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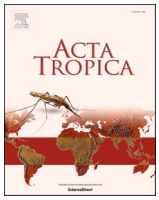

Strongyloides stercoralis in a dog litter: Evidence suggesting a transmammary transmission

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ABSTRACT

Strongyloides stercoralis is a soil-transmitted helminth with an unusual life cycle, causing canine and human strongyloidiasis, mainly endemic in tropical and subtropical areas. Following percutaneous or oral transmission of infective third-stage larvae in the vertebrate host, the parasite can cause autoinfection, leading to life-long infection. At present, the transmammary transmission was only assessed in experimentally infested dogs. Here, we provide observational evidence of *S. stercoralis* transmammary transmission in puppies suckling from a truffle dog from Central Italy, from where its presence was neglected.

1. Introduction

With over 50 species, *Strongyloides* (Rhabditida: Strongyloididae) nematodes are globally distributed soil-transmitted helminths (STHs), causing strongyloidiasis in a wide variety of vertebrate hosts (Thamsborg et al., 2017; Ko et al., 2019). In particular, *Strongyloides stercoralis,* the species of zoonotic concern, affects human and canine populations mostly within tropical and subtropical areas characterised by low-income and poor hygiene standards (Schär et al., 2013). About 613.9 million people throughout the world are infected and autochthonous human cases are also reported in temperate countries of Europe and North America (Schar et al., 2013; Buonfrate et al., 2020).

Unlike the other STHs, *Strongyloides* spp. are amphizoic parasites, alternating a sexual free-living generation and a parasitic one, represented only by females shedding eggs by parthenogenesis (Viney, 2016). Eggs of the parasitic generation hatch when still in host intestine, and the majority of hatched first-stage larvae (L1) are passed in the faeces and develop to infective third-stage larvae (L3i) in the soil. However, some larvae can develop within the same host and cause autoinfection, both in humans and dogs, leading to a chronic, often life-long, asymptomatic infection. In immunocompromised hosts, clinical complicated and potentially fatal hyperinfection may occur as a result of autoinfection, with extra-intestinal and extra-pulmonary larval localization (Keiser and Nutman, 2004). The L3i of *Strongyloides* spp. enter the new host percutaneously or orally. However, a transmammary route has been demonstrated for *Strongyloides westeri* in foals (Abbas et al., 2021), *Strongyloides ransomi* in pigs (Stewart et al., 1976), *Strongyloides papillosus* in ewes (Nwaorgu et al., 1990) and *Strongyloides fuelleborni* in infants (Brown et al., 1977). In addition, dogs experimentally infected with *S. stercoralis* transmitted the infection to their pups by the transmammary route (Shoop et al., 2002). Dogs are reservoirs of zoonotic *S. stercoralis* mainly in poor socioeconomic context, with prevalence up to 60% in some communities (Jaleta et al., 2017; Otranto et al., 2017; Barratt et al., 2019; Bradbury et al., 2021). Conversely, in temperate areas and high-income countries such as Europe, the seroprevalence of infection in humans is as low as 3.8% (Starr and Montgomery 2011; Buonfrate et al. 2018; Ottino et al., 2020). Likewise, canine strongyloidiasis has been widely reported, with isolated foci or case reports in Europe (Štrkolcová et al., 2017), including Italy (Riggio et al., 2013; Zanzani et al., 2014; Paradies et al., 2017; Iatta et al., 2019). Knowledge about the transmission patterns is pivotal for understanding the epidemiology of the infection and transmammary route, from dam to puppies, has only been demonstrated in experimental studies (Shoop et al., 2002). This study aims to report infection by *S. stercoralis* in suckling puppies, therefore suggesting for the first time the occurrence of parasite transmammary transmission in a litter, arising from a naturally infected mother.

