



UNIVERSITÀ DEL PIEMONTE ORIENTALE

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SCHOOL OF MEDICINE

Department of Health Science

Master's degree in Medical Biotechnology (LM-9)

President: Prof. Francesca BOCCAFOSCHI

Master Thesis

**Seroepidemiology of human toxocariasis in a selected
population in Eastern Piedmont**

Tutor

Dr. Paolo RAVANINI



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Academic year 2025/2026



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*Ai miei zii Pierluigi e Loris,
che avrei tanto voluto fossero qui con me oggi,
ma sono certa che mi stiano guardando*

SUMMARY

Rational of the study: Toxocariasis and strongyloidiasis are classified as Neglected Tropical Diseases (NTDs) but they remain relevant even in non-tropical, industrialized countries such as Italy. Unluckily, awareness of these parasitic infections remains limited, particularly in agricultural settings, and only a few epidemiological studies have been conducted in our country. The aim of this study was to investigate the seroepidemiology of *Toxocara Canis* and *Strongyloides stercoralis* in a selected population from Eastern Piedmont and to provide an updated picture of the circulation of these neglected parasitic infections. A statistical comparison between occupationally exposed individuals and a control group was performed to determine whether occupational activity and exposure to rural environments represent significant risk factors.

Planning of the study: The study was conducted at the Microbiology and Virology Laboratory of the Maggiore della Carità University Hospital in Novara. Ethical approval was obtained from the Ethics Committee of the Spallanzani Institute and subsequently from the Supra-Regional Ethics Committee of Novara. Participant identification and sample selection were based on a previous study investigating a *Hantavirus* epidemiological survey conducted in the Piedmont region. A total of 66 participants were included in this study, equally divided between the occupationally exposed group (n = 33) and the control group (n = 33), according to occupational activity and potential exposure to rural environments. The exposed group consisted of farmers and livestock breeders, whereas the control group included individuals whose occupations did not involve contact with agricultural or livestock environments.

Serological monitoring was performed using highly sensitive ELISA method for the detection of specific IgG antibodies against *Strongyloides stercoralis* and Western blot (immunoblotting) for the detection of anti-*Toxocara canis* IgG antibodies.

Results: Results showed a significantly higher seroprevalence of *Toxocara canis* among farmers than among controls. Specifically, 11 of the 33 farmers tested positive (seroprevalence of 33.3%) compared with 2 of the 33 controls (seroprevalence 6.0%), and this difference was statistically significant ($p = 0.016$; $\chi^2 = 5.8022$). Seropositive cases of *Strongyloides stercoralis* were exclusively confined to the occupationally exposed group, with 3 of the 33 farmers testing positive (seroprevalence of 9.1%), whereas no positive cases were identified in the control group. However, this difference did not reach statistical significance ($p = 0.076$; $\chi^2 = 3.1429$). Cross-analysis of the serological results identified a single case of co-infection in a patient belonging to the farmers' group.

Conclusions: Based on these findings, greater awareness of parasitic zoonoses among healthcare professionals and agricultural workers appears warranted. Encouraging good hygiene practices and regular deworming of domestic animals may contribute to reduce the risk of transmission. Future multicentre studies involving different geographical areas and accounting for agricultural and environmental factors are needed to provide a more comprehensive understanding of the epidemiology of *Toxocara canis* and *Strongyloides stercoralis* in Italy. As these infections often proceed asymptotically or with non-specific symptoms, serological monitoring is recommended as the diagnostic tool for identifying previously unrecognized cases, particularly in populations at increased risk of exposure. Moreover, the implementation of highly specific diagnostic assays will allow a more accurate assessment of the burden of these infections, thereby supporting improved surveillance, targeted prevention strategies, and appropriate clinical management.

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INTRODUCTION

1. General information on Nematodes

Nematodes are among the most abundant animals on earth and constitute a large and distinct phylum of worm-like invertebrates.¹

The great majority of nematodes are free-living, inhabiting most essential niches in soil, fresh, and saltwater, as well as other, more specialized ones. Only a small fraction of the total number of species is parasitic, and only some of these infect the human host.²

Nematodes are commonly referred to as roundworms due to their appearance, characterized by a thin, elongated and cylindrical body. They are unsegmented organisms with a vermiform body, that tapers at both the anterior and posterior ends.³

Nematodes possess a body cavity known as the pseudocoelom or coelomic cavity, which is filled with fluid (haemolymph), that functions as a hydrostatic skeleton and contains the internal organs. Their body is covered by the integument, consisting of a robust and flexible external acellular cuticle which is shed four times during growth. The cuticle is produced by the underlying hypodermis. The longitudinal musculature lies between the hypodermis and the coelomic cavity.^{3,4}

Nematodes can be classified according to their dietary requirements into:

- Haematophages
- Histophages
- Chemivores

Nematodes exhibit marked sexual dimorphism, and males are generally smaller than females.⁴

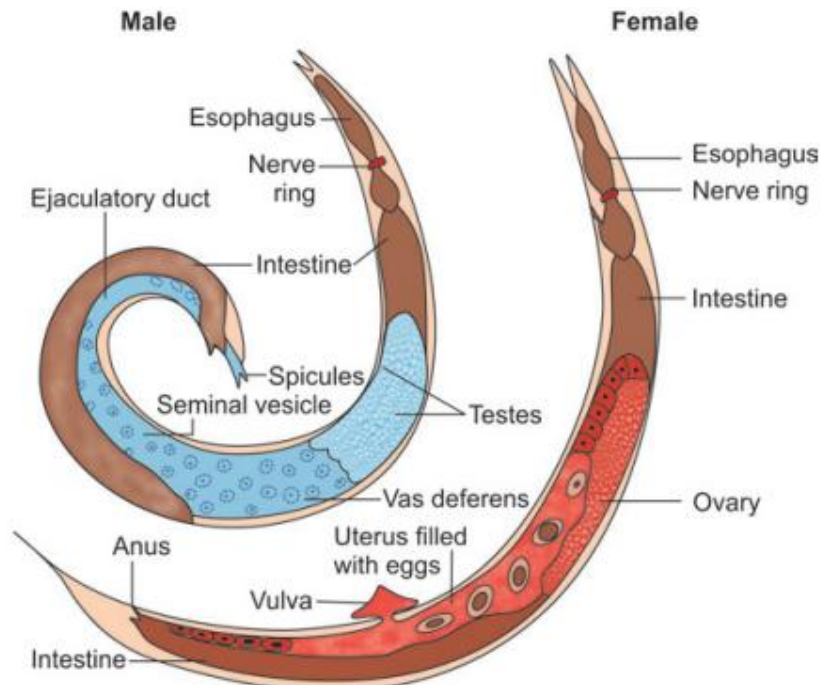


Figure n°1 - schematic diagram of adult male and female nematode (Sastry A.S., Bhat S., 2014)

1.1. Life cycle and infection

Most nematodes are oviparous (female produce segmented eggs) but there are also ovoviparous nematodes (embryos/larvae hatch from eggs that have matured within the uterus).⁴

Development is characterised by a series of moults during which the cuticle is shed. The life cycle comprises an egg and four larval stages: L1, L2, L3 and L4. L5 stage is considered adult. The infective form is the third-stage larva (L3).⁴

The host in which the parasite reproduces sexually is called the “definitive host”. Hosts in which the parasite must undergo one or more larval stages to

reach the definitive host are referred to as “intermediate hosts”. The “paratenic host” is one in which the parasite’s larva can survive without developing into other stages whilst awaiting the definitive host. This latter type of host can bridge an ecological gap between the intermediate and definitive hosts.⁴

The life cycle of nematodes can be divided into:

- Direct, where no intermediate hosts are involved. In this case, hatching of the egg is followed by two moults (L1→L2→L3). Infection of the definitive host generally occurs through ingestion or percutaneous entry by third-stage larvae (L3); this type of cycle is also called monoxenic.
- Indirect, where the first moults occur in an intermediate host. The definitive host becomes infected either by ingesting the intermediate host or through contamination of micro-wounds caused by the bite or sting of an arthropod carrying infective larvae. This type of cycle is called heteroxenic.⁴

After infection, a further two moults are required for the adult to develop. Reproduction allows another life cycle to begin.⁴

Humans can act as either definitive or accidental hosts for various nematodes, and infection occurs via specific routes of entry, which depend on the infective stage and the type of life cycle:

- Ingestion of embryonated eggs (faecal-oral cycle): this is the most common route, whereby humans accidentally ingest infective eggs containing larvae at the L2 or L3 stage from the external environment. This occurs through contamination of water, food or hands (e.g. *Ascaris lumbricoides*, *Toxocara canis* as an accidental host);

