



# Ultrasonography for early diagnosis of *Toxocara canis* infection in puppies

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## Abstract

*Toxocara canis* is one of the most common intestinal parasites in dogs and represents a highly infectious zoonotic parasite worldwide. Adult worms live in the bowel of dogs, and infections in puppies are commonly acquired transplacentally. The biology of the parasite and the commonly used diagnostic method, based on faecal examination, often prevent an early diagnosis of toxocariasis in puppies. Ultrasonographic diagnosis of intestinal ascariasis could be a feasible alternative method to diagnose *T. canis* infection in puppies during the prepatent period. The present study evaluated the diagnostic accuracy of ultrasonography for the diagnosis of intestinal toxocariasis in new-born puppies during the prepatent period. Fifty-four new-born puppies were prospectively recruited in the study. Each dog underwent intestinal ultrasonography and copro-microscopic examination every 5 days, starting from the 10th day post-birth. Intestinal ultrasonography visualized adult *T. canis* nematodes in the puppies' small intestine from the 10th day post-birth. On the 15th day after birth, ultrasonography showed 100% specificity and 85.4% sensitivity in diagnosing *T. canis* infection, despite negative results deriving from the copro-microscopic examination performed at the same time point. Our results showed that ultrasonography can be used as test for early diagnosis of *T. canis* infection in new-born puppies during the prepatent period. Early ultrasonographic diagnosis of *T. canis* infection in puppies could help control the disease in dogs and reduce the zoonotic risk for the human population.

**Keywords** Intestine · Ultrasound · Toxocariasis · Dog · Roundworms

## Introduction

*Toxocara canis* (*T. canis*) is considered one of the most common intestinal helminth in dogs worldwide (Parsons 1987; Overgaauw 1997a; Riggio et al. 2013; Szwabe and Błaszczowska 2017; Wright et al. 2016; Joffe et al. 2011; Barutzki and Sohaper 2003; Overgaauw 1997b; Gillespie et al. 1991; Lassen and Jokelainen 2016). This nematode is also an important zoonotic pathogen, as human toxocariasis can manifest in various clinical syndromes known as visceral

larva migrans, ocular larva migrans, neurotoxocariasis, and covert toxocariasis (Ma et al. 2018).

Adults worms live in the intestine of dogs from where they release a large number of non-infectious eggs into the environment and where they develop to an infectious stage and may remain infective for years (Parsons 1987; Overgaauw 1997a; Jacobs, et al. 1977). The prevalence of patent *T. canis* infection is usually higher in puppies than in adult dogs (Overgaauw 1997a; Jacobs, et al. 1977). A survey in Sardinia showed an overall prevalence for *T. canis* of 11% in client owned dogs and a prevalence of 20% in puppies younger than 6 months (Tamponi et al. 2017). The clinical manifestations depend on the age of the animal and on the number, location and stage of development of the worms. Neonatal puppies can get pneumonia secondary to larval pulmonary migration; however, they usually show gastrointestinal symptoms such as diarrhoea, emesis, stunted growth, abdominal distension, constipation and, in severe cases, bowel obstruction (Epe 2009).

The vertical larval transmission is considered the most important transmission route of *T. canis* in puppies (Burke and

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Roberson 1985). Transplacental infection is well documented and occurs from the 42nd day of gestation by the migration of somatic larvae from the pregnant bitch to the foetus (Scothorn, et al. 1965; Lloyd, et al. 1983). Patency of transplacental infection develops from the 21st day postpartum (Parsons 1987; Yutuc 1954). Neonatal puppies can also become infected by transmammary transmission of larvae from the lactating bitch (Lloyd, et al. 1983; Stone and Girardeau 1967).

Faecal examination is considered the gold standard test for the diagnosis of *T. canis* infection in dogs (Parsons 1987; European Scientific Counsel Companion Animal Parasites 2017). Faecal examination is usually performed by flotation techniques using a solution with a high specific gravity (1.2–1.3) and the following identification of the characteristic eggs. Such techniques are sensitive for diagnosis in young animals, due to the shedding of large numbers of eggs, but sensitivity may be lower in older animals which shed eggs intermittently and/or in low numbers (Macpherson 2013).