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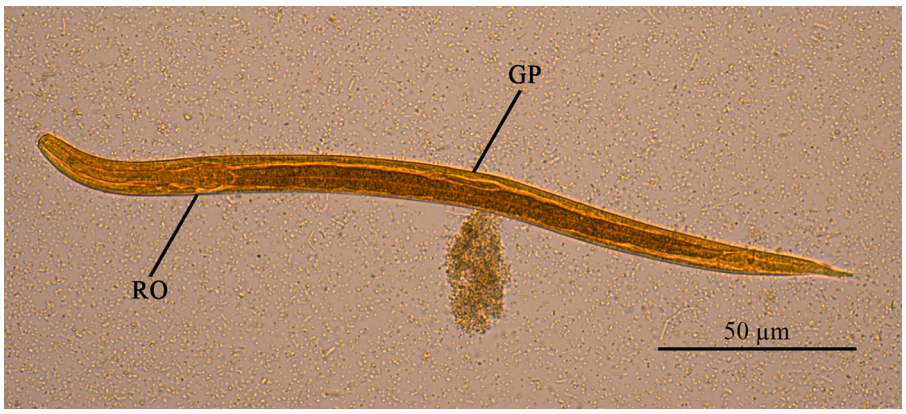

Fig. 1. *Strongyloides stercoralis* first stage larva (iodine stained) from a dog faecal sample. GP: genital primordium, RO: rhabditiform oesophagus.

2. Material and methods

In February 2021, two 2-month old puppies (both males) living in a village 40 km northeast of Rome (42°01'06.98''N-12°55'33.43''E, Lazio Region, central Italy) were screened through a routine coprological examination at the Laboratory of Parasitology at the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Italy. The puppies were part of a litter of 4 Lagotto Romagnolo breed, usually employed in truffle hunting, and lived in a courtyard of a house with the dam and another dog of the same breed. All the dogs were kept in a pen with a concrete basement. The owner used to remove the faeces daily and to clean thoroughly the pen with bleach weekly. The faecal samples of the two puppies were analyzed by direct microscopy, Baermann and flotation methods for the detection of intestinal and bronchopulmonary parasites.

Briefly, for the Baermann examination, 3 grams of faeces were placed on double layer gauze and then in a Baermann funnel with 50 ml warm tap water. After 24 hours, the liquid was transferred into a 15 ml tube and centrifuge at 600 g for 5 min and the sediment placed on a microscope slide for examination. Faecal flotation with 28 ml of zinc sulphate solution ($ZnSO_4$, specific gravity = 1.35) was performed on 10 g of faeces to diagnose other intestinal parasites. After the recovery of *S. stercoralis* in the faecal samples of the first two puppies, the faeces of the bitch, the remaining two puppies and the only dog cohabiting with them were collected and analysed. In total, 6 dogs, 4 brothers, the mother and a dog cohabiting with them, were analysed.

Ten larvae from the first two puppies (4 larvae each) and the bitch (2 larvae) were morphologically and morphometrically analyzed. Larval morphological identification followed the description by Toledo et al. (2015). Due to the uncommon finding, identification was confirmed via molecular analyses at the Department of Veterinary Medicine of the University of Bari. Genomic DNA was extracted from a single larva isolated by Baermann method from each positive dog and preserved in 70% ethanol, using a commercial kit (QIAamp, DNA Micro Kit, GmbH, Hilden, Germany) in accordance with the manufacturer's instruction. A conventional PCR targeting the cytochrome c oxidase subunit 1 (*cox1*) gene was performed, according to the protocol by Hasegawa et al. (2010) and the amplicons were purified and sequenced using the Taq Dye Doxy Terminator Cycle Sequencing Kit (v.2, Applied Biosystems, Foster City, CA) in an automated sequencer (ABI-PRISM 377). Sequences were compared with those available in the GenBank database by Basic Local Alignment Search Tool (BLASTn, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results

L1 larvae morphological identification as belonging to *S. stercoralis* relied on the dimensions (length: 190-260 µm, width: 9-11 µm), on the presence of a rhabditiform oesophagus occupying the anterior third of the body and of a clearly visible genital primordium (Toledo et al., 2015) (Fig. 1).

The *cox*1 gene sequences of 623bp confirmed *S. stercoralis* species identification, with a nucleotide identity of 100% with a free-living adult of *S. stercoralis* (GenBank accession n. AJ558163). No other parasites or helminthic eggs were isolated by flotation method. Also the faecal samples from the further two puppies and the bitch were positive for *S. stercoralis*. Although larval number was not recorded at Baermann technique, at visual estimation the number of larvae observed in two of the puppies was much larger than in the other two puppies as well as in the bitch. The only dog cohabiting with all the animals above scored negative. None of the dogs presented any clinical symptoms. The positive dogs were treated for 10 days (with 100mg mebendazole pro die) and after 15 day post treatment they scored negative for *S. stercoralis* by the Baermann method.