- Ingestion of larvae in tissues: humans ingest raw or undercooked meat from an intermediate or paratenic host containing encysted infective larvae, generally L3 (e.g. *Trichinella spiralis* in pork or wild boar meat);
- Active penetration of the skin: infective larvae (often filariform L3) that have developed in a moist environment actively penetrate intact skin, usually on the feet (e.g. *Strongyloides stercoralis*);
- Vector-borne transmission: through the bite of arthropods, L3 larvae are injected directly into the bloodstream or subcutaneous tissues by the feeding activity of a blood-sucking insect acting as a vector (e.g. filariae).⁵

Nematodes can also be classified according to their host environment as intestinal (geohelminths) or tissue-dwelling nematodes.⁶

Geohelminths are parasitic nematodes of the intestine whose eggs, once expelled by the host, require a period of maturation in the soil before they can infect a new host. Transmission occurs via indirect faecal-oral routes, mainly through the ingestion of water or food contaminated by soil containing mature eggs.⁶

Tissue dwelling nematodes are parasitic nematodes whose definitive habitat is not confined to the intestinal lumen, but involves the host's organs, tissues and blood vessels. They have more complex routes of infection and life cycles, and do not necessarily require the egg to mature in the soil. Possible routes of infection in humans for this group include vector-borne transmission, whereby L3 larvae are injected directly into the bloodstream by a blood-feeding insect (vector), or the ingestion of raw or undercooked meat, which causes larval migration into the tissues of the intermediate/paratenic host, followed by encystment.⁶

2. *Strongyloides stercoralis*

Strongyloides stercoralis is a nematode responsible for the disease known as strongyloidiasis. It has been estimated to affect over 600 million people worldwide and its clinical importance is due to its ability to cause chronic infection that can become life-threatening in the setting of immunosuppression.⁷

The World Health Organization (WHO) has recognized strongyloidiasis as a neglected tropical disease (NTD) with defined management targets.⁸

S. stercoralis most commonly infects humans through the cutaneous route by filariform larvae. Primary infection can also occur by ingestion of the filariform larvae, either by consumption of contaminated food or water, or sexually, by anal–oral contact.⁷

Cases of transmission following kidney transplantation have also been reported.⁹

Several conditions increase the risk of strongyloidiasis. These include infection with human T-lymphotropic virus 1 (HTLV-1), hypogammaglobulinemia, alcoholism, and malnutrition. Institutionalized patients also seem to be more commonly affected.⁷

Of note, although persons with HIV can have disseminated strongyloidiasis or hyperinfection syndrome, observational studies have not shown an increased risk in this population. (<https://www.cdc.gov/strongyloides/hcp/clinical-care/index.html>)

Activities that increase exposure to soil, such as walking barefoot, contact with human waste or sewage, and occupations that involve working with contaminated soil, like farming and coal mining, increase the risk of infection.¹⁰



Figure n°2 - Parasitic female *S. stercoralis* (Despommier, D., Griffin, R., Gwadz, R.W., et al., 2019)

2.1. Life cycle and pathogenesis

The life cycle of *S. stercoralis* is more complex than that of other nematodes, including both a free-living and a parasitic cycle. Once in the external environment, rhabditoid larvae (non-infectious) can develop through two different pathways: a direct cycle and an indirect (free-living) cycle.^{4,11}

In the direct cycle, the rhabditoid larvae feed and grow in the soil and transform into filariform larvae. The latter are unable to feed in the external environment but can penetrate the human body through the mucous membranes or intact skin. Filariform larvae can, in fact, survive in the external environment for up to two weeks.⁴

In the indirect sexual cycle, which takes place in warm, moist soil, the rhabditoid larvae expelled in the faeces undergo four moults and develop into free-living adults. All this occurs within 36 hours. The free-living adults have a different morphology from that of the parasites. Sexual reproduction occurs exclusively in the free-living cycle. The eggs hatch within a few hours, releasing rhabditoid larvae, which, after two moults, transform into filariform larvae.⁴

The larvae penetrate the skin and are carried via the bloodstream to the heart and lungs. From the pulmonary capillaries, they break through into the alveoli, travel up the trachea, descend the oesophagus and settle in the duodenal mucosa. It is also possible that the larvae may reach the duodenum directly by migrating through other tissues.⁴

When in the small intestine, the larvae mature into adult females, that reproduce by parthenogenesis and produce eggs, which hatch into rhabditiform larvae. Larvae are excreted in stools.⁷

The first rhabditoid larvae appear in human faeces 28 days after infection.⁴

The rhabditiform larvae then give rise to the infectious filariform larvae, either in soil or in the own host's intestine. The latter causes the unique phenomenon of autoinfection, which can be internal or external. In external autoinfection, rhabditoid larvae develop into filariform larvae in faecal matter in the perineal region, migrating into the skin and causing transient, recurrent skin eruptions (larva currens). Internal autoinfection occurs when rhabditoid larvae transform within the intestinal lumen into filariform larvae, which invade the tissues. These conditions lead to a chronic and frequently subclinical infection. A low level of internal autoinfection is responsible for the persistence of this parasitic infection in a latent form for years, even in the absence of external exposure. In people with delayed fecal transport, autoinfection can be significant and cause large worm burdens. In those where fecal soilage of the skin occurs for prolonged periods of time, penetration of filariform larvae can cause local irritation as well as autoinfection.^{7,12}

Autoinfection can result in chronic infection lasting for several decades, up to 75 years after initial exposure. Autoinfection usually occurs in patients with

impaired cell-mediated immunity, leading to hyperinfection syndrome and disseminated strongyloidiasis.¹¹

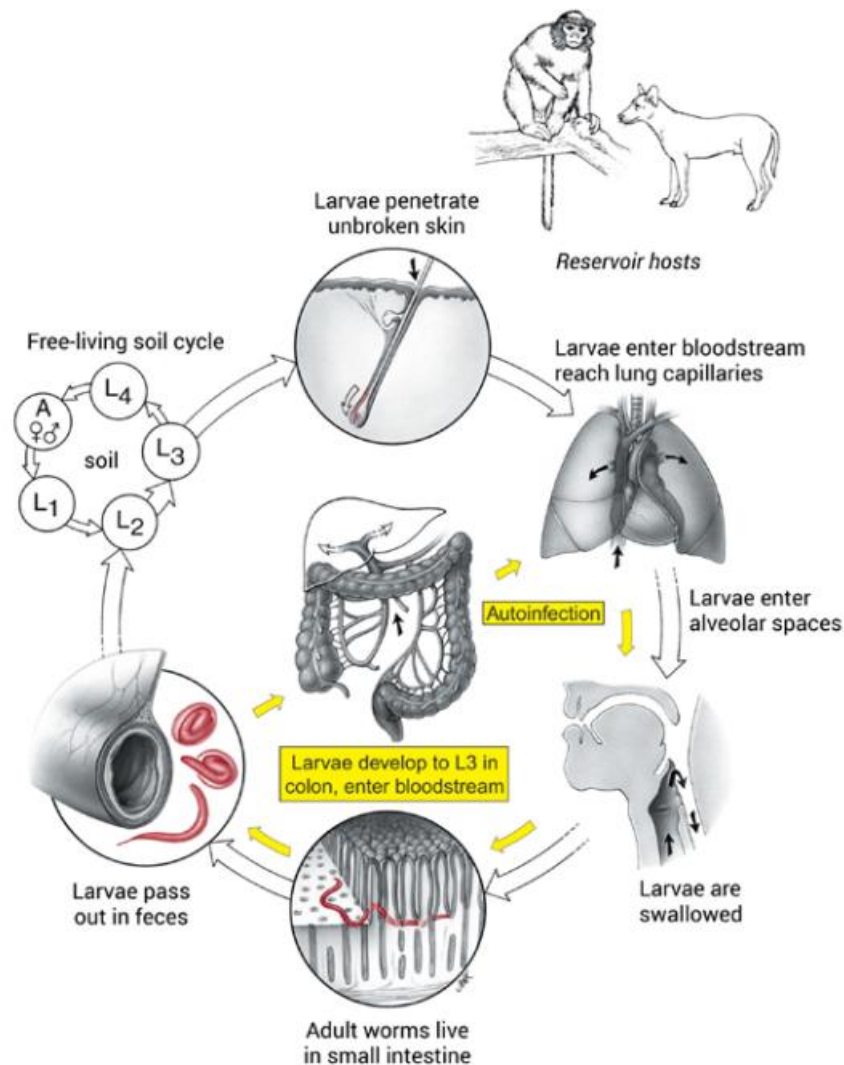


Figure n°3 - lifecycle of *Strongyloides stercoralis* (Despommier, D., Griffin, R., Gwadz, R.W., et al., 2019)

2.2. Epidemiology

Strongyloides stercoralis is endemic worldwide, but is most prevalent in tropical and subtropical climates, especially in areas with inadequate sanitary conditions. Community-based studies have estimated a prevalence of over 70% in countries such as Peru, Kenya, Namibia, and Papua New Guinea. In the