Considering the biology of the parasite and the commonly used diagnostic methods, an early diagnosis of *T. canis* infection in puppies is not always possible. For this reason, and also to reduce environmental contamination, the early treatment of puppies, even without a specific diagnosis, is recommended by CAPC (Companion Animal Parasite Council 2014) and ESCCAP (European Scientific Counsel Companion Animal Parasites 2017). On the other hand, other institutions such as the EMA (European Medicines Agency 2017) recommend owners and practitioners treat animals only after a full diagnosis, as the indiscriminate use of anthelmintics could increase the possibility of anthelmintic resistance onset (Traversa 2012).

Alternative methods for the diagnosis of endoparasites have been used for other animal species (Varcasia et al. 2011; Dore et al. 2014); it would thus be interesting to study the feasibility of new tools, such as ultrasonography, for the early diagnosis and management of *T. canis* infection in puppies, in order to reduce the environmental contamination and zoonotic risk.

The ultrasonographic appearance of intestinal roundworms has already been described in dogs (Wigger, et al. 2007), cats (Wigger, et al. 2007), foals (Nielsen et al. 2016) and humans (Peck 1990; Kara 2013). In longitudinal section, individual worm appeared as a non-shadowing “train track” structure, characterized by two parallel hyperechoic lines separated by a narrow inner hypoechoic zone (Hoffmann et al. 1997; Wigger, et al. 2007; Nielsen et al. 2016). In transverse section, the worm had a small doughnut-like appearance with a circular hyperechoic non-shadowing wall and a hypoechoic centre (Mahmood et al. 2001). Despite this, to date, no studies have been carried out to prospectively evaluate the accuracy of ultrasonography for the early diagnosis of toxocariasis in puppies.

The aim of the present study was therefore to evaluate the diagnostic accuracy of ultrasonography for the diagnosis of intestinal *T. canis* infection in new-born puppies during the prepatent period.

## Materials and methods

A prospective observational study was carried out in the Municipality of Sassari (Sardinia, Italy) from January 2018 to June 2018. The study protocol was approved by the Ethical Committee of the University of Sassari (Prot. N. 2218 of 15/01/2018), and all dog owners signed an informed consent form before the enrolment of their animals. In order to reduce the stress induced by maternal and littermate separation, the intestinal ultrasonography (IUS) scan lasted a maximum of 5 min. Examinations were performed in the environment of the puppies, either at the owner’s home or at the breeder’s kennels. Puppies born from bitches that had not received any anthelmintic treatment in the previous 3 months before parturition were enrolled in the study. New-born puppies were submitted to physical examination, IUS and copro-microscopic examination (CE) every 5 days, starting from the 10th day post-birth (PB). Fresh faeces were collected from each dog within 12 h before, during the exam or within 12 h after the IUS examination. Copro-microscopic examination was performed using the Wisconsin technique as described elsewhere (Tamponi et al. 2017). Briefly, individual faecal samples were examined with centrifugation/flotation (626 g for 10 min) using a zinc sulphate ( $ZnSO_4$ ) flotation solution (specific gravity 1.2). Samples were considered positive when at least one *T. canis* egg was found. Copro-microscopic exams were performed by an experienced operator (CT) unaware of the results of the IUS tests. When puppies scored positive at the CE test, they were excluded from the study and underwent appropriate pharmacological treatment. In the case of negative CE results, both tests, IUS and CE, were repeated until day 40 PB.

All IUS examinations were performed by a single experienced operator (AC) with a single portable ultrasound unit (My Lab Alpha, Esaote, Florence, Italy) equipped with a multi-frequency (3–13 MHz) linear transducer (SL1543, Esaote, Florence, Italy). During the scan, the puppies were gently restrained by a student and positioned in dorsal or left lateral recumbence. The IUS test was defined as positive if at least one of the “train track” structures, characterized by two parallel hyperechoic lines separated by a narrow inner hypoechoic zone, was visualized in the small bowel lumen. The level of infestation was defined as mild if one or two parasite sections were visualized in only one intestinal section, moderate if one or two parasite sections were recognized in two or more intestinal sections along the small bowel and severe if at least one small intestine section contained three or more parasite sections.

