Journal of Equine Veterinary Science 149 (2025) 105573

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.elsevier.com/locate/jevs

Case Report

What is the "train track" in the retained equine testis?

S. Di Giorgio [a], S. Monti [a], V. Palmieri [a,*], G. Marino [a], E. Napoli [a], C. Vullo [b], G. Catone [a]

[a] Department of Veterinary Sciences, University of Messina, Viale G. Palatucci, Messina, 98168, Italy
[b] Department of ChiBioFarAm, University of Messina, Viale Ferdinando Stagno D'Alcontres, Messina, 98166, Italy

ARTICLE INFO

Keywords:
Cryptorchidism
horse
Migration
Strongylus vulgaris
Ultrasonography

ABSTRACT

A 3-year-old Quarter horse stallion with unilateral left cryptorchidism was referred for the absence of the left testis in the scrotal bursa and an enlarged right testis. The ultrasonographic examination revealed the presence of the left testis at the abdominal level. Two small hyperechogenic lines were observed running parallel and resembling a cylindrical cavitary foreign body, within a non-homogeneous area with blurred margins. The testis was removed by laparoscopy and macroscopically, the testis's cut section revealed the presence of two live adult nematodes emerging from 2 mm blood-filled tracks within the testicular parenchyma. The parasites were isolated and washed in sterile saline solution and morphologically classified as *Strongylus vulgaris*. Histologically, the tracks were characterized by a lymphoplasmacytic infiltrates with abundant haemorrhage invading the surrounding structures. *S. vulgaris* erratic migration has been reported in the retained testis of stallions, but this localization is considered uncommon; the case here reported is one of the few cases reported in the literature highlighting the rarity of *S. vulgaris* aberrant localization into the equine cryptorchid testis. This case also reports the ultrasonographic findings related to the parasite presence and track.

1. Introduction

Cryptorchidism is recognized as the most common congenital defect of sexual development in male horses, resulting from the failure of one (unilateral) or both testes (bilateral) to descend normally into the scrotum [1,2]. This condition arises due to disruptions in the normal process of testicular descent. Transinguinal migration of a testis is apparently mediated by intra-abdominal pressure, which assists in pushing the testis trough the inguinal canal. Testosterone is not obligatory for correct inguinoscrotal migration of testes. However, normally testosterone stimulates growth of the vaginal process, secretion of calcitonin gene-related peptide by the genitofemoral nerve. This peptide provides directional guidance to the gubernaculum, ultimately leading to its regression and constriction of the inguinal canal, aiding testicular descent [1].

Unilateral cryptorchidism occurs more frequently than bilateral cases, with an estimated prevalence of 9 % to 14 % [3]. Bilateral cryptorchidism, however, often results in infertility due to the absence of both testes in the scrotum, leading to impaired spermatogenesis [2]. The retained testis can be found in different positions: within the inguinal canal, abdominal cavity, or subcutaneously at the level of the inguinal ring [1]. The left abdominal retention occurring more frequently than right abdominal retention (75 % vs. 42 %) and right inguinal retention occurring more frequently than left inguinal retention (58 % vs. 25 %) [3]. The aetiology of cryptorchidism is multifactorial, involving genetic, epigenetic and environmental factors, including the pregnant mare's endogenous hormonal status [4].

The diagnosis of cryptorchidism combines the collection of amnestic data (single scrotal testis, absence of prior castration attempts, and stallion-like behaviour) with physical examination, including inguinal and rectal palpation, supplemented by ultrasound findings to locate the retained testis [5].

The cryptorchidism causes hypofertility, and cryptorchid horses often display undesirable behaviours [6] such as persistent stallion-like and/or aggressive behaviour.

Surgical removal of the retained testis is often necessary [7]. Inguinal, parainguinal or laparoscopic techniques have been described with the latter being currently considered the gold standard [8]. Standing laparoscopic is the surgical treatment of choice, in case of abdominal retention, because it avoids the costs and risks of general anaesthesia, and the hemodynamic difficulties associated with the simultaneous recumbency and pneumoperitoneum [9]. In the retained testis, testicular degeneration, tumour alteration, and torsion can occur, while nematode infestation (*Strongylus* spp. or *Setaria equina*) can cause

* Corresponding author.
E-mail address: valentina.palmieri1@studenti.unime.it (V. Palmieri).

https://doi.org/10.1016/j.jevs.2025.105573
Received 27 October 2024; Received in revised form 14 April 2025; Accepted 14 April 2025
Available online 15 April 2025

parasitic orchitis [4,10,11].