United States and Europe, most cases are considered imported. However, local transmission has been reported, especially in rural areas.^{7,13}

The global incidence of strongyloidiasis was estimated to range from 30 to 100 million cases, with increased infection in immunocompromised patients. However, recent studies concluded that the true prevalence of *S. stercoralis* would be as high as 600 million cases worldwide and was severely underestimated due to variations in the applied diagnostic schemes.¹⁴

The Committee to Advise on Tropical Medicine and Travel (CATMAT) categorized the epidemiological risk for Strongyloides infection and exposure as follows: high risk (>10%) for birth, residence, or long-term travel (defined as cumulative six-month exposure, or contact of skin with sand or soil in a high risk area during shorter-term travel) in Southeast Asia, Oceania, Sub-Saharan Africa, South America, and the Caribbean; moderate risk (3–10%) in Mediterranean countries, Middle East, North Africa, Indian sub-continent, and Asia; and low risk (<3%) in Australia, North America, or Western Europe.¹²

Buonfrate D., Baldissera M., Abrescia F. et al. multicenter case-control study was conducted between February 2013 and July 2014 and it involved three Italian Regions: Veneto (Negrar, San Bonifacio, and Treviso sites), Lombardia (Brescia, Mantova sites), and Friuli Venezia Giulia (Trieste, Udine sites). Screening for *S. stercoralis* infection was done with serology, confirmation tests were a second serological method or stool agar culture.

The high number of screened individuals permitted to obtain a valuable estimate of the prevalence of strongyloidiasis in the studied regions in the north of Italy: 8 and 17%, respectively, in Italians and immigrants with eosinophilia, 1 and 2% in those with a normal eosinophil count, irrespective of signs/symptoms of the infection. The geographical pattern of infection

prevalence is consistent with a higher transmission in agricultural areas of Po valley during the first decades of the past century, with a downward trend over time likely due to improvement of hygiene and sanitary conditions.¹⁵

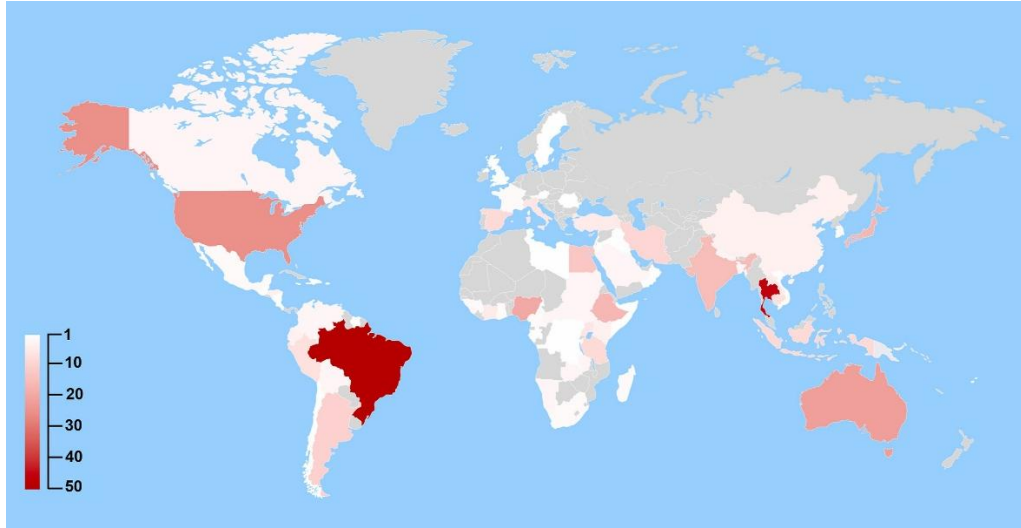


Figure n°4 - Estimated seroprevalence of *Strongyloides stercoralis* infection worldwide (Yang, R., Xu, M., Zhang, L., et al., 2025)

2.3. Clinical picture of strongyloidiasis

S. stercoralis can cause a wide spectrum of disease presentations depending on the host's immunity and parasite load. The clinical syndromes include acute strongyloidiasis, chronic strongyloidiasis, hyperinfection syndrome, and disseminated infection.¹²

2.3.1. Acute strongyloidiasis

Acute strongyloidiasis is mainly reported in travelers who returned from endemic areas. The symptoms are mainly related to reaction at the site of larval entry and migration, which normally occur immediately to several weeks after the infection. Skin manifestations include a serpiginous lesion at the entry site.¹²

When filariform larvae migrate into the pulmonary tract, they can cause point bleeding and inflammatory cell infiltration with cough, dyspnoea, haemoptysis, and wheezing.¹¹

Gastrointestinal symptoms begin about 2 weeks after infection and include diarrhoea, constipation, anorexia, nausea, vomiting, abdominal pain, fever, anaemia and malaise.¹¹

Eosinophilia is commonly seen in immunocompetent patients with strongyloidiasis and might provide some protection, especially in early-stage infection.¹¹

2.3.2. Chronic strongyloidiasis

Most of the patients affected by chronic strongyloidiasis are asymptomatic or only experience mild symptoms. Most common are the gastrointestinal symptoms that include sensations of abdominal bloating, and epigastric pain that intensify with eating, sporadic vomiting, diarrhea, constipation, and borborygmus. These symptoms are not specific so it can be challenging to suspect strongyloidiasis unless accompanied by other indications or symptoms.¹⁰

Another common reported sign of chronic infection is larva currens, a serpiginous urticarial rash secondary to intradermal migration of *Strongyloides* larvae at 5-15 centimeters per hour. The most commonly involved sites are buttock, perineum, and thighs. Repeated autoinfection is the cause of larva currens.¹⁰

Hyperinfection syndrome is defined by an increase in parasite load from the autoinfection life cycle, usually (but not always) caused by impairment of immune status.¹²



Figure n°5-Disseminated larva currens on the inferior abdomen (Gardini G., Froeschl G., Petra Rosa Puzzi P.R., 2024)

2.4. Prevention and control

Implementation of control programmes for *Strongyloides stercoralis* infection is a target within the World Health Organization Roadmap to 2030.¹¹

Personal hygiene education should be given to patients, persons at risk, and the general population in endemic areas. In rural and remote communities in tropical regions, access to clean sanitation, water, and footwear has been fundamental to preventing strongyloidiasis. In both children and adults at risk, early detection and prompt treatment are key to eliminating or reducing *S. stercoralis* infection.¹¹

Hospitalized patients with hyperinfection should be managed with contact isolation, and their family members should also be screened for infection. Moreover, individuals who are planning to undergo transplantation or are receiving immunosuppressive therapy should be tested for *S. stercoralis*.

Eventually, strongyloidiasis should be considered in patients presenting with eosinophilia.¹¹

2.5. Laboratory diagnosis

Strongyloidiasis should be suspected in any patient with unexplained eosinophilia especially if they have had epidemiologic exposure in areas of high disease prevalence. Patients at increased risk of developing hyperinfection, such as those undergoing transplantation or starting medications, such as corticosteroids or with evidence of infection with HLTV-1 should be screened. Diagnosis can happen with parasitological or serological testing.⁷

Direct visualization of larvae is the gold-standard for diagnosis of strongyloidiasis. The intermittent parasite shedding and low infectious burden lead to a sensitivity of single specimen stool microscopy is as low as 21%. Examining multiple stool samples collected on different days significantly improves detection rates, with sensitivity approaching 100% when up to seven specimens are analyzed. In cases of hyperinfection, detection is less challenging due to a larger number of worms.⁷

Serology based on the ELISA (Enzyme-Linked Immunosorbent Assay) test has demonstrated high diagnostic performance, with a sensitivity of 85%, a specificity of 97%, and a negative predictive value (NPV) of 100%. This indicates that serological methods can be considered superior to stool-based tests, making them more reliable for epidemiological assessment in populations at higher risk of infection.¹⁰

As previously mentioned, serodiagnosis by ELISA using crude antigens has shown high sensitivity and specificity. However, ELISA tests that detect

antibodies cannot distinguish between past and current infection. Furthermore, it is difficult to know whether low-level autoinfection is continuing. This may because antibody levels remain detectable for years after anti-*S. stercoralis* treatment. The antibody test also shows cross-reactivity with other helminth infections, such as filariasis, acute schistosomiasis, and *Ascaris lumbricoides* infection.¹¹

In conclusion the diagnosis of strongyloidiasis should rely on both parasitological and serological methods, as no single test is sufficiently sensitive and specific in all clinical settings.