## Statistical analysis

Statistical analysis was performed using Stata 14, (StataCorp, College Station, TX) and Epi-Info® 6.0 (CDC/WHO, Atlanta,

GA, USA). Categorical data were summarized as frequencies and proportions. Differences in the location of the parasites within the small intestine and the degree of infestation observed by IUS, at each time point, were assessed by the chi-square test, and values of  $P < 0.05$  were considered significant. Parasite thicknesses at each time point were summarized as mean and standard deviation (SD) or as median and interquartile ranges, depending on their parametric distribution. The IUS results performed at each instance were related to the CE results obtained throughout the study. Puppies were thus classified as true positive (TP), true negative (TN), false positive (FP) or false negative (FN). The accuracy of IUS as a test for early diagnosis of ascariasis in puppies was evaluated by calculating the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the method at day 10, 15 and 20 PB.

## Results

Fifty-four puppies, 24 males and 30 females, from nine different litters, were included in the study. Twenty-one belonged to private breeders, 14 to public kennels and 19 were client-owned puppies. The breeds included were German Shepherd ( $n = 16$ ), Cavalier King Charles Spaniel ( $n = 7$ ), Dachshund ( $n = 7$ ), Boxer ( $n = 7$ ), Crossbreed ( $n = 7$ ), Pomeranian ( $n = 6$ ) and German Shorthaired Pointer ( $n = 4$ ). Anamnestic data and physical examination did not show symptoms or clinical signs related to *T. canis* infection during the study period.

### CE results

An overall prevalence of 76% (41 out of 54 puppies) for *T. canis* infection was found in the examined animals (95% CI 62–86.5%). Among them, 29.7% (16 out of 54) tested positive to *T. canis* on day 20 PB and 46.3% (25 out of 54) on day 25 PB (Table 1). No puppy tested positive on day 10 PB or on day 15 PB (Table 1). Thirteen puppies out of 54 (24%) tested negative for *T. canis* infection at each time point, up to day 40 PB (Table 1). No other nematodes were detected in the faeces of the examined puppies. A total of 54 puppies underwent the CE test on days 10, 15 and 20 PB, 38 on day 25 PB and 13 on days 30, 35 and 40 PB (Table 1) because, when they scored positive at CE, they were excluded from the study and underwent appropriate pharmacological treatment. The 41 positive puppies came from six litters out of nine (67%). The negative puppies (six Pomeranian and seven Cavalier King Charles Spaniel) came from three litters (33%) raised by the same private breeder. No significant difference related to infection was observed between different breeds examined.

### IUS results

Intestinal ultrasonography was technically feasible for all puppies within the established time limits. A total of 239 IUS examinations were performed during the study period. In all the examinations, we obtained a good-to excellent quality of bowel images (Fig. 1). Overall, 42.7% of IUS tests (101 out of 239) tested positive to the presence of roundworms in the small intestinal lumen.

A total of 54 puppies underwent the IUS test on days 10, 15 and 20 PB, 28 on day 25 PB and 13 on days 30, 35 and 40 PB (Table 1). Overall, 68.5% of puppies (37 out of 54) tested positive and 31.5% (17 out of 54) tested negative to roundworm infection at the IUS test. Among them 7.4% (4 out of 54) tested positive at day 10 PB, 64.8% (35 out of 54) at day 15 PB and 68.5% (37 out of 54) at day 20 PB (Table 1).

### Infestation grade

At day 10 PB, the infestation grade was considered mild in all four puppies. At day 15 PB, it was classified as mild in 11.4% (4 out of 35), moderate in 85.7% (30 out of 35) and severe in only 1 puppy out of 35 (2.9%) (Fig. 2). At day 20 PB, the infestation grade was considered mild in 1 puppy out of 37 (2.7%), moderate in 64.9% of puppies (24 out of 37) and severe in 32.4% (12 out of 37) (Fig. 3). At day 25 PB, it was classified as moderate in 28% (7 out of 25) and severe in 72% of puppies (18 out of 25) (Fig. 4). Results concerning the infestation grade are summarized in Table 2.

