the IFAT (i.e., up to 60% in CRS2), this technique could be useful for screening animal populations also considering the chronic status of the disease and their lethality in immunocompromised individuals. The diagnostic performance of IFAT provided by ROC analysis indicated that the specificity increased for higher titers (≥1:320), which could be used for a certain diagnosis of strongyloidiosis without the support of any faecal-based tests. Data confirming that the specificity of IFAT increases for high titers have been reported also in humans (Bisoffi *et al*. 2014; Buonfrate *et al*. 2017b).

The study on the 4-week follow-up on dogs positive to *S. stercoralis* at the parasitological examination (Table 2) indicated the inconsistent shedding of L1, suggesting the importance of multiple collections of faeces for a reliable diagnosis, mostly if animals screened positive at the serology. The parasitological positivity of dogs at T0 overlaps the results of rt-PCR, except for six animals, which, scored positive to IFAT only with titers ≥1:80 (Table 2).

The only two dogs scored positive to *S. stercoralis* at T0 and negative at 3 and/or 4 weeks follow-up at both Baermann and rt-PCR may depend only on the sensitivity of the parasitological tests. Of the two dogs treated with ivermectin soon after the strongyloidiosis diagnosis, one dog died despite the treatment, whereas the other dog discontinued to shed L1 in the faeces during the follow-up period as detected by Baermann and rt-PCR, suggesting the usefulness of both tests in monitoring the treatment efficacy (Mansfield and Schad, 1992; Yang *et al*. 2013).

The gastrointestinal worms remain an important and common finding in shelter dogs, including those of zoonotic concerns (Martínez-Carrasco *et al*. 2007; Martins *et al*. 2012; Neves *et al*. 2014; Schär *et al*. 2014). The overall prevalence of intestinal helminths (71.2%) reflects the living conditions in kennel where overcrowding and environmental contamination favour parasite transmission and maintenance of infestations (Otranto *et al*. 2017). In addition, the high prevalence of *S. stercoralis* along with *T. canis* (49.1%) and Ancylostomatidae (3.4%) is of concern as it represents a potential hazard for shelter workers. Therefore, more effective treatment strategies and improvement in measures for the prevention and control of these infections are required. The detection of *Tritrichomonas foetus* 'feline genotype' in dogs is unexpected (Šlapeta *et al*. 2012; Gookin *et al*. 2017). The single case of *T. foetus* in a shelter dog by microscopy and molecularly identified is of relevance, because the epidemiology of feline trichomonosis and the spectrum of

reservoir hosts is not known. In conclusions, the employment of rt-PCR and Baermann tests is an optimal approach to detect *S. stercoralis* patent infection, and therefore the reservoir dogs, although in the case of intermittent larval shedding missing diagnoses may occur. Therefore, IFAT assay, highly sensitive for screening and specific for diagnosis with IgG titers ≥1:320, should be used in combination with parasitological exams for diagnosis of strongyloidiosis. Under the above circumstances and considering the tight human-dog bounds, an improvement in the control and prevention of parasite infections is mandatory. The finding of an effective therapy in dogs, the monitoring of larval shedding and antibodies titers in infected animals after treatment, would deserve further investigations.

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References

Anselmi, M, Buonfrate, D, Guevara Espinoza, A, Prandi, R, Marquez, M, Gobbo M, Montresor, A, Albonico, M, Racines Orbe, M, Martin Moreira, J and Bisoffi, Z (2015) Mass administration of ivermectin for the elimination of onchocerciasis significantly reduced and maintained low the prevalence of *Strongyloides stercoralis* in Esmeraldas, Ecuador. *PLoS Neglected Tropical Diseases* **9**, e0004150. doi: 10.1371/journal.pntd.0004150.

Bisoffi Z, Buonfrate D, Sequi M, Mejia R, Cimino RO, Krolewiecki AJ, Albonico, M, Gobbo, M, Bonafini, S, Angheben, A, Requena-Mendez, A, Muñoz, J and Nutman, TB (2014) Diagnostic accuracy of five serologic tests for *Strongyloides stercoralis* infection. *PLoS Neglected Tropical Diseases* **8**, e2640. doi: 10.1371/journal.pntd.0002640.