This study reports a rare case of retained testis associated with an erratic localization of *Strongylus vulgaris* in a 3-years-old Quarter horse stallion; moreover, the present case reports ultrasonographic findings associated with the presence of nematodes in the retained testis.

2. Clinical report

The horse owner was informed of the study's purposes and signed a proper informed consent form for the use of their horse's data in this study.

A 3-year-old Quarter horse stallion was referred for the absence of the left testis in the scrotal bursa and an enlarged right testis. External palpation confirmed the absence of testicular, epididymal or ductal tissues in the left hemiscrotum or next to the external inguinal ring. Diagnosis of abdominal retained testis was made through rectal palpation and transrectal ultrasonography, performed with a 7.5 MHz transrectal linear transducer, for the determination of the exact location of the retained testis. The left abdominal retained testis was located just laterally to the bladder and identified for the characteristic hyperechogenic appearance of the tunica albuginea and the presence of the central vein, measuring approximately 5 × 3 cm. The ultrasound findings were characterized by the presence of a heterogeneously hypoechogenic area (0.8 cm in diameter) surrounded by testicular tissue. In this area, two hyperechoic lines were seen running parallelly and resembling a cavitary cylindrical foreign body (Fig. 1). The right testicle was in scrotal position and the parenchyma was homogeneous in echotexture.

A standing laparoscopic cryptorchidectomy was performed. Sedation and analgesia were achieved with acepromazine (0.03 mg/kg IM), followed 20 min by detomidine (0.01 mg/kg EV) and butorphanol (0.2 mg/kg EV) and maintained by constant infusion of detomidine (0.02 mg/kg/h). The skin, subcutaneous tissue, and muscles were infiltrated with 2 % lidocaine (20 mL) in the paralumbar fossa for local analgesia. A 10 mm diameter, 20 cm long cannulas trocar and blunt obturators were introduced from the 2 cm skin incision, in the paralumbar fossa and a pneumoperitoneum was created connecting the cannula to a CO_2 insufflator to reach an intrabdominal pressure of 10-15 mmHg. A rigid, 30-degree, 10 mm diameter and 57 cm long (Storz, Tuttlingen, Germany), telescope was introduced from the cannula and connected to a video camera to search the testis. A second 2 cm skin incision was made ventral to the first to insert another 10 mm trocar cannula and infiltration with lidocaine 2 % (10 mL) was done into the mesorchium at multiple locations using a laparoscopic injection needle. Then a LigaSure Atlas® device was inserted in the second trocar and held in place to allow sealing of the testicular vessels, mesorchium and vas deferens. The gonad was grasped with laparoscopic grasping forceps and the incision through the skin was enlarged to remove the testis. The right testis was routinely castrated using a closed approach. The horse received antibiotic therapy of Procaine Benzylpenicillin + Dihydrostreptomycin (4.5 mL/100 kg of body weight 5 days SID IM; Combiotic – Huvepharma, Sofia, Bulgaria) and flunixin meglumine (1.1 mg/Kg 3 days SID EV; Meflosyl - Zoetis Italia, Italy) after surgery as postoperative treatment. No postoperative complications were reported.