4. Discussion

In the described case, the occurrence of first-stage larvae in the faeces of the dam and in the four puppies sucking milk strongly supports the hypothesis of a transmammary transmission of *S. stercoralis* to puppies. Other elements supporting this hypothesis are the absence of the parasite in the cohabiting dog and the concrete basement of the pen and its management, not favouring the development and survival of free-living larvae. Considering that truffles dogs sniff in moist ground after raining (i.e., a suitable environment for the development of *S. stercoralis*), the dog herein analysed may have been infected during the field activities in the wooded areas of Lazio. The absence of clinical signs often occurs in immunocompetent hosts, both animals and humans, where the first-stage larvae develop into adult females leading to chronic asymptomatic infections (Thamsborg et al., 2017). Although the transmission of *Strongyloides* spp. mainly occurs percutaneously and orally, the transmammary route has been reported for *S. westeri, S. ransomi,* and *S. papillosus* (Abbas et al., 2021; Stewart et al., 1976; Nwaorgu et al., 1990), as well as *S. fuelleborni* in infants (Brown et al., 1977). Although the presence of L3i in the milk has not been herein demonstrated, our data are consistent with previous experimental evidence (Shoop et al., 2002), in that *S. stercoralis* passes through milk in lactating animals.

In addition, the risk of infection in humans should not be underestimated, considering that infection by STHs, including *S. stercoralis*, may occur in people in close contact with soil contaminated by animal faeces, such as shelter workers or dog breeder (Paradies et al., 2019; Stufano et el. 2022). In the case of *S. stercoralis*, the infection in humans may be of particular relevance, in consideration of the risk of developing disseminated strongyloidiasis in immunocompromised hosts, although rare (Rivasi *et al.*, 2006). Indeed, immunosuppressant factors such as the frequent steroid administration for many different health conditions, may trigger for the development of hyperinfection by *S. stercoralis*

(Cappella *et al.*, 2019; Ortega-Díaz *et al.*, 2020) that can be fatal if not promptly treated (Ursini *et al.*, 2013).

5. Conclusions

Data herein presented demonstrate that puppies may be infected and spread *S. stercoralis*, representing a source of infection primarily for owners or breeders. This report appears particularly relevant, due to the lack of knowledge and awareness about this parasite in central Italy, where its occurrence in dogs was not reported until now. Therefore, both physicians and veterinarians may not be aware of *S. stercoralis*' presence and consider this potentially clinically relevant parasite in differential diagnosis.

Studies in Humans and animals

Not applicable.

CRediT authorship contribution statement

Claudio De Liberato: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Roberta Iatta:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Maria Alessia Scarito:** Investigation. **Goffredo Grifoni:** Investigation. **Giampiero Dante:** Investigation. **Domenico Otranto:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abbas, G., Ghafar, A., Koehler, A.V., Bauquier, J., Wilkes, E.J.A., Jacobson, C., Beasley, A., Hurley, J., Cudmore, L., Carrigan, P., Tennent-Brown, B., El-Hage, C., Nielsen, M.K., Gauci, C.G., Hughes, K.J., Beveridge, I., Jabbar, A., 2021. Molecular detection of *Strongyloides* sp. in Australian thoroughbred foals. Parasit. Vectors 14, 444. https://doi.org/10.1186/s13071-021-04966-1.

Barratt, J.L.N., Lane, M., Talundzic, E., Richins, T., Robertson, G., Formenti, F., Pritt, B., Verocai, G., Nascimento de Souza, J., Mato Soares, N., Traub, R., Buonfrate, D., Bradbury, R.S., 2019. A global genotyping survey of *Strongyloides stercoralis* and *Strongyloides fuelleborni* using deep amplicon sequencing. PLOS Negl. Trop. Dis. 13, e0007609.

Bradbury, R.S., Pafčo, B., Nosková, E., Hasegawa, H., 2021. *Strongyloides* genotyping: a review of methods and application in public health and population genetics. Int. J. Parasitol. 51, 1153–1166.