2.6. Treatment and management

Traditionally, treatment of strongyloidiasis has been based on thiabendazole. Ivermectin is a broad-spectrum antiparasitic that causes muscle paralysis in invertebrates and it has shown fewer side effects and is more effective than thiabendazole in treating strongyloidiasis.^{7,11}

Albendazole is considered an alternative therapy. Combination treatment with both albendazole and ivermectin would improve the efficiency of mass drug administration targeting *S. stercoralis*.¹¹

3. *Toxocara canis* and *Toxocara cati*

Toxocara canis and *Toxocara cati* are intestinal ascarid nematodes of canids and felids, respectively, and they're responsible of human toxocariasis (HT). *T. canis* is able to infect different canids, such as dogs, foxes, wolves, jackals and coyotes. *T. cati* is found in a total of 21 species of wild and domestic felids of all ages.^{16,17}

This zoonosis is mainly transmitted via the oro-fecal route: adult *Toxocara* worms live in the small intestine of wild or domestic hosts, and their eggs are excreted in feces. These eggs can contaminate soil, water, and food and may be ingested by humans. Another way of ingestion is through raw meat of paratenic hosts, such as chickens. Currently, cases of human toxocariasis have been reported due to the consumption of raw chicken liver, duck liver, sheep liver and cattle liver.^{16,18}

Additionally, direct contact with the fur of cats infected with *T. cati* has been reported as a possible transmission route, since infective embryonated eggs have been identified in the perianal area, extremities, and lower part of the tail.

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This type of zoonosis is widespread throughout the world and mainly affects children, who become infected by accidentally ingesting infectious *Toxocara* eggs found in the environment or, occasionally, by eating invertebrates such as earthworms.¹⁶

Symptoms of human toxocariasis might include fever, headaches, coughing, and abdominal or limb pain. Several syndromes can occur such as visceral larva migrans (VLM), ocular toxocariasis (OT), neurotoxocariasis (NT) (e.g., eosinophilic meningoencephalitis) and covert/common toxocariasis (CT).

From an epidemiological perspective, there are still gaps in understanding, despite the clinical and public health significance of human toxocariasis, particularly in the tropical and subtropical regions of the world and in disadvantaged communities in temperate climates. Recent studies indicate that toxocariasis is a neglected disease and is not subject to mandatory reporting. In some communities, despite the culling or treatment of dogs and cats to reduce

environmental contamination from infectious eggs, the prevalence of anti-*Toxocara* antibodies in human serum may remain high.¹⁶



Figure n°6 - *Toxocara canis* adult male (above) and female (below) ((Despommier, D., Griffin, R., Gwadz, R.W., et al., 2019)

3.1. Life cycle and pathogenesis

The development of adult worms of *T. canis* occurs primarily in puppies less than 6 months of age. Worms develop in the small intestine and the produced eggs are eliminated with feces. After 14-35 days in the external environment, L3 larvae (L3s) develop inside the eggs and must be ingested by an host in order to continue their life cycle. Once in the small intestine, the L3 hatches and crosses the intestinal wall, and migrates to different organs through the lymph or blood. Subsequent migration varies depending on whether the host is a young dog (<3 months), an adult dog, a pregnant dog, or a paratenic host, such as pigs, rats, mice, gerbils, sheep, chickens, or humans.²⁰

Embryonated *Toxocara* eggs, containing infective third stage larvae, can be ingested by humans via contaminated soil, water or food or through raw or undercooked meat from paratenic hosts such as chickens.¹⁹

For this reason, sandboxes, playgrounds, beaches, parks, and town squares contaminated with the feces of infected dogs and cats are the main source of human transmission. Moreover, vertical transmission can also occur in humans, as seen in dogs and cats.²¹

At this point the larvae penetrate the intestinal wall and are transported by the circulatory system to different organs including liver, lungs, central nervous system and/or musculature. The development of the larvae in the tissues may come to a halt for several months.^{16,19}

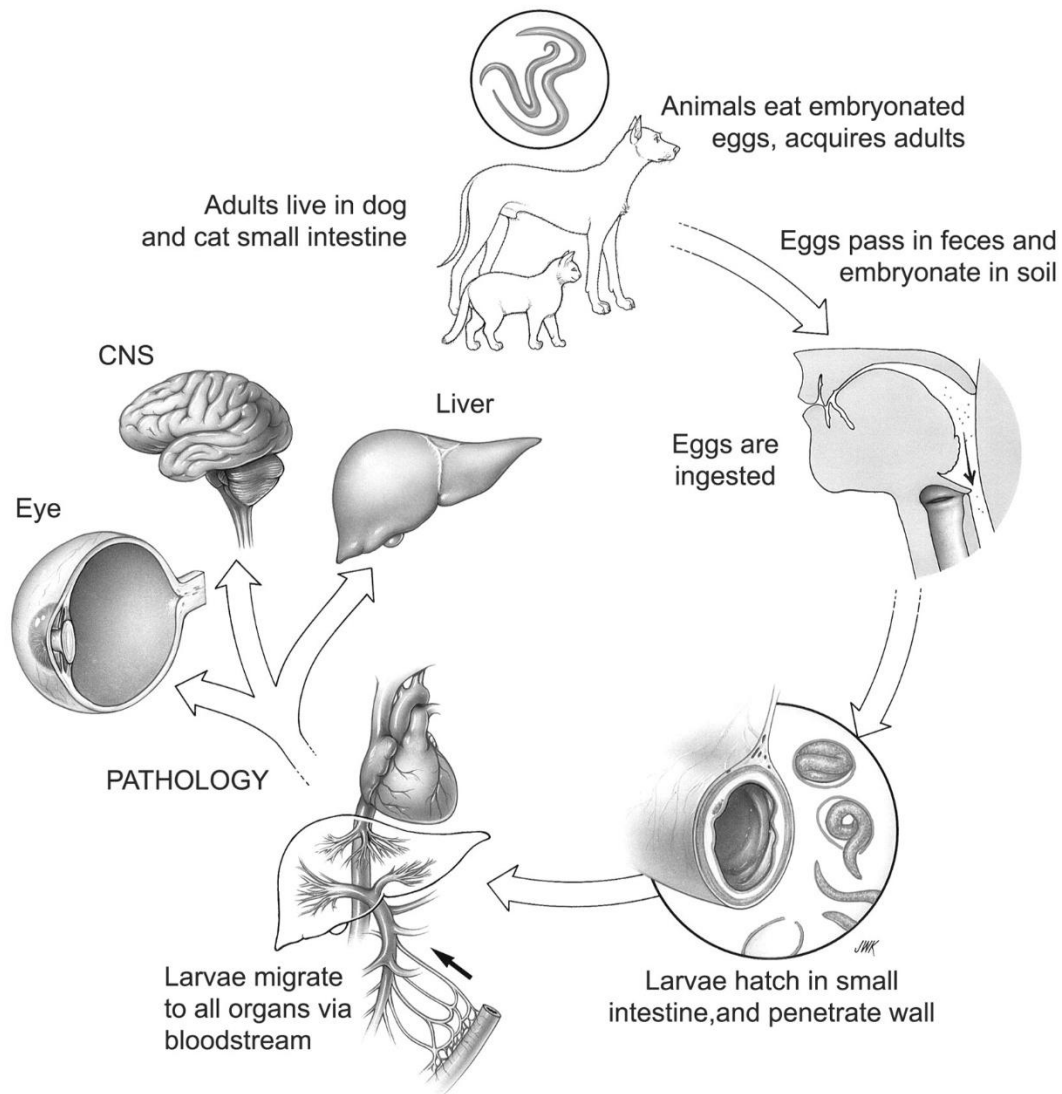


Figure n°7- *Toxocara* lifecycle (Despommier D., 2003)

3.2. Epidemiology

Toxocara canis and *Toxocara cati* are distributed all over the world. Epidemiological data are still limited for most countries all around the world. However, over the past 36 years, nation-wide surveys of humans have estimated prevalences of specific anti-*Toxocara* serum antibodies at 0.7% in New Zealand, 1.6% in Japan, 2.4% in Denmark, 6.3% in Austria, 7% in Sweden, 14% in the USA and 31% in Ireland, but prevalences are over 20% in some ethnic and socioeconomically disadvantaged groups, (22% in Iran and 81% in Nepal). In particular, a seroprevalence of 85% has been recorded in school children in Manado, Indonesia, and 87% on the Marshall Islands.¹⁶

Rostami A, Ma G, Wang T, et al. concluded that 19% of the human population is seropositive for *Toxocara*. Lowest seroprevalence rate was found in the Eastern Mediterranean region with a mean of 8.2%, while the highest was found in Africa with a mean of 37.7%. In the European region they estimated a seroprevalence of 10.5%. Regions with low human development index, income level, latitude and high environmental temperature and precipitation was more likely to show high seropositivity. The authors also highlighted that *Toxocara* seropositivity was associated with factors such as male gender, rural residence, younger age, frequent contact with dogs, cats, or soil, and the consumption of raw meat or untreated water.¹⁶