Macroscopically, the retained testis, measuring 5,6 × 3,5 cm, appeared softer and smaller compared to the scrotal one. On cut surface, two mm blood-filled tracks that extended from the tunica albuginea toward the centre of the testis were observed containing two alive nematodes (Fig. 2). The parasites were gently isolated using tweezers under a stereomicroscope (Stereo Discovery-V12, Zeiss, Jena, Germany); the specimens were subsequently rinsed twice in sterile saline solution and stored in 70 % ethanol for morphological identification. In order to perform the parasites identification, the nematodes were cleared in glycerine for 24 h, mounted on temporary slides in glycerine, and examined under a light microscope (Axioskop2, Zeiss, Jena, Germany). Species identification was performed based on a morphometric key [12–14]. Briefly were collected two adult parasites brownish grey in colour, a male and a female, the male was 1.5 cm in length while the female was longer 2.2 cm. The anterior section of the parasite was robust and cylindrical, featuring distinct, coarse, and broad leaf crowns at the base, measuring 10–12 μm in width (average 11.5 ± 0.5 μm). These crowns narrowed slightly to 8–10 μm (average 8.5 ± 0.4 μm) before branching into 8–10 coarse filaments (mean 9 ± 1 filaments), each extending 35–40 μm (mean 37 ± 0.6 μm) from the tip. The buccal capsules, with an oval shape, was characterized by the presence of two ear-shaped teeth at its base [14]. The posterior end of the male featured a prominent copulatory bursa, with a triangular dorsal lobe and well-defined dorsal rays. The female genital cone was slender, with numerous small bosses distributed along its ventral and lateral surfaces. The female also had a long, slender tail measuring 600–800 μm in length (mean 650 ± 13.5 μm) from the anus to the tip of the tail. The findings were consistent with the species *Strongylus vulgaris* [14].

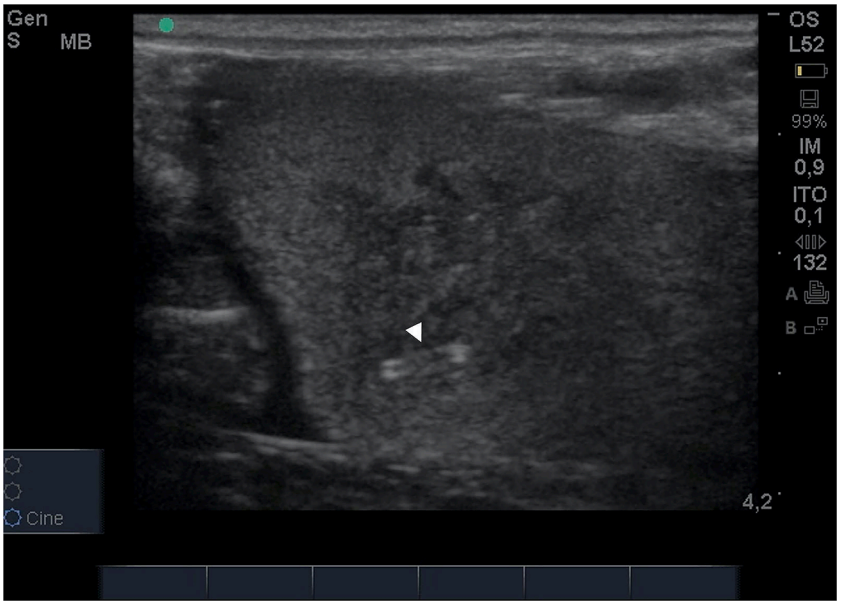

Fig. 1. The intraabdominal testes imaged with transrectal ultrasonography. Two parallel hyperechoic lines were seen in a hypo-anechoic area (arrowhead).

Fig. 2. Two parasites (*Strongylus vulgaris)* (arrowheads) and their haemorrhagic tracks in the testicular parenchyma on cut surface.

The excised testes were fixed in 10 % neutral buffered formalin for 48 h. The fixed testes were subjected to gradient alcohol dehydration: 70 %, 80 %, 95 % ethanol for 2-3 h each, xylene clearing for 4 h and immersion in paraffin wax for 4 h. Five micrometer-thick sections were cut using a microtome and mounted on glass slides. The sections were then stained with hematoxylin and eosin (H&E) for histopathological evaluation and observed with an optic microscope (Axioskop2, Zeiss, Jena, Germany).

The left testes presented chronic inflammation characterized by aggregation of inflammatory cells, predominantly lymphocytes and plasma cells and mild fibrotic areas, due to the passage of the parasite. In addition, there was an abundant haemorrhage invading surrounding structures where hypoplastic seminiferous tubules were recognizable (Figs. 3–4).

3. Discussion

The present study reported a rare case of retained testis in a horse in which the presence of erratic nematodes belonging to the species *S. vulgaris* were suspected using ultrasonography and confirmed after surgical removal.

Unilateral cryptorchidism is very common in horses [3] with the approach changing if the retained testis is inguinal or abdominal. To confirm presence or location, a deep external and transrectal palpation is generally performed associated to ultrasound [5]. Ultrasound may be useful to detect eventual complications of retained testis such as: degeneration, tumour change, torsion and parasitic orchitis.