Brown, R.C., Girardeau, H.F., 1977. Transmammary passage of *Strongyloides* sp. larvae in the human host. Am. J. Trop. Med. Hyg. 26, 215–219. https://doi.org/10.4269/ajtmh.1977.26.215.

Buonfrate, D., Bisanzio, D., Giorli, G., Odermatt, P., Fürst, T., Greenaway, C., French, M., Reithinger, R., Gobbi, F., Montresor, A., Bisoffi, Z., 2020. The global prevalence of *Strongyloides stercoralis* infection. Pathogens 9, 468.

Buonfrate, D., Requena-Mendez, A., Angheben, A., Cinquini, M., Cruciani, M., Fittipaldo, A., Giorli, G., Gobbi, F., Piubelli, C., Bisoffi, Z., 2018. Accuracy of molecular biology techniques for the diagnosis of *Strongyloides stercoralis* infection-a systematic review and meta-analysis. PLOS Negl. Trop. Dis. 12 https://doi.org/10.1371/journal.pntd.0006229.

Cappella, E.D., Piscaglia, A.C., Cadioli, A., Manoni, S., Silva, R., Buonfrate, D., 2019. *Strongyloides stercoralis* infection in San Marino republic: first epidemiological data from an observational study. Epidemiol. Infect. 147, e211.

Hasegawa, H., Sato, H., Fujita, S., Nguema, P.P., Nobusue, K., Miyagi, K., Kooriyama, T., Takenoshita, Y., Noda, S., Sato, A., Morimoto, A., Ikeda, Y., Nishida, T., 2010. Molecular identification of the causative agent of human strongyloidiasis acquired in Tanzania: dispersal and diversity of *Strongyloides* spp. and their hosts. Parasitol. Int. 59, 407–413.

Iatta, R., Buonfrate, D., Paradies, P., Cavalera, M.A., Capogna, A., Iarussi, F., Šlapeta, J., Giorli, G., Trerotoli, P., Bisoffi, Z., Otranto, D., 2019. Occurrence, diagnosis and follow-up of canine strongyloidiosis in naturally infected shelter dogs. Parasitology 146, 246–252.

Jaleta, T.G., Zhou, S., Bemm, F.M., Schär, F., Khieu, V., Muth, S., Odermatt, P., Lok, J.B., Streit, A., 2017. Different but overlapping populations of *Strongyloides stercoralis* in dogs and humans - dogs as a possible source for zoonotic strongyloidiasis. PLOS Negl. Trop. Dis. 11, e0005752.

Keiser, P.B., Nutman, T.B., 2004. *Strongyloides stercoralis* in the immunocompromised population. Clin. Microbiol. Rev. 17, 208–217.

Ko, P.P., Sakaguchi, K., Yoshida, A., Maruyama, H., Nonaka, N., Nagayasu, E., 2019. First molecular identification of *Strongyloides vituli* in cattle in Japan and insights into the evolutionary history of *Strongyloides* parasites of ruminants. Parasitol. Int. 72, 101937 https://doi.org/10.1016/j.parint.2019.101937.

Nwaorgu, O.C., Onyali, I.O., 1990. *Strongyloides papillosus*: prenatal and transmammary infection in ewes. Rev. Elev. Med. Vet. Pays Trop. 43, 503–504.

Ortega-Díaz, M., Puerta Carretero, M., Martín Navarro, J.A., Aramendi Sánchez, T., Alcázar Arroyo, R., Corchete Prats, E., de Sequera Ortiz, P., Albalate Ramón, M., Jaldo Rodríguez, M.T., Medina Zahonero, L., 2020. Immunosuppression as a trigger for hyperinflammatory syndrome due to *Strongyloides stercoralis* in membranous nephropathy. Nefrologia 40, 345–350.

Otranto, D., Dantas-Torres, F., Mihalca, A.D., Traub, R.J., Lappin, M., Baneth, G., 2017. Zoonotic parasites of sheltered and stray dogs in the era of the global economic and political crisis. Trends Parasitol 33, 813–825.