Bisoffi, Z, Buonfrate, D, Angheben, A, Boscolo, M, Anselmi, M, Marocco, S, Monteiro, G, Gobbo, M, Bisoffi, G and Gobbi, F (2011) Randomized clinical trial on ivermectin versus thiabendazole for the treatment of strongyloidiasis. *PLoS Neglected Tropical Diseases* **5**, e1254. doi: 10.1371/journal.pntd.0001254.

Bisoffi, Z, Buonfrate, D, Montresor, A, Requena-Mendez, A, Munoz, J, Krolewiecki, AJ, Gotuzzo, E, Mena, MA, Chiodini, PL, Anselmi, M, Moreira, J and Albonico, M (2013) *Strongyloides stercoralis*: a plea for action. *PLoS Neglected Tropical Diseases* **7**, e2214. doi: 10.1371/journal.pntd.0002214.

Boscolo, M, Gobbo, M, Mantovani, W, Degani, M, Anselmi, M, Monteiro, GB, Marocco, S, Angheben, A, Mistretta, M, Santacatterina, M, Tais, S and Bisoffi, Z (2007) Evaluation of an indirect immunofluorescence assay for strongyloidiasis as a tool for diagnosis and follow-up. *Clinical and Vaccine Immunology* **14**, 129–133.

Buonfrate, D, Baldissera, M, Abrescia, F, Bassetti, M, Caramaschi, G, Giobbia, M, Mascarello, M, Rodari, P, Scattolo, N, Napoletano, G and Bisoffi, Z; CCM Strongyloides

Study Group (2016) Epidemiology of *Strongyloides stercoralis* in northern Italy: results of a multicentre case-control study, February 2013 to July 2014. *Euro Surveillance* **21**. doi: 10.2807/1560-7917.ES.2016.21.31.30310.

Buonfrate, D, Formenti, F, Perandin, F and Bisoffi, Z (2015) Novel approaches to the diagnosis of *Strongyloides stercoralis* infection. *Clinical Microbiology and Infection* **21,** 543-552. doi: 10.1016/j.cmi.2015.04.001.

Buonfrate, D, Paradies, P, Iarussi, F, Formenti, F, Perandin, F, Otranto, D and Bisoffi, Z (2017a) Serological and molecular tests for the diagnosis of *Strongyloides stercoralis* infection in dogs. *Parasitology Research* **116,** 2027-2029. doi: 10.1007/s00436-017-5468-0.

Buonfrate, D, Perandin, F, Formenti, F and Bisoffi, Z (2017b) A retrospective study comparing agar plate culture, indirect immunofluorescence and real-time PCR for the diagnosis of *Strongyloides stercoralis* infection. *Parasitology* **144**, 812-816. doi: 10.1017/S0031182016002559.

Buonfrate, D, Requena-Mendez, A, Angheben, A, Cinquini, M, Cruciani, M, Fittipaldo, A, Giorli, G, Gobbi, F, Piubelli, C and Bisoffi, Z (2018) Accuracy of molecular biology techniques for the diagnosis of *Strongyloides stercoralis* infection - A systematic review and meta-analysis. *PLoS Neglected Tropical Diseases* **12**, e0006229. doi: 10.1371/journal.pntd.0006229.

Cabezas-Fernández, MT, Salas-Coronas, J, Lozano-Serrano, AB, Vazquez-Villegas, J, Cabeza-Barrera, MI and Cobo, F (2015) Strongyloidiasis in immigrants in Southern Spain. *Enfermedades Infecciosas y Microbiologia Clinica* **33**, 37-39. doi: 10.1016/j.eimc.2014.06.010.

Cervone, M, Giannelli, A, Otranto, D and Perrucci, S (2016) *Strongyloides stercoralis* hyperinfection in an immunosuppressed dog from France. *Revue Vétérinaire Clinique* **51**, 55-59.

Dillard, K, Saari, SAM and Antitila, M (2007) *Strongyloides stercoralis* infection in a Finnish kennel. *Acta Veterinaria Scandinavica* **49,** 37.

Epe, C, Ising-Volmer, S and Stoye, M (1993) Parasitological fecal studies of equids, dogs, cats, and hedgehogs during the years 1984-1991. *Deutsche Tierarztliche Wochenschrift* **100**, 426-428.