Testicular degeneration causes generally smaller retained testis because of temperature and block of spermatogenesis [15]. The

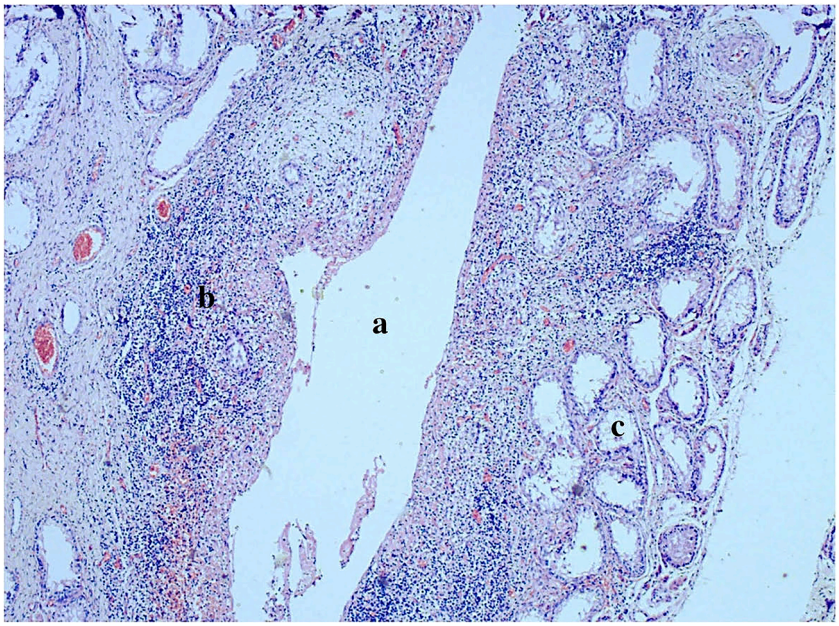

Fig. 3. Histological appearance of the track of *Strongylus vulgaris* in the equine testis. (a) The optically empty space represents the parasite track; (b) chronic lymphoplasmacellular infiltrate around the parasite track; (c) hypoplastic seminiferous tubules (H&E, 5x).

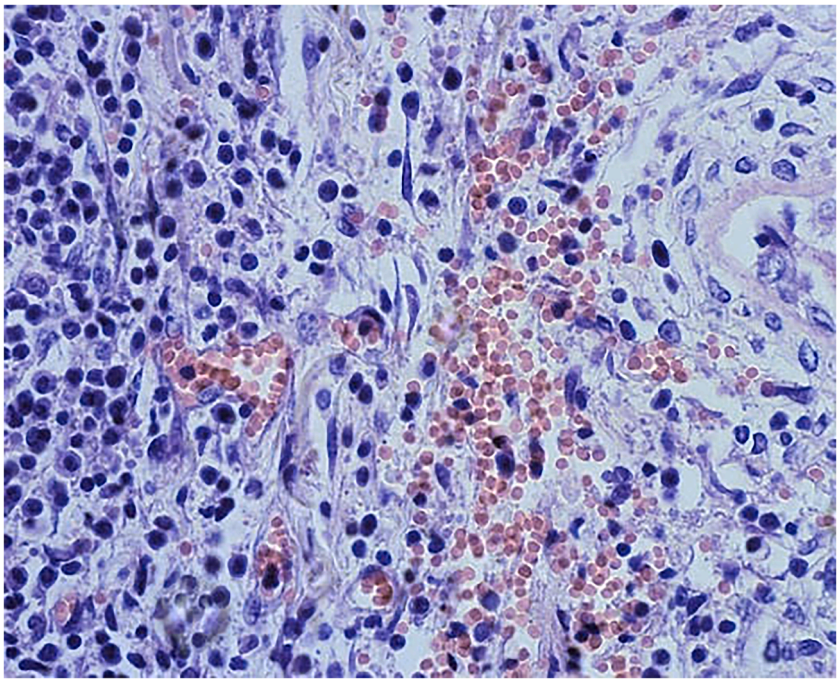

Fig. 4. High magnification of inflammatory cells (lymphocytes, plasma cells) and abundant haemorrhage around the track of parasite (H&E, 20x).

occurrence of testicular tumours in cryptorchid horses is more frequent, probably between 4 and 11 times higher, compared to a non-cryptorchid male [1] due to a detrimental effect of temperature. However, this association is not so evident because cryptorchid horses are generally castrated when young. Testicular teratomas have been reported in undescended testes [16]. Due to their congenital origin, these tumours can increase testicular size, potentially causing cryptorchidism by obstructing normal testicular descent. Torsion of the spermatic cord of the retained testis is not so common in the horse [17] and may provoke colic. Jejunal entrapment and strangulation of a retained testis has been described [18].