Ottino, L., Buonfrate, D., Paradies, P., Bisoffi, Z., Antonelli, A., Rossolini, G.M., Gabrielli, S., Bartoloni, A., Zammarchi, L., 2020. Autochthonous human and canine *Strongyloides stercoralis* infection in Europe: report of a human case in an Italian teen and systematic review of the literature. Pathogens 9, 439.

Paradies, P., Iarussi, F., Sasanelli, M., Capogna, A., Lia, R.P., Zucca, D., Greco, B., Cantacessi, C., Otranto, D., 2017. Occurrence of strongyloidiasis in privately owned and sheltered dogs: clinical presentation and treatment outcome. Parasit. Vectors 10, 345.

Paradies, P., Buonfrate, D., Iatta, R., Iarussi, F., Giorli, G., Palmieri, V.O., Sasanelli, M., Perandin, F., Šlapeta, J., Capogna, A., Otranto, D., Bisoffi, Z., 2019. Efficacy of ivermectin to control *Strongyloides stercoralis* infection in sheltered dogs. Acta Trop. 190, 204–209.

Riggio, F., Mannella, R., Ariti, G., Perrucci, S., 2013. Intestinal and lung parasites in owned dogs and cats from central Italy. Vet. Parasitol. 193, 78–84.

Rivasi, F., Pampiglione, S., Boldorini, R., Cardinale, L., 2006. Histopathology of gastric and duodenal *Strongyloides stercoralis* location in fifteen immunocompromised subjects. Arch. Pathol. Lab. Med. 130, 1792–1798.

Schär, F., Trostdorf, U., Giardina, F., Khieu, V., Muth, S., Marti, H., Vounatsou, P., Odermatt, P., 2013. *Strongyloides stercoralis*: global distribution and risk factors. PLOS Negl. Trop. Dis. 7, e2288.

Shoop, W.L., Michael, B.F., Eary, C.H., Haines, H.W., 2002. Transmammary transmission of *Strongyloides stercoralis* in dogs. J. Parasitol. 88, 536–539. https://doi.org/10.1645/0022-3395(2002)088.

Starr, M.C., Montgomery, S.P., 2011. Soil-transmitted helminthiasis in the United States: a systematic review—1940–2010. Am. J. Trop. Med. Hyg. 85, 680–684.

Stewart, T.B., Stone, W.M., Marti, O.G., 1976. *Strongyloides ransomi*: prenatal and transmammary infection of pigs of sequential litters from dams experimentally exposed as weanlings. Am. J. Vet. Res. 37, 541–544.

Stufano, A., Foti, C., Lovreglio, P., Romita, P., De Marco, A., Lia, R.P., Otranto, D., Iatta, R., 2022. Occupational risk of cutaneous larva migrans: a case report and a systematic literature review. PLOS Negl. Trop. Dis.

Štrkolcová, G., Goldová, M., Bocková, E., Mojžišová, J., 2017. The roundworm *Strongyloides stercoralis* in children, dogs, and soil inside and outside a segregated settlement in Eastern Slovakia: frequent but hardly detectable parasite. Parasitol. Res. 116, 891–900.

Thamsborg, S.M., Ketzis, J., Horii, Y., Matthews, J.B., 2017. *Strongyloides* spp. infections of veterinary importance. Parasitology 144, 274–284.

Toledo, R., Muñoz-Antoli, C., Esteban, J.G., 2015. Strongyloidiasis with emphasis on human infections and its different clinical forms. Adv. Parasitol. 88, 165–241.

Ursini, T., Polilli, E., Fazii, P., Ieraci, A., Sindici, G., Parruti, G., 2013. Late diagnosis of central nervous system involvement associated with lethal dissemination of *Strongyloides stercoralis* in an advanced HIV patient from Nigeria. Int. J. Infect. Dis. 17, 280–282.

Viney, M., 2016. Strongyloides. Parasitology 19, 1–4.

Zanzani, S.A., Di Cerbo, A.R., Gazzonis, A.L., Genchi, M., Rinaldi, L., Musella, V., Cringoli, G., Manfredi, M.T., 2014. Canine fecal contamination in a metropolitan area (Milan, North-Western Italy): prevalence of intestinal parasites and evaluation of health risks. Sci. World J. 2014, 132361 https://doi.org/10.1155/2014/132361.