A study has been carried out in Northern Italy and reported a *Toxocara* seroprevalence of 4%, while another one conducted in the Marche region indicated a seroprevalence of 1.6%.²²

Nicoletti A, Cicero CE, Mantella A et al. study was conducted in Sicily, in particular in Catania, and investigated the seroprevalence of *T. canis* in adult

population (over 14 years) of the city. In this context, authors found a seroprevalence of 8.0%.²²

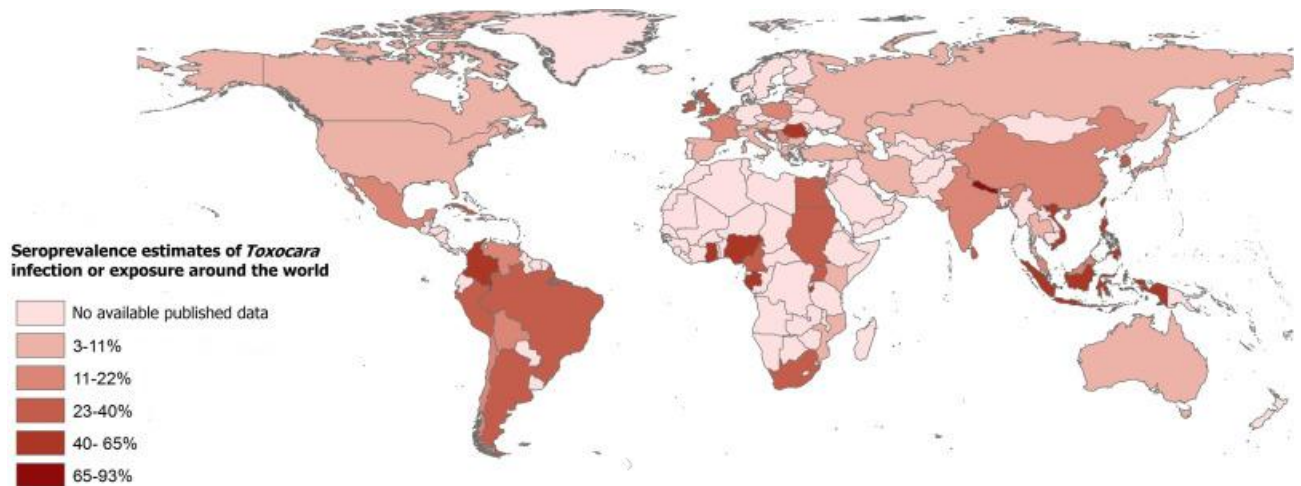


Figure n°8 - Estimated seroprevalences of Toxocara infection or exposure around the world (Rostami, A., Ma, G., Wang, T., et al., 2019)

3.3. Clinical picture of toxocariasis

It's possible to categorize toxocariasis into four clinical forms according to the organs affected: visceral larva migrans (VLM), ocular toxocariasis (OT), covert or common toxocariasis (CT), and neurotoxocariasis (NT). The severity of disease depends on the parasite burden, the duration of larval migration, and age- and immune-mediated responses of the affected individuals.²³

3.3.1. Visceral larva migrans (VLM)

Of the four main clinical forms of human toxocariasis, VLM is the most advanced one, even if it's relatively uncommon.¹⁶

VLM is the consequence of a systemic migration of *Toxocara* larvae through the tissue of human viscera and it results from high intensity or repeated infections by *T. canis* larvae. It mostly affects children aged 2-7 years.²³

Infections in adult individuals have been reported in East Asia (e.g. South Korea and Japan), through ingestion of raw beef, lamb, chicken, or ostrich liver.^{24,25}

The most commonly affected organ in VLM is the liver with the formation of granulomatous lesions and hepatitis. Less often, larvae may also invade other organs, such as heart, lungs, kidneys and muscle, resulting in myocarditis, myalgia with eosinophilic polymyositis, respiratory symptoms, arthritis and nephritis. Rash, pruritus, eczema, panniculitis, urticaria and vasculitis, have also been detected in some VLM cases.²³

3.3.2. Ocular toxocariasis (OT)

OT is relatively uncommon and it refers to human toxocariasis only affecting the eye. It is mostly reported to occur in children between three and 16 years of age. Uni-ocular visual impairment might occur, accompanied by chronic endophthalmitis/retinitis and/or posterior/peripheral granulomata.¹⁶

Other abnormalities include vitreous opacities, yellowish-white intraretinal lesions in the optic disc with papilledema, live intraocular worm, papillitis, and a tractional retinal detachment, and diffuse unilateral subacute neuroretinitis.²³

The extent of this impairment is linked to migrating or dead larvae and/or resultant immune reactivity against them as well as location in the eye. Blindness can result from tractional retinal detachment, vitritis and/or cystoid macular oedema.¹⁶

3.3.3. Covert/common toxocariasis (CT)

CT representing ‘common toxocariasis’ in adults and ‘covert toxocariasis’ in children, has non-specific symptoms, thus it’s challenging to diagnose clinically. Clinical signs, such as weakness, pruritus, rash, pulmonary dysfunction, pulmonary insufficiency and/or abdominal pain can be observed in adults, while fever, anorexia, headache, nausea, abdominal pain, vomiting, wheezing, lethargy, sleepiness and behavioural disorders, pulmonary symptoms and/or limb pain, might be seen in children. Eosinophilia and elevated IgE levels are common.¹⁶

3.3.4. Neurotoxocariasis (NT)

NT is reported to occur mainly in middle-aged people and less in children.¹⁶

NT is caused by invasion of *Toxocara* larvae to the brain and spinal cord, leading to cerebral lesions and neurological damage, mainly in the cerebral and cerebellar white matter, with cerebral blood vessels occlusion. The associated clinical symptoms include myelitis, encephalitis, mental confusion and/or meningitis. Headache, fever, photophobia, weakness, dorsalgia, confusion, tiredness, visual impairment, epileptic seizures, neuropsychological disturbances, dementia and depression are also reported. Motor impairment can also be observed in clinical NT cases, such as ataxia, rigor, para- or tetraparesis dysaesthesia, urinary retention, and faecal incontinence.²³

3.4. Prevention and control

The rapid increase in the number of dogs and cats, especially uncontrolled feral and stray populations and their close proximity to humans, has increased the

risk of human infection with *Toxocara*. The lack of an effective method to kill *Toxocara* eggs makes it impossible to eradicate this parasite from the environment. For these reasons, in order to prevent infection it's important to prevent initial contamination of the environment. There are different measures to implement in order to interrupt transmission of *Toxocara* eggs from animals to humans, such as de-worming household pets frequently and from a young age. In particular, prophylactic anthelmintics should be given to puppies, kittens, or pregnant dogs, since they are most likely to transmit the disease. Owners should also safely collect and hygienically dispose of pet faeces, before the eggs become infective. Prevention of human infections can also be achieved by washing hands after touching or playing with pets, or following exposure to potentially contaminated sites.²³

It's also crucial to investigate *T. canis* infection in a variety of food-producing animals since they can be paratenic hosts for *T. canis*. Every stage of the production chain should be rigorously controlled and tested, beginning with the farm and ending with the consumer.¹⁸

It is essential to maintain the cleanliness of the breeding site to prevent infestation by infective eggs. Regular fecal testing should be conducted on the food-producing animals to detect any infections. Consumers should ensure that meat is fully cooked, and it is advisable to avoid consuming raw or undercooked meat products.¹⁸

3.5. Laboratory diagnosis

Diagnosis of toxocariasis is problematic, particularly in tropical countries where polyparasitism is common.²⁶

In humans, the diagnosis of *Toxocara* infection or toxocariasis is usually achieved by serology, occasionally combined with imaging methods to detect granulomata (with encapsulated larvae) in tissues.¹⁶

The preferable laboratory approach includes enzyme-linked immunosorbent assays (ELISAs), utilising *Toxocara* excretory/secretory (TES) antigens as well as commercially produced test kits, and western blotting (WB) to reduce false positive results related to background or cross reactivity with other parasites. WB, based on the use of fractionated, native, TES antigens of *T. canis* larvae has been shown to increase specificity, with reactivity to bands of low molecular weight (24–35 kDa) shown previously to be specific for *T. canis*.^{16,26}

However, it is not possible to unambiguously distinguish between *T. canis* and *T. cati* infections using current serological or immunological techniques such that optimised, species-specific diagnostic tools are required to support future epidemiological investigations.¹⁶

The sensitivity and specificity of serodiagnostic tests for human toxocariasis depend on the antigens used, the isotype of antibodies (e.g., IgG, IgG subclass or IgM) being detected and the extent of optimisation of the assays.¹⁶

For the diagnosis of NT, the detection of specific antibodies in serum as well as eosinophils in cerebrospinal fluid (CSF) need to be evaluated. The detection of specific anti-*Toxocara* antibody in blood (although false-negative results are common) and vitreous or aqueous humour should be assessed for the diagnosis of OT.¹⁶

3.6. Treatment and management

The treatment of human toxocariasis is challenging: some drugs don't reach and penetrate *Toxocara* larvae in tissues and their efficacy cannot be rigorously

assessed in patients. Nonetheless, anthelmintic treatment is recommended for acute toxocariasis, principally to prevent larvae from reaching the central nervous system.¹⁶

Albendazole (ABZ) and mebendazole (MBZ) are commonly used for oral treatment of VLM, despite limited efficacy.¹⁶

Moreover, nonsteroidal anti-inflammatory drugs or corticosteroids, such as dexamethasone or prednisolone, can be administered to reduce clinical signs linked to allergic responses to *Toxocara* antigens.¹⁶

The current standard treatment for OT with active intraocular inflammation includes systemic corticosteroid in combination with ABZ.²³

OBJECTIVE OF THE THESIS

The main aim of this thesis project is to estimate the seroprevalence of IgG antibodies directed against the zoonotic nematodes *Toxocara canis* and *Strongyloides stercoralis* within a selected population in the Eastern Piedmont region.