Setaria equina, although occasionally free in the vaginal cavity or encapsulated in the tunica vaginalis of the testis, causes periorchitis and serous hemorrhagic lesions [11].

Occasionally, *Strongylus edentatus* can migrate to the equine testis and provoke characteristic inflammatory changes, however it is rare to find alive parasites in the scrotal testes. Prior to use of macrocyclic lactones, migrating *S. edentatus* were seen to cause orchitis, epididymitis and periorchitis especially in cryptorchid testes [19].

Especially, in young horses, *S. edentatus* and *S. equina* can be detected in the vaginal cavity and in the parenchyma of the scrotal gonads and more commonly in the cryptorchid testes parenchyma [11]. The larvae of *S. edentatus* and *S. equina* could enter into the testis through the spermatic artery or directly from the peritoneum, passing under the vaginal tunica of the testicular cord [11,20]. Smith in 1973 [20] reports that the larvae of *S. edentatus* reach the cryptorchid testes because the passage of the larvae is facilitated as they have a shorter mesorchium and spermatic cord. In the same manner, as reported elsewhere the larvae of *S. vulgaris* migrate in the mesenteric cranial artery and its main branches [13] and for proximity the 4 stage larvae (L4) should penetrate the retained testis. However, the capability of *S. edentates, S. vulgaris* and *S. equina* to enter the vaginal cavity is limited due to the distance, narrow inguinal ring, and inappropriate temperatures.

For these reasons, only traumatic injuries or granulomatous lesions can be detected in the testicles of the scrotum as a sign of a transitory invasion of these nematodes or because of their death in this abnormal location [11].

Parasitic invasion of equine testes is an extremely rare phenomenon, with species such as *S. vulgaris* and *S. edentatus* occasionally misdirecting their migration. These erratic migrations result in severe pathological changes, particularly in undescended testes. In this report, we focus on an unusual case of *S. vulgaris* localization in a retained equine testis, highlighting its ultrasonographic features and associated pathological findings

Ultrasound is increasingly being applied in veterinary medicine due to the versatility of the method or even the availability of small, portable, battery-powered US systems and the rapidity with which diagnostic results can be obtained and, finally, the possibility of examining non-sedated animals in the field. Whereas the main limitation of US is the strong dependence on the skills and experience of the operator.

Parasites can be recognised in ultrasound images based on their morphology, size and location [21]. The ultrasonographic appearance of nematodes is due to the structure of the parasite which, in longitudinal section, appears as a linear “train track” structure composed of two parallel hyperechoic lines, that represent the cuticle, separated by a narrow hypo/anechoic line. In cross section, they have a “donut” appearance characterized by a hyperechoic circular wall with a hypo-anechoic centre [21–23].

The presence of “train tracks” in retained testis of the stallion can be suggestive a nematode invasion of the gonad.

4. Conclusion

This study describes the laparoscopic removal of a retained testicle parasitized by *S. vulgaris*, and the useful suggestion given by the ultrasonographic evaluation.

The ultrasonographic findings of "train-track" structures, though suggestive, require histological confirmation for a definitive diagnosis. These tracks, composed of hyperechoic parallel lines, align with the structural morphology of nematodes. However, ultrasound alone cannot confirm the presence of live parasites.

CRediT authorship contribution statement

S. Di Giorgio: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **S. Monti:** Writing –

review & editing, Data curation. **V. Palmieri:** Writing – review & editing, Conceptualization. **G. Marino:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis. **E. Napoli:** Writing – review & editing, Project administration, Methodology. **C. Vullo:** Methodology, Investigation, Formal analysis, Data curation. **G. Catone:** Project administration, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

Preliminary results were presented as an Abstract at the 28th International Congress of SIVE, Venice, Italy, 3-4 February 2023.