Euzeby, J (1981) Diagnostic experimental des helminthoses animals. In: Livre 1 – *Informations Techniques des Services Veterinaires, Ministere de l'Agriculture*, Paris: pp. 349.

Ferreira-Júnior, A, Goncalves-Pires, MRF, Silva, DAO, Goncalves, ALR and Costa-Cruz JM (2006) Parasitological and serological diagnosis of *Strongyloides stercoralis* in domesticated dogs from southeastern Brazil. *Veterinary Parasitology* **136**, 137-145.

Gates, MC and Nolan, TJ (2009) Endoparasite prevalence and recurrence across different age groups of dogs and cats. *Veterinary Parasitology* **166**, 153-158. doi: 10.1016/j.vetpar.2009.07.041

Gonçalves, AL, Machado, GA, Gonçalves-Pires, MR, Ferreira-Júnior, A, Silva, DA and Costa-Cruz, JM (2007) Evaluation of strongyloidiasis in kennel dogs and keepers by parasitological and serological assays. *Veterinary Parasitology* **147**, 132-139.

Gookin, JL, Hanrahan, K and Levy, MG (2017) The conundrum of feline trichomonosis: The more we learn the 'trickier' it gets. *Journal of Feline Medicine and Surgery* **19,** 261-274.

Hasegawa, H, Sato, H, Fujita, S, Nguema, PP, Nobusue, K, Miyagi, K, Kooriyama, T, Takenoshita, Y, Noda, S, Sato, A, Morimoto, A, Ikeda, Y and Nishida, T (2010) Molecular identification of the causative agent of human strongyloidiasis acquired in Tanzania: dispersal

and diversity of *Strongyloides* spp. and their hosts. *Parasitology International* **59**, 407-413. doi: 10.1016/j.parint.2010.05.007.

Jaleta, TG, Zhou, S, Bemm, FM, Schär, F, Khieu, V, Muth, S, Odermatt, P, Lok, JB and Streit, A (2017) Different but overlapping populations of *Strongyloides stercoralis* in dogs and humans-Dogs as a possible source for zoonotic strongyloidiasis. *PLoS Neglected Tropical Diseases* **11,** e0005752. doi: 10.1371/journal.pntd.0005752.

Koga, K, Kasuya, S, Khamboonruang, C, Sukhavat, K, Ieda, M, Takatsuka, N, Kita, K and Ohtomo, H (1991) A modified agar plate method for detection of *Strongyloides stercoralis*. *The American Journal of Tropical Medicine and Hygiene* **45**, 518-521.

Mansfield, L and Schad, GA (1992). Ivermectin treatment of naturally acquired and experimentally induced infections in dogs. *Journal of the American Veterinary Medical Association* **201**, 726–730.

Martínez-Carrasco, C, Berriatua, E, Garijo, M, Martínez, J, Alonso, FD and de Ybáñez, RR (2007) Epidemiological study of non-systemic parasitism in dogs in southeast Mediterranean Spain assessed by coprological and post-mortem examination. *Zoonoses and Public Health* **54**, 195-203.

Martins, CM, Barros Cda, C, Bier, D, Marinho, AP, Figueiredo, JM, Hoffmann, JL, Molento, MB and Biondo, AW (2012) Dog parasite incidence and risk factors, from sampling after one-year interval, in Pinhais, Brazil. *Revista Brasileira de Parasitologia Veterinaria* **21**, 101-106.

Neves, D, Lobo, L, Simões, PB and Cardoso, L (2014) Frequency of intestinal parasites in pet dogs from an urban area (Greater Oporto, northern Portugal). *Veterinary Parasitology* **200**, 295-298. doi: 10.1016/j.vetpar.2013.11.005.

Nutman, TB (2017) Human infection with *Strongyloides stercoralis* and other related *Strongyloides* species. *Parasitology* **144**, 263-273. doi: 10.1017/S0031182016000834.

Otranto, D, Dantas-Torres, F, Mihalca, AD, Traub, RJ, Lappin, M and Baneth, G (2017) Zoonotic parasites of sheltered and stray dogs in the era of the global economic and political crisis. *Trends in Parasitology* **33**, 813-825. doi: 10.1016/j.pt.2017.05.013.