### Parasite location

At day 10 PB, parasite location was the duodenum in 50% (2 out of 4), jejunum in 25% (1 out of 4) and both intestinal tracts in the remaining 25% of puppies (1 out of 4). At day 15 PB, it was the duodenum in 8.6% (3 out of 35), jejunum in 11.4% (4 out of 35) and both intestinal tracts in 80% of puppies (28 out of 35). At 20 days PB, parasites were localized in the duodenum in 8.1% (3 out of 37), jejunum in 21.6% (8 out of 37) and both intestinal tracts in 70.3% of puppies (26 out of 37). At day 25 PB, parasite location was the jejunum in 24% of puppies (6 out of 25) and both the duodenum and jejunum in 76% (19 out of 25). Results concerning the parasite location are summarized in Table 3.

### Parasite thickness

Parasite median thickness at 10 days PB was 0.73 mm (range of 0.7–0.92 mm). At 15 days PB, it was 0.84 mm (range 0.7–1.1 mm). Mean parasite thickness at 20 days PB was 1.05 mm (SD 0.14 mm, range 0.74–1.4 mm). At 25 days PB, mean thickness was 1.26 mm (SD 0.51 mm, range 1.2–1.3 mm).

**Table 1** Coprological examination and intestinal ultrasonography results at each time point

Day PB	Puppies examined <sup>a</sup>	Positives <sup>b</sup> CE, % (n)	Negatives <sup>b</sup> CE, % (n)	Positives <sup>b</sup> IUS, % (n)	Negatives <sup>b</sup> IUS, % (n)
10th	54	0	100 (54)	7.4 (4)	92.6 (50)
15th	54	0	100 (54)	64.8 (35)	35.2 (19)
20th	54	29.7 (16)	70.3 (38)	68.5 (37)	31.5 (17)
25th	38	46.3 (25)	24 (13)	46.3 (25)	24 (13)
30th	13	0	24 (13)	0	24 (13)
35th	13	0	24 (13)	0	24 (13)
40th	13	0	24 (13)	0	24 (13)

Day PB day post-birth, n number of puppies, CE coprological examination test, IUS intestinal ultrasonography test

<sup>a</sup> Puppies examined at each time point

<sup>b</sup> Percentages were calculated on the overall sample (n = 54)

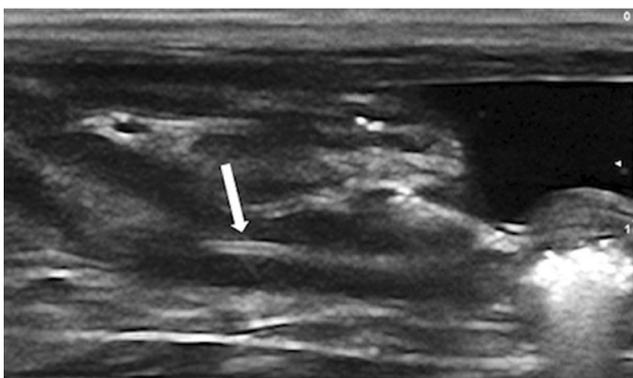
### Diagnostic accuracy of intestinal ultrasonography

Table 4 summarizes the number of TP, FP, FN and TN together with the results of the sensitivity, specificity, PPV and NPV with their respective 95% CI, calculated on the overall study period and also on days 10, 15 and 20 PB. Overall, the sensitivity, specificity, PPV and NPV of IUS for the diagnosis of intestinal roundworms infection were 90.2%, 100%, 100% and 76.5% respectively. The specificity and the PPV of IUS in detecting roundworm infection in puppies were 100% at each time point considered. At day 15 PB, no puppy tested positive to CE, whereas 35 puppies (65%) tested positive to IUS. Intestinal ultrasonography, performed on day 15 PB, demonstrated a sensitivity, specificity, PPV and NPV of 85.4%, 100%, 100% and 68.4% respectively. Results concerning the diagnostic accuracy of IUS are summarized in Table 4.