The research focuses on a group of individuals defined according to specific vulnerability and susceptibility criteria, with the aim of reassessing the incidence and epidemiology of the two parasitic infections in non-tropical settings.

Serological monitoring was carried out by determining the frequency of exposure to the parasites using two specific diagnostic techniques. In particular, *Strongyloides stercoralis* specific IgG antibodies were assessed with the highly sensitive ELISA (enzyme-linked immunosorbent assay) method.

Anti-*Toxocara canis* IgG antibodies were detected using Western blot (immunoblotting), the gold-standard method for serological diagnosis because it allows the identification of characteristic antigenic bands.

In parallel, the study focused on analysing occupational and behavioural risk factors, investigating the correlation between serological positivity and epidemiological variables, such as prolonged contact with soil, ownership of pets or farm animals, and origin from endemic areas.

Particular attention was paid to agricultural workers, as this group is considered to be at high risk due to potential exposure to animal faeces and contaminated soil.

The research involved a statistical comparison between the group of vulnerable individuals and the control group, characterised by differing probabilities of

infection, in order to determine whether occupational activity and/or living environment represent significant risk factors.

In conclusion, the study aims to provide an up-to-date epidemiological picture of the circulation of these two neglected parasitic infections in the Eastern Piedmont region.

MATERIALS AND METHODS

1. Sampling of patients involved in the study

Participant identification and sample selection were based on a previous study investigating a *Hantavirus* epidemiological survey conducted in the Piedmont region.

Biological samples were handled in accordance with regulations concerning privacy and personal data protection. All participants provided informed consent, and samples were processed anonymously through the assignment of alphanumeric identification codes. The project was approved under CUP F43C22000330001. Ethical approval was obtained from the Ethics Committee of the Spallanzani Institute and subsequently from the Supra-Regional Ethics Committee of Novara (approval no. 9-2023, 17 July 2023). The study was conducted in accordance with the principles of the Declaration of Helsinki.

A total of 66 participants were included in this study, comprising 44 men and 22 women, with a mean age of 47.7 years (range: 22–87 years). Participants resided in the provinces of Novara, Biella, Vercelli, Pavia, and Turin. Particular attention was given to areas where rice cultivation represents the primary agricultural activity. Sample collection was carried out between July 2023 and April 2025.

The samples were equally divided between the experimental group ($n = 33$) and the control group ($n = 33$). Group comparisons were performed taking age and sex into account.

The study population was divided into two groups according to occupational activity and potential exposure to rural environments. The experimental group consisted of farmers and livestock breeders, who were considered a high-risk population because of their frequent exposure to soil and agricultural settings. The control group comprised individuals whose occupations did not involve contact with agricultural or livestock environments.

A venous blood sample was collected from each participant using 9 mL collection tubes. Samples were centrifuged, and the resulting serum was aliquoted and stored at -20°C until laboratory analysis.

Analyses were carried out at the Microbiology and Virology Laboratory of the Maggiore della Carità University Hospital in Novara. Serological diagnosis was performed using highly sensitive and specific methods to detect antibody response against *Toxocara canis* and *Strongyloides stercoralis*. Antibody reactivity was assessed by detecting specific IgG antibodies.

Anti-*Toxocara canis* IgG antibodies were detected by Western blot using the commercially available TOXOCARA Western Blot IgG kit (LDBIO Diagnostics, Lyon, France). In parallel, anti-*Strongyloides stercoralis* IgG antibodies were measured using an indirect enzyme-linked immunosorbent assay (ELISA) with the commercially available *Strongyloides ratti* IgG ELISA kit (Bordier Affinity Products S.A., Crissier, Switzerland).

2. Indirect ELISA for the detection of anti-*Strongyloides stercoralis* IgG antibodies

Serological diagnosis of human strongyloidiasis was performed using an indirect enzyme-linked immunosorbent assay (ELISA) with the commercial *Strongyloides ratti* IgG ELISA kit (Bordier Affinity Products S.A., Crissier, Switzerland).

The assay quantitatively detects IgG antibodies against *Strongyloides stercoralis* and is based on the antigenic similarities between *Strongyloides ratti* and *Strongyloides stercoralis*. The ELISA kits provide 96 wells coated with somatic antigens from *Strongyloides ratti* larvae. Each strip consists of 8 separable wells, a suitable format for laboratories with a low workload.

All reagents were stored at 2-8°C and used according to the manufacturer's instructions.

Optical density (OD) values were measured using a microplate spectrophotometer at a wavelength of 405 nm. Results were interpreted by calculating the index value, which is the ratio between the OD of each sample and the assay cut-off value. Samples were classified as positive or negative according to the manufacturer's interpretation criteria.

The assay is based on the binding of specific IgG antibodies present in serum samples to *S. ratti* somatic antigens coated on the surface of the microplate wells. When specific antibodies are present, antigen–antibody complexes are formed during incubation, while the unbound antibodies are removed during the washing step.

The presence of parasite-specific antibodies is detected using a protein A-alkaline phosphatase conjugate. A second washing step removes any unbound conjugate.

The addition of a chromogenic substrate generates a colorimetric reaction producing a yellow-colored product in the presence of alkaline phosphatase. The reaction is subsequently stopped by the addition of potassium phosphate stop solution.

The intensity of the color developed is measured spectrophotometrically at 405 nm and directly proportional to the concentration of anti-*Strongyloides* IgG antibodies in the sample.

All components of the *Strongyloides ratti* IgG ELISA kit were brought to room temperature prior to use. Some reagents included in the *Strongyloides ratti* IgG ELISA kit need to be prepared according to the manufacturer's instructions before use. In particular, dilution buffer (10x) concentrate and washing solution (10x) concentrate were diluted 1/10 in distilled water.

10 µl of control sera were diluted in 190 µl of dilution buffer solution (final dilution 1/20).

Conjugate was diluted 1/50 in dilution buffer solution.

In order to obtain substrate solution, phosphatase substrate tablet(s) were dissolved in undiluted enzyme buffer. The stopping solution is ready to use.

Serum samples were diluted 1/201 in dilution buffer solution. In particular, each serum sample was diluted by mixing 10 µL of serum with 2 mL of dilution buffer, obtaining a final volume of 2010 µL.

Following preparation, reagent vials were tightly capped and briefly vortex-mixed to ensure complete homogenization and to minimize the risk of contamination.



Figure n°9 - *Strongyloides ratti* IgG ELISA kit

Followed procedure:

- Pre-incubation step: each well was filled with 200 μ L of dilution buffer in order to rehydrate antigens and incubated at room temperature for 5 min. The buffer was then removed,
- Samples incubation: control sera and samples were arranged in a specific order within the microplate. Wells were set up as follows and the microplate was sealed with Parafilm and incubated at 37°C for 30 min.
 - Blank: 100 μ L of dilution buffer (no serum)
 - Negative control: 100 μ L of diluted negative control serum
 - Cut-off control: 100 μ L of diluted cut-off control serum
 - Positive control: 100 μ L of diluted positive control serum
 - Serum samples: 100 μ L of diluted serum samples
- Washing step: wells were washed four times with 250 μ L of wash solution
- Conjugate incubation: 100 μ L of diluted protein A-alkaline phosphatase conjugate were added to each well. The plate was sealed and incubated at 37°C for 30 min

- Washing step: conjugate was removed and wells were washed four times with 250 μ L of wash solution
- Substrate incubation: 100 μ L of substrate solution were added to each well. The plate was incubated at 37°C for 30 min
- Stopping reaction: the enzymatic reaction was stopped by adding 100 μ L of stop solution to each well
- Spectrophotometric reading: absorbance (A) was measured at 405 nm using a microplate spectrophotometer.