Papazahariadou, M, Founta, A, Papadopoulos, E, Chliounakis, S, Antoniadou-Sotiriadou, K and Theodorides, Y (2007) Gastrointestinal parasites of shepherd and hunting dogs in the Serres Prefecture, Northern Greece. *Veterinary Parasitology* **148**, 170-173.

Paradies, P, Iarussi, F, Sasanelli, M, Capogna, A, Lia, RP, Zucca D, Greco, B, Cantacessi, C and Otranto, D (2017) Occurrence of strongyloidiasis in privately owned and sheltered dogs: clinical presentation and treatment outcome. *Parasites & Vectors* **10**, 345. doi: 10.1186/s13071-017-2275-5.

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Requena-Méndez, A, Chiodini, P, Bisoffi, Z, Buonfrate, D, Gotuzzo, E and Muñoz, J (2013) The laboratory diagnosis and follow up of strongyloidiasis: a systematic review. *PLoS Neglected Tropical Diseases* **7,** e2002. doi: 10.1371/journal.pntd.0002002.

Riggio, F, Mannella, R, Ariti, G and Perrucci, S (2013) Intestinal and lung parasites in owned dogs and cats from central Italy. *Veterinary Parasitology* **193,** 78-84. doi: 10.1016/j.vetpar.2012.11.026.

Rutjes, AW, Reitsma, JB, Coomarasamy, A, Khan, KS and Bossuyt, PM (2007). Evaluation of diagnostic tests when there is no gold standard. A review of methods. *Health Technology Assessment* **11**, iii, ix-51.

Schär, F, Inpankaew, T, Traub, RJ, Khieu, V, Dalsgaard, A, Chimnoi, W, Chhoun, C, Sok, D, Marti, H, Muth, S and Odermatt, P (2014) The prevalence and diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village. *Parasitology International* **63**, 597-603. doi: 10.1016/j.parint.2014.03.007.

Schär, F, Trostdorf, U, Giardina, F, Khieu, V, Muth, S, Marti, H, Vounatsou, P and Odermatt, P (2013) *Strongyloides stercoralis*: Global distribution and risk factors. *PLoS Neglected Tropical Diseases* **7**, e2288. doi: 10.1371/journal.pntd.0002288.

Šlapeta, J, Craig, S, McDonell, D and Emery, D (2010) *Tritrochomonas foetus* from domestic cats and cattle are genetically distinct. *Experimental Parasitology* **126**, 209–213. doi: 10.1016/j.exppara.2010.04.024.

Šlapeta, J, Müller, N, Stack, C, Walker, G, Lew-Tabor, A, Tachezy, J and Frey, CF (2012) Comparative analysis of *Tritrichomonas foetus* (Riedmüller, 1928) cat genotype, *T. foetus* (Riedmüller, 1928) cattle genotype and *Tritrichomonas suis* (Davaine, 1875) at ten DNA loci. *International Journal for Parasitology* **42**, 1143–1149. doi: 10.1016/j.ijpara.2012.10.004.

Thamsborg, SM, Ketzis, J, Horii, Y and Matthews, JB (2017) *Strongyloides* spp. infections of veterinary importance. *Parasitology* **144**, 274-284. doi: 10.1017/S0031182016001116.

Verweij, JJ, Canales, M, Polman, K, Ziem, J, Brienen, EA, Polderman, AM and van Lieshout, L (2009) Molecular diagnosis of *Strongyloides stercoralis* in faecal samples using real-time PCR. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **103**, 342-346. doi: 10.1016/j.trstmh.2008.12.001.

Viney, M (2017) *Strongyloides*. *Parasitology* **144**, 259-262. doi: 10.1017/S0031182016001773

Yang, M, Gebeyehu, EB, Jung, SJ, Kwon, OD and Kwak, D (2013) Treatment of naturally acquired *Strongyloides stercoralis* infection in a dog with ivermectin. *Journal of Animal and Plant Sciences* **23**, 337–339.

671 **Figure legends**

672 **Fig. 1.** ROC curve for IFAT assessed against the faecal (**A**) or faecal and serological CRS
673 (**B**) composite reference standard

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675 **Fig. 2.** Dog infected by *Strongyloides stercoralis*