### Discussion

To the best of our knowledge, this is the first survey that evaluates the accuracy of ultrasonography for the early

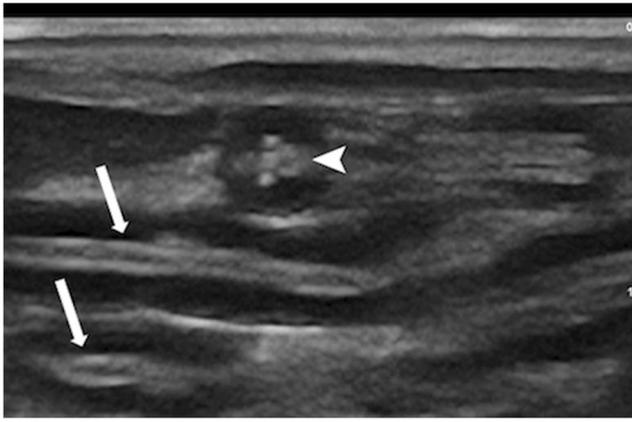
diagnosis of toxocariasis in puppies. Our results showed that IUS could be a valuable test for the early diagnosis of *T. canis* infection in new-born puppies during the prepatent period. Intestinal ultrasonography in puppies is easy to perform and can reliably detect adult roundworms given their ultrasonographic appearance (Hoffmann et al. 1997; Wigger, et al. 2007; Nielsen et al. 2016) characterized by two parallel hyperechoic lines, which represent the cuticle, separated by a narrow inner hypoechoic zone, which is the alimentary canal of the parasite (Mahmood et al. 2001). Because of their dimensions, adult *T. canis* worms can be easily recognized by ultrasonography; in fact, adult males and females measure up to 11 cm and 20 cm in length and up to 2.5 mm and 3 mm in width, respectively (Parsons 1987). Interestingly, the results of our study showed that IUS was able to visualize adult *T. canis* nematodes in the puppies' small intestine even from day 10 and also at days 15 and 20 PB, when the CE test was negative. The most important result of the present study is that IUS had a 100% specificity and 85.4% sensitivity in detecting adult *T. canis* worms on the 15th day PB. On the 20th day after birth, the sensitivity of the IUS increased up to 90% although at this time, 39% of the total positive puppies (16 out of 41) also



**Fig. 1** Ultrasongraphic image of the longitudinal section of a *T. canis* parasite (arrow) in the small bowel of a puppy on day 10 post-birth



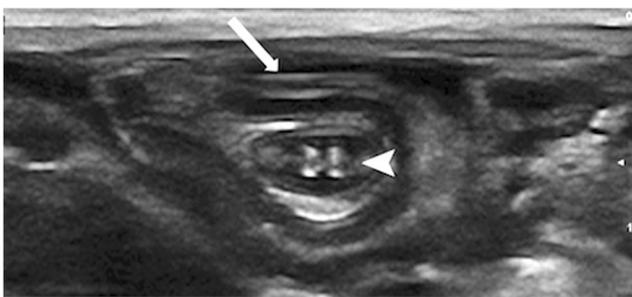
**Fig. 2** Ultrasongraphic image of a longitudinal section of a *T. canis* parasite (arrow) in the small bowel of a puppy on day 15 post-birth



**Fig. 3** Ultrasonographic image of longitudinal (arrow) and transversals (arrowhead) sections of *T. canis* parasites in the small bowel of a puppy on day 20 post-birth

tested positive at CE. In our opinion, the falsely negative puppies detected by ultrasonography on days 15 and 20 PB (six and four respectively) were due to the presence of gas and ingesta in the gastrointestinal tract which made it difficult to visualize the parasites. In fact, the IUS exam was performed often few minutes after feeding, thus increasing the presence of ultrasonographic artefacts. On the 10th PB, the sensitivity of the IUS was very low (9.8%), making the method inaccurate to rule out the presence of *T. canis* infection at this time point.