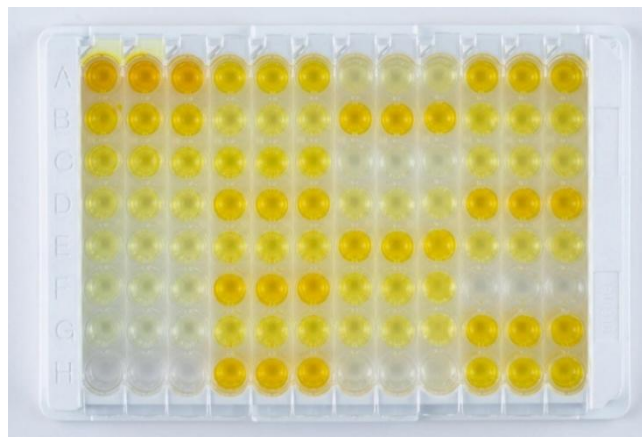


Figure n°10- ELISA test: change in the intensity of the yellow color following the third incubation with the chromogenic substrate

Absorbance measurements for each sample were performed using a BioTek ELx800 microplate reader (BioTek Instruments Inc., Winooski, VT, USA; Agilent Technologies).

The instrument is an automated system designed for ELISA assays, with a spectrophotometric range of 400-750 nm. It operates on the principle of absorbance spectrophotometry based on the Beer-Lambert law, measuring the intensity of transmitted light through the sample to determine optical density (OD). The optical system employs a tungsten halogen lamp as the light source, ensuring high measurement accuracy (<1% at 2.0 OD), which is crucial for reproducible photometric readings.

The instrument has a microplate carrier that transports plates into a dark chamber, ensuring isolation of wells and minimizing interference from ambient light. It is also capable of orbital shaking to prevent bubble formation in the wells, which could interfere with absorbance measurements.

Protocol selection and wavelength settings are controlled via a lateral panel equipped with a keypad and display. In this study, a wavelength of 405 nm was used, as specified in the assay protocol.



Figure n°11 - BioTek ELx800 microplate reader

Spectrophotometric readings were processed automatically by the microplate reader, which corrected the optical density (OD) values by subtracting the absorbance of the serum-free blank from all measured samples.

The test was considered valid only if the results met the criteria set out in the method guidelines:

- Absorbance (A) Positive Control (C+): > 1.200
- Absorbance (A) Cut-off: 18% of C+
- Absorbance (A) Negative Control: < 10% of C+
- Absorbance (A) Serum-free blank: < 0.350

Sample results were interpreted by calculating an index value, defined as the ratio between the absorbance of the sample and the absorbance of the cut-off serum:

$$Index = \frac{\text{Absorbance of the sample}}{\text{Absorbance of cut - off serum}}$$

The calculated index values were interpreted as follows:

- Negative (Index < 1.0): indicates the absence of a diagnostically significant concentration of anti-*Strongyloides stercoralis* IgG antibodies
- Positive (Index > 1.0): indicates a diagnostically significant concentration of anti-*Strongyloides stercoralis* IgG antibodies, suggesting previous or current exposure to the parasite
- Borderline: defined as the ‘grey area’ where values are close to the positivity threshold. The protocol recommends repeating the test after 2-4 weeks using a new blood sample.

3. Immunoblotting for the detection of anti-*Toxocara canis* IgG antibodies

TOXOCARA Western Blot IgG kit (LDBIO Diagnostics, Lyon, France) is a qualitative immunoblot assay designed for the detection of IgG in the serum. The assay is based on ready-to-use numbered nitrocellulose strips pre-coated with *Toxocara canis* antigens separated according to their molecular weight. Each strip is intended for the analysis of a single serum sample. The kit used in this study contained reagents for 24 in vitro diagnostic tests.

All analyses were performed according to the manufacturer's instructions. For diagnostic evaluation purposes, the assay was carried out in a semi-automated mode.

The ready-to-use nitrocellulose strips included in the kit are coated with excretory-secretory (ES) antigens of *Toxocara canis*.

Each serum sample is incubated with a separate nitrocellulose strip to allow the formation of specific antigen–antibody complexes. The presence of any specific anti-*Toxocara canis* IgG in the serum sample leads to the formation of an antigen-antibody complex.

This complex is detected by adding a secondary antibody, consisting of alkaline phosphatase-conjugated anti-human IgG antibodies. Following the addition of the chromogenic substrate, specific antigen-antibody reactions can be visualized as purple transverse bands on the strip.



Figure n°12 - TOXOCARA Western Blot IgG kit

The instrument used to carry out the semi-automated diagnostic testing for *Toxocara canis* is the Dynablot Plus (Mikrogen Diagnostik, Neuried, Germany), developed by Dynex.

The instrument is designed for the processing of strip-based immunoassays, including immunoblots and western blots, and automates the incubation and washing steps of the protocol, thereby reducing the need for manual intervention and increasing the accuracy of the results.

Its main feature is ‘walk-away’ automation, whereby, once the samples and reagents have been loaded onto the instrument, the automatic protocol is initiated. Dispensing is carried out via seven precision capillaries, which ensure high reproducibility, limiting systematic error to less than 10 per cent and minimizing the risk of contamination.

It has a processing capacity of up to 44 strips simultaneously per cycle. The workstation comprises a tilting platform known as a ‘rocking shaker’.



Figure n°13 - Dynablot Plus - Dynablot Plus Strip processor – Mikrogen

Followed procedure:

- All reagents from the TOXOCARA Western Blot IgG kit were equilibrated to room temperature prior to use. The Washing solution was diluted 1/10 with distilled water according to the manufacturer's instructions.

- Ready-to-use nitrocellulose strips were removed from the card support and carefully cut using sterile forceps, handling them by the numbered end to avoid damage to the antigen-coated membrane.
- The incubation tray was placed on the oscillating platform of the Dynablot Plus. 1mL of Serum Dilution Buffer was dispensed into each channel, and the strips were positioned in the corresponding wells with the numbered side facing upward. The tray was gently agitated for approximately 2 minutes to ensure complete rehydration of the strips.
- Subsequently, 10 µL of serum samples and positive controls were added to the appropriate wells containing the strips.
- The Dynablot Plus instrument was then operated according to the predefined immunoblot program. All reagents, including diluted washing solution, serum diluent buffer, anti-human IgG conjugate, and substrate, were automatically aspirated from the kit vials via a capillary system.
- After automated processing, strips were removed using forceps, placed on paper, and air-dried. During drying, signal intensity decreased as expected.
- Results were interpreted only after complete drying of the strips. Strips were aligned and visually compared with the positive control according to the manufacturer's interpretation criteria.

According to the manufacturer's interpretation criteria, the simultaneous presence of two bands within the 24-35 kDa molecular weight range was considered indicative of the presence of specific anti-*Toxocara canis* antibodies. Additional reactive bands may be observed in the 70-90 kDa and 100-200 kDa ranges; however, these are not considered specific for the

diagnosis of toxocariasis, as they may result from cross-reactions with other helminth infections.

The banding pattern of each test strip from the selected subjects was compared with that of the positive control, paying particular attention to the position and intensity of the bands. The positive control serves as a reference for the correct occurrence of enzymatic reactions and for the identification of specific bands in the selected samples, enabling the correct interpretation of the transverse bands on the test strips.

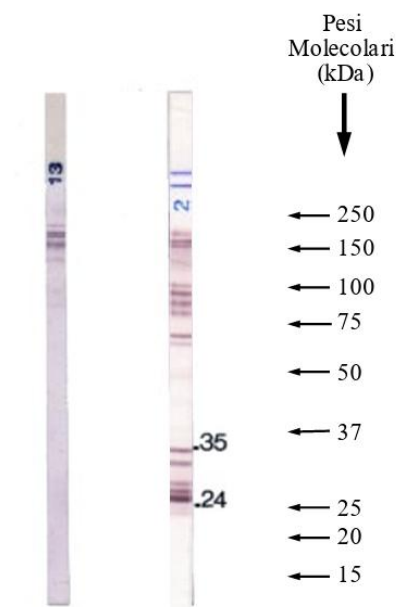


Figure n°14 - examples of negative (left) and positive (right) results

RESULTS

1. Experimental results

This chapter presents and discusses the data obtained from the serological diagnostic activity carried out at the Microbiology and Virology Laboratory of the Azienda Ospedaliero-Universitaria Maggiore della Carità of Novara.

The primary aim of this study was to evaluate the seroprevalence and diagnostic accuracy of toxocariasis and strongyloidiasis, two parasitic infections caused by the nematodes *Toxocara canis* and *Strongyloides stercoralis*, respectively. The investigation was conducted on 66 serum samples in total, equally divided to ensure a statistically balanced comparison between two specific study cohorts. The group of farmers, selected as an occupationally vulnerable population with potential exposure to infection, consisted of 33 samples. The remaining 33 samples constituted the control group, representing the general population.