References

[1] Amann RP, Veeramachaneni DNR. Cryptorchidism in common eutherian mammals. Reproduction 2007;133:541–61.
[2] Eriksson S, Jäderkvist K, Dalin AM, Axelsson J, Lindgren G. Prevalence and genetic parameters for cryptorchidism in Swedish-born Icelandic horses. Livest. Sci. 2015; 180:1–5.
[3] Stickle RL, Fessler JF. Retrospective study of 350 cases of equine cryptorchidism. J. Am. Vet. Med. Assoc. 1978;172:343–6.
[4] Amann PR, Veeramachaneni DN. Cryptorchidism and associated problems in animals. Anim. Reprod 2006:3108–20.
[5] Straticò P, Varasano V, Guerri G, Celani G, Palozzo A, Petrizzi L. A retrospective study of cryptorchidectomy in horses: diagnosis, treatment, outcome and complications in 70 cases. Animals 2020;10:2446.
[6] Trotter GW. Normal and cryptorchid castration. Vet. Clin. N. Am. Equine Pract. 1988;4:493–513.
[7] Schumacher J. Equine surgery. 2nd ed. Philadelphia: WB Saunders Co.; 1999.
[8] Hendrickson D. Laparoscopic cryptorchidectomy and ovariectomy in horses. Vet Clin North Am Equine Pract 2006;22:777–98.
[9] Barnes G. Cardiovascular response to elevation of intrabdominal hydrostatic pressure. Am. J. Physiol. 1985:248.
[10] Parker JE, Rakestraw PC. Intra-abdominal testicular torsion in a horse without signs of colic. Journal of the American Veterinary Medical Association 1997;3: 375–7.
[11] Marino G, Zanghi A, Quartuccio M, Cristarella Mazzullo G, Catone G. Equine testicular lesions related to invasion by nematodes. J Equine Vet Sci 2009;29: 728–33.
[12] Lichtenfels J, Kharchenko V, Dvojnos G. Illustrated identification keys to strongylid parasites Strongyllidae nematoda of horses zebras and asses equidae. Vet Parasitol 2008;156:4–161.
[13] Marchiondo AA, Cruthers LR, Fourie JJ. Parasiticide screening, 2. Academic Press; 2019. p. 136–41.
[14] Otranto D, Wall R. Veterinary parasitology. 5th Edition, 29. John Wiley & Sons, Ltd; 2024.
[15] Bøgh IB, Baltsen M, Byskov AG, Greve T. Testicular concentration of meiosis-activating sterol is associated with normal testicular descent. Theriogenology 2001;55:983–92.
[16] Leonardi L, Bertoletti A, Bellezza E, Pettinari I, Gialletti R. Dentigerous equine teratoma in a stallion: surgical management and clinicopathology. Vet Sci 2021;8: 84.
[17] Raisi A, Davoodi F. Testicular torsions in veterinary medicine. Vet Res Commun 2022;46:303–13.
[18] Haider W, Brehm W, Keller H. Colic due to jejunal strangulation in a cryptorchid stallion. Tierarztl. Prax 1996;24:476–8.
[19] Edwards JF. Pathologic conditions of the stallion reproductive tract. Anim Reprod Sci 2008;107:197–207.
[20] Smith JA. The occurrence of larvae of *strongylus edentatus* in the testicles of stallions. Vet Rec 1973;93:604–6.
[21] Corda A, Corda F, Secchi V, Pentcheva P, Tamponi C, Tilocca L, Varcasia A, Scala A. Ultrasonography of parasitic diseases in domestic animals: a systematic review. Animals 2022;12:1252.
[22] Hoffmann H, Kawooya M, Esterre P, Ravaoalimalala VE, Roth J, Thomas AK, Roux J, Seitz HM, Doehring E. In vivo and in vitro studies on the sonographical detection of ascaris lumbricoides. Pediatr. Radiol. 1997;27:226–9.
[23] Corda A, Tamponi C, Meloni R, Varcasia A, Parpaglia MLP, Gomez-Ochoa P, Scala A. Ultrasonography for early diagnosis of *Toxocara canis* infection in puppies. Parasitol. Res. 2019;118:873–80.