Our findings showed that the parasite thickness and infestation grade observed by IUS increased from the 10th to the 25th day PB probably because of the parasite's growth from larval to adult stages, which made them more easily recognizable in the ultrasound images. The mean adults thickness of *T. canis* previously reported (Parsons 1987) is considerably higher (2.5 mm males and 3 mm females) than that measured by ultrasound in the present study (range 1.2–1.3 mm). This difference could be explained by the following reasons: firstly, since the coexistence of parasites in different stages of development is possible (Parsons 1987), it appears probable that



**Fig. 4** Ultrasonographic image of longitudinal (arrow) and transversals (arrowhead) sections of *T. canis* parasites in the small bowel of a puppy on day 25 post-birth

that during the ultrasound examination, pre-adult parasites were measured instead of adults.

Secondly, the presence of gas and ingesta in the gastrointestinal tract could impair the correct imaging of parasites thickness. Thirdly, because parasite thickness was often measured from longitudinal section, the diameter may have been underestimated when the ultrasound beam went through the worm body in an off-centred plane. Finally, since the outermost layer of the nematode cuticle is composed by lipids and glycoproteins (Page and Johnstone 2007), it may have not been displayed by ultrasonography.

As previously described (Parsons 1987), intestinal ultrasonography revealed that the most common parasite location was the small bowel, particularly the duodenum and the jejunum. In fact, the simultaneous presence of parasites in both intestinal tracts was the most common finding at each time point considered.

According to several authors, patency of transplacental infection develops with the release of large amounts of eggs in the intestinal lumen by adult female worms from 16 to 40 days postpartum (Overgaauw 1997a; Parsons 1987; Yutuc 1954); in our study, 29.7% of puppies start excreting eggs at day 20 and 46.3% at day 25 postpartum. The early diagnosis and treatment of *T. canis* infection in puppies could reduce the egg's environmental contamination, the spread of infection in dogs, the negative consequences of the infection in puppies, the resistance to anthelmintic drugs and the zoonotic risk.

Puppies can be heavily infected by *T. canis* worms in utero which can cause severe illness before diagnosis is possible by faecal examination. Clinical disease caused by these parasites in puppies ranges from reduced growth to death. Symptoms of severe *T. canis* infection include diarrhoea, constipation, vomiting, coughing, nasal discharge, abdominal distension and neurological signs. Death can be due to secondary obstruction and rupture of the intestine, gallbladder, bile duct or pancreatic duct (Parsons 1987).

Humans acquire toxocariasis through the accidental ingestion of infective eggs from contaminated soil, water, raw vegetables or fruit (Ma et al. 2018). Humans can also become infected by ingesting encapsulated larvae present in raw or undercooked meat or viscera from paratenic hosts (Taira et al. 2004; Salem and Schantz 1992; Yoshikawa et al. 2008) and by direct contact with dogs (Roddie et al. 2008). Human toxocariasis varies widely within and between countries and is higher in developing countries and in socio-economically disadvantaged people (Ma et al. 2018). Human seroprevalence has been estimated at 3.8% in northern Italy (Genchi et al. 1990), 2.4% in Denmark (Stensvold et al. 2009), 6.3% in Austria (Poepl et al. 2013), 13% in Serbia

**Table 2** Infestation grade assessed by intestinal ultrasonography at each time point

Day PB	Positives IUS	Infestation grade <sup>a</sup>			P value
		Mild, % (n)	Moderate, % (n)	Severe, % (n)	
10th	4	100 (4)	0	0	nd
15th	35	11.4 (4)	85.7 (30)	2.9 (1)	< 0.0001*
20th	37	2.7 (1)	64.9 (24)	32.4 (12)	< 0.0001*
25th	25	0	28 (7)	72 (18)	0.0018*

Day PB day post-birth, IUS intestinal ultrasonography test, n number of puppies, nd not determined because of the small simple size

\*Significant difference

<sup>a</sup> Percentages were calculated on the number of puppies positives at intestinal ultrasonography test at each time point