FARMER'S GROUP		
N°	<i>Toxocara</i>	<i>Strongyloides</i>
N002	NEG	NEG
N006	POS	NEG
N021	NEG	POS
N037	POS	NEG
N041	NEG	NEG
N045	POS	NEG
N046	POS	NEG
N047	POS	NEG
N048	POS	NEG
N049	POS	POS
N050	NEG	NEG
N051	NEG	NEG

CONTROL GROUP		
N°	<i>Toxocara</i>	<i>Strongyloides</i>
N001	NEG	NEG
N003	POS	NEG
N005	NEG	NEG
N007	NEG	NEG
N008	NEG	NEG
N009	NEG	NEG
N010	POS	NEG
N011	NEG	NEG
N013	NEG	NEG
N014	NEG	NEG
N015	NEG	NEG
N016	NEG	NEG

N066	NEG	NEG	N017	NEG	NEG
N068	NEG	NEG	N022	NEG	NEG
N069	NEG	NEG	N023	NEG	NEG
N070	POS	NEG	N024	NEG	NEG
N072	NEG	NEG	N025	NEG	NEG
N077	NEG	NEG	N027	NEG	NEG
N078	NEG	NEG	N028	NEG	NEG
N079	POS	NEG	N030	NEG	NEG
N080	NEG	NEG	N031	NEG	NEG
N082	POS	NEG	N032	NEG	NEG
N086	NEG	NEG	N033	NEG	NEG
N088	NEG	POS	N035	NEG	NEG
N091	NEG	NEG	N036	NEG	NEG
N092	NEG	NEG	N042	NEG	NEG
N093	POS	NEG	N081	NEG	NEG
N095	NEG	NEG	N083	NEG	NEG
N097	NEG	NEG	N084	NEG	NEG
N099	NEG	NEG	N085	NEG	NEG
N100	NEG	NEG	N089	NEG	NEG
N101	NEG	NEG	N098	NEG	NEG
N108	NEG	NEG	N109	NEG	NEG

Table n°1-Seroprevalance results of *Toxocara canis* and *Strongyloides stercoralis*

Immunoblot analysis was performed to detect specific anti-*Toxocara canis* antibodies. The results showed a markedly higher seroprevalence among farmers than among the control group:

- Farmers' group: 11 of the 33 farmers samples tested positive, corresponding to a seroprevalence of 33.3%.
- Control group: 2 of the 33 subjects samples were positive, corresponding to a seroprevalence of 6.0%.

Strongyloides stercoralis antibodies were detected by the ELISA assay and the results showed a lower seropositivity than the one observed for *Toxocara*

infection. Nevertheless, positive cases were almost exclusively confined to the occupationally exposed group:

- Farmers' group: 3 of the 33 subjects tested positive, corresponding to a seroprevalence of 9.1%.
- Control group: No positive cases were detected (0/33). This indicates the absence of evidence of *S. stercoralis* infection among non-agricultural individuals in this study population.

Cross-analysis of the serological results identified a single case of co-infection. The subject identified by the code N049, belonging to the farmers' group, tested positive for both *Toxocara canis* and *Strongyloides stercoralis*.

	<i>Toxocara canis</i>		<i>Strongyloides stercoralis</i>	
	Farmers	Controls	Farmers	Controls
Positive	11	2	3	0
Negative	22	31	30	33
Seroprevalence	33.3%	6.0%	9.1%	0%

2. Statistical analysis

Statistical analysis of the results, using chi-square test, revealed significant differences in positivity rates between the two groups. With regard to *Toxocara canis*, a p-value of <0.05 was observed (p=0.016; $\chi^2= 5.8022$), highlighting a significant association between occupational exposure and *Toxocara canis* seropositivity.

Although *Strongyloides stercoralis* seropositivity was registered only in farmer's group, the difference was not statistically significant, with a p-value >0.05 (p=0.076; $\chi^2= 3.1429$).

DISCUSSION

This study investigated the seroepidemiology of toxocariasis and strongyloidiasis in a selected population from Eastern Piedmont. Although both infections are classified as Neglected Tropical Diseases (NTDs), the findings confirm that they remain relevant even in non-tropical, industrialized countries such as Italy.

The results provide insight into the seroprevalence of *Toxocara canis* and *Strongyloides stercoralis* among agricultural workers and allow comparison with the current epidemiological situation in Italy.

In particular, the study conducted by Nicoletti et al. (2019) in Catania reported a seroprevalence of 8% for *Toxocara canis* in a cohort of 287 adults. The seroprevalence observed in the control group of the present study (6%) was comparable to that reported by Nicoletti et al., suggesting that it may reflect the background prevalence of *Toxocara* infection in the non-occupationally exposed population. The difference is even more pronounced when compared with historical studies conducted in Italy, which reported seroprevalence rates of 1.6% in Central Italy and 4.0% in Northern Italy.²²

According to the study by Buonfrate et al. (2013-2014), the seroprevalence of strongyloidiasis among native Italians with eosinophilia is approximately 8%, whereas it decreases to about 1% in those without eosinophilia. The 9,1% seroprevalence observed in the present study is comparable to that reported for high-risk clinical groups in Italy. Scientific literature has shown that, in Italy, *Strongyloides stercoralis* infection is frequently associated with individuals born before the 1950s who lived in rural areas during the period when the infection was endemic.¹⁵

Serological analysis highlighted a significantly higher prevalence of *Toxocara canis* antibodies among farmers than among the control group. The seroprevalence was 33.3% (11 positive out of 33) in farmers and 6% (2 positive out of 33) in controls, representing a difference that was statistically significant ($p=0.016$).

These findings support the initial hypothesis that prolonged contact with soil and soil-handling activities, which are characteristic of agricultural work, can be important risk factors for exposure to embryonated *Toxocara* eggs.

Moreover, the seroprevalence observed in the control group (6%) suggests that environmental exposure to *Toxocara* is not negligible in the general population. This may be explained by the widespread environmental contamination caused by infected dogs and cats. The presence of feces from domestic and stray animals in public parks, gardens, and playgrounds can contaminate the soil with *Toxocara* eggs, extending the risk of human exposure well beyond the agricultural setting.

This study identified only 3 cases of farmers positive for anti-*Strongyloides stercoralis* IgG antibodies, corresponding to a seroprevalence of 9.1% (3 positive out of 33), whereas no seropositive cases (0 out of 33) were identified in the control group.

Even in that case the specificity of the findings in the occupationally exposed group, whilst not reaching a statistically significant level ($p=0.076$), suggests a correlation with the rural working environment, where *Strongyloides* larvae can penetrate intact skin through contact with contaminated soil.

The patient N049, who tested positive for both *Toxocara canis* and *Strongyloides stercoralis* was of particular interest. This single case suggests

that individuals working in agricultural environments may be exposed to multiple soil-transmitted parasites.

This patient is elderly, suggesting that, in the 1940s and 1950s, soil contamination from sewage and faeces was considerably more common than today, facilitating the persistence and transmission of soil-transmitted helminths. At that time, the prevalence of *Strongyloides stercoralis* in Italy - particularly in the rural and rice-growing areas of northern Italy - was very high.¹⁵

This case highlights how each patient's medical and occupational history is a key factor in order to interpret serological results, confirming that prolonged exposure to a rural environment increases the likelihood of infection.

One limitation of this study is the small number of samples, which limited the statistical power to detect significant differences in the seroprevalence of *Strongyloides stercoralis*. For this reason, it would be useful to continue this investigation by increasing the number of subjects recruited in order to assess the epidemiological prevalence of the parasite.

Although the sample size was sufficient to demonstrate a statistically significant difference in the seroprevalence of *Toxocara canis*, a larger study population would further strengthen and consolidate the findings obtained.

Another limitation is the restricted geographical scope of the study, which was conducted exclusively in the agricultural area of Eastern Piedmont. Consequently, the observed seroprevalence rates may not be representative of other regions of Italy. For this reason, future multicentre studies involving different geographical areas and accounting for agricultural and environmental

factors are needed to provide a more comprehensive understanding of the epidemiology of *Toxocara canis* and *Strongyloides stercoralis* in Italy.

Based on these findings, greater awareness of parasitic zoonoses among healthcare professionals and agricultural workers appears warranted. Encouraging good hygiene practices and regular deworming of domestic animals may contribute to reduce the risk of transmission. As these infections often proceed asymptotically or with non-specific symptoms, serological monitoring is recommended as the diagnostic tool for identifying positive cases. Future implementation of species-specific diagnostic assays will allow a more accurate assessment of the burden of *Toxocara canis* and *Strongyloides stercoralis* infections in our region, thereby supporting improved surveillance, targeted prevention strategies, and appropriate treatment approaches.

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