(Gabrielli et al. 2017), 12.1% in Estonia (Lassen et al. 2016), 8% in the Netherlands (Mughini-Gras et al. 2016), 4.2% in Poland (Krotten et al. 2016), 7% in Sweden (Ljungström and van Knäpen 1989), 5% in the USA (Liu et al. 2018), 1.6% in Japan (Ma et al. 2018) and 19.6% in Malaysia (Hakim, et al. 1993). The wide geographical distribution and potential association of toxocariasis with allergic (Li et al. 2014; Pinelli and Aranzamendi 2012) and neurological disorders have raised considerable public concern (Deshayes, et al. 2016; Fan et al. 2015). In order to reduce the prevalence

of the disease, the negative consequences of the infection in dogs and the zoonotic risk, ESCCAP recommendations (European Scientific Counsel Companion Animal Parasites 2017) state that puppies should be treated with appropriate anthelmintics from the 14th day PB and every 15 days up to 2 weeks after weaning and then with monthly treatment until 6 months of age. The frequent use of anthelmintics in companion animals could promote the possible onset of drug resistance; even to date, only incomplete efficacy of Pyrantel against *T. canis* and hookworms has been observed (Riggio et al. 2013). The

**Table 3** Parasite location assessed by intestinal ultrasonography

Day PB	Positives IUS	Parasite location <sup>a</sup>			P value
		Duodenum, % (n)	Jejunum, % (n)	Duodenum jejunum, % (n)	
10th	4	50 (2)	25 (1)	25 (1)	nd
15th	35	8.6 (3)	11.4 (4)	80 (28)	< 0.0001*
20th	37	8.1 (3)	21.6 (8)	70.3 (26)	< 0.0001*
25th	25	0	24 (6)	76 (19)	0.0002*

Day PB day post-birth, IUS intestinal ultrasonography test, n number of puppies, nd not determined because of the small simple size

\*Significant difference

<sup>a</sup> Percentages were calculated on the number of puppies positives at intestinal ultrasonography test at each time point

**Table 4** Diagnostic performance of intestinal ultrasonography for the diagnosis of *Toxocara canis* infection in puppies

Day PB	TP	FN	FP	TN	Sensitivity % (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)
10th	4	37	0	13	9.8 (2.7–23.1)	100 (75.3–100)	100 (39.8–100)	26 (14.6–40.3)
15th	35	6	0	13	85.4 (70.8–94.4)	100 (75.3–100)	100 (90–100)	68.4 (43.4–87.4)
20th	37	4	0	13	90.2 (77–97.3)	100 (75.3–100)	100 (90.5–100)	76.5 (50.1–93.2)

Day PB day post-birth, TP true positives puppies, FN false negatives puppies, FP false positives puppies, TN true negatives puppies, PPV positive predictive value, NPV negative predictive value

early ultrasonographic diagnosis of *T. canis* infection herein described could also represent an easy and non-invasive method for monitoring toxocariasis in puppies in natural and experimental infections, also before patency. Further studies may be also in comparison with ELISA Coproantigens detection tests like that proposed by Elsemore et al. (2017) could give more information on the possibility to detect infections with single parasites or a low parasite burden by IUS.

The method described in the present study offers an excellent diagnostic accuracy, but it also has some disadvantages such as the higher cost, as an ultrasonographic exam would be more expensive than the cost for blind treatments starting from the age of 2 weeks, as recommended by ESCCAP (European Scientific Counsel Companion Animal Parasites 2017). Secondly, the need to perform the exam at the owner's home or breeder's kennel requires the use of portable ultrasound equipment and a longer time, which could further increase the cost of the examination. Finally, although in our study the dogs apparently did not undergo any stress during the procedures, the handling of animals at this age could be a stress factor for bitches and puppies.

In summary, we have described a feasible ultrasonographic method to diagnose *T. canis* infection in puppies during the prepatent period. Considering the technological progress of diagnostic ultrasonography and the diffusion of portable ultrasonographic equipment among veterinary practitioners, intestinal ultrasonography could be used as a diagnostic test for the early detection of toxocariasis in puppies from the 15th day after birth. Early ultrasonographic diagnosis of *T. canis* infection in puppies could help to control the disease in dogs and thus protect the human population.

### Compliance with ethical standards

The study protocol was approved by the Ethical Committee of the University of Sassari (Prot. N. 2218 of 15/01/2018), and all dog owners signed an informed consent form before the enrolment of their animals.

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