



# Neurobehavioral Disorders and Pathological Changes in the Brain of Mice Are Caused by Chronic *Toxocara canis* Larval Invasion with Low to Moderate Inoculum

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Received: 5 June 2024 / Accepted: 23 July 2024

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## Abstract

*Toxocara canis* larvae are one of the most overlooked agents of nervous system infection in paratenic hosts. Previous studies in mouse models have shown that infection with various (mainly high) numbers of larvae leads to neurobehavioral disturbances and pathological changes. Our study investigated whether the infection with low and moderate numbers of larvae could affect the physical condition, motor skills, and pathogenesis in the brains of experimentally infected mice.

Two groups of BALB/c mice were orally infected with 10 and 100 *T. canis* larvae per animal and examined regularly until the 97th week after infection. General appearance, specific antibody responses, and motor/balance skills were assessed. The number and viability of larvae in the liver, spleen, lungs, and brain were assessed by quantitative compressed biopsy technique, while the pathological changes of the brain infection were studied histologically.

As a result, changes were observed in overall appearance, activity, as well as motor and balance ability. The infections were associated with an increased IgG antibody response to the specific anti-*T. canis* excretory/secretory antigen and tissue damage in the brain characterized by necrosis, cell infiltrations, including foamy cells, and hemorrhages.

The study demonstrated the effects of low and moderate *T. canis* infection in a paratenic host during the chronic phase of infection, which lasted up to 97 weeks for the first time.

**Keywords** Neurobehavioral disorders · Pathological changes · Mice · *Toxocara canis* · Moderate to low inoculum

## Introduction

The roundworm *Toxocara canis* commonly parasitizes various canids, but its eggs can also be a source of infection for paratenic (non-final) hosts, including humans. In these hosts, the third-stage larvae (L3) hatch from the eggs, penetrate the intestinal wall, and migrate via the bloodstream to different tissues, where they can survive for years [1]. Infected paratenic hosts may become a reservoir of infection not only for the definitive but also for the next paratenic host [1, 2].

Although *Toxocara* spp. infections in humans may remain clinically inapparent, they can rarely manifest as larval toxocarosis (LT): (i) the most common form is visceral larva migrans (VLM), followed by (ii) ocular larva migrans (OLM), (iii) covert/common toxocarosis, and (iv) neural larval toxocarosis or neurotoxocarosis (NLM) [1–6]. The severity of the infection is closely related to the number of parasites deposited [7, 8].

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Compared to VLM, knowledge on the pathogenesis of NLM is scarce (e.g., Deshayes et al. [9]), even though this form of infection can also cause behavioral changes in infected individuals, including humans [10–13]. Studies on animals experimentally infected with *Toxocara* eggs or larvae under defined conditions have helped to elucidate these changes [12]. The larvae have been shown to migrate naturally, accumulate, and damage the host nervous tissue under certain circumstances [14–17]. Most available data show that the risk of central nervous system (CNS) invasion by *Toxocara* larvae [16, 18] and the associated development of neurodegenerative changes [12, 15, 19–21] increases with higher parasite infection dose, i.e., more than 1,000 eggs/larvae per mouse.

Although infections with lower parasite doses (up to 100 eggs/larvae per animal) are thought to more closely mimic the natural conditions that lead to NLM, they are still not fully understood due to the significantly lower number of studies [18, 22, 23]. Nevertheless, it seems that these types of infection [24, 25] may also lead to pathophysiological processes that could affect the behavioral changes of the affected individuals.

It has been documented that the presence of larvae in host tissues led to the host immune response characterized by increased leukocyte infiltration, particularly of eosinophil counts, as well as specific cytokine and antibody production (e.g., interleukin 4, interleukin 10, interferon-gamma, immunoglobulin E) [26, 27], and hepatosplenomegaly [8, 17, 28, 29]. In terms of the brain damage during the *T. canis* infection, substantial studies showed that the tissue damages are also associated with enhanced expression of selected biomarkers (e.g., transforming growth factor  $\beta$ 1, glial fibrillary acidic protein,  $\beta$ -amyloid precursor proteins) [15] widely used to delineate pathophysiological mechanisms in various brain injuries. Some pathophysiological changes in experimental cerebral toxocarosis in mice inoculated with a high dose of 1,000 eggs appear to be relevant to the abnormal behavioral and motor changes, e.g. stereotyped unidirectional circling movements, progressive limb ataxia, tumbling movements, incoordination and balance problems, reduced righting reflex, spatial awareness and exploratory behavior [6, 25, 30–33].

In contrast, the aim of our study was to mimic the conditions of natural *T. canis* infections, as the number of larval invasions into the brain was low, to clarify whether even moderate to low parasite infections can affect the motor activities of the paratenic hosts.

## Materials and methods

### Material for Infection and Serological Studies

Adults of *Toxocara canis* were obtained from the feces of naturally infected shelter dogs previously treated with one tablet of praziquantel (Drontal™, Bayer, Germany). From the adults, unembryonated eggs were isolated and their maturation was performed in vitro using a method by Kolbeková et al. [14]. From fully embryonated eggs, the larvae were hatched according to a method by Fan et al. [34]. The obtained larvae in the L3 stage were kept in RPMI 1640 medium (R8758, Sigma-Aldrich, Germany) with 100 IU/ml penicillin and 250  $\mu$ g/ml streptomycin at 37 °C and 5% CO<sub>2</sub> with regular weekly changes of the medium until used in experimental infections. The collected medium was used for the preparation of larval excretory-secretory antigens (TcES).

Before the infection of animals, viable larvae were washed with a sterile medium, and the number of larvae per 1 ml was counted by sampling method using a binocular stereomicroscope. Then, the larvae were concentrated to the desired infection dose (ID), i.e., 100 (moderate) or 10 larvae (low) per mouse.

Preparation of TcES antigens and further determination of protein concentration was performed according to Novák et al. [35] and Savigny et al. [36]; antigen aliquots with a protein concentration of 0.0875  $\mu$ g/ml were stored at -20 °C until used.

### Infection Experiments

Female BALB/c mice aged 6 weeks were used for the experiments (Velaz Ltd., Prague, Czech Republic). Mice were infected by oral administration of defined *T. canis* ID in 10–50  $\mu$ l of medium per mouse using sterile pipet tips. A total of 7 mice infected with 100 larvae each were included in group G/100; group G/10 included 4 mice infected with 10 larvae each. The control group G/0 consisted of three uninfected mice that received only tap water and were kept under the same conditions as in the previous cases.

### Infection Response Assessment

The whole blood was collected from the tail tip of all experimental animals at week 7 post infection (p.i.) and sera were stored at -20 °C until use. The mouse infection was confirmed by the detection of specific anti-TcES IgG antibodies by ELISA according to Novák et al. [35]. Briefly, the wells of microplates (NUNC Maxi Sorp, Massachusetts, USA) were coated with 100  $\mu$ l of bicarbonate coating buffer (pH 9.6) containing TcES diluted at 1:750 (final protein

concentration 0.0875 µg/ml, i.e. 0.00875 µg/well) for 1 h at 37 °C, then overnight at 4 °C. Free binding sites were blocked using 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) containing 0.02% Tween 20 (PBS/T/BSA) for 1 h at room temperature (RT). After three cycles of washing with PBS containing 0.05% Tween 20 (PBS/T), 100 µl of mouse sera (pre-diluted at 1:800 in PBS/T/BSA) were added and incubated for 1 h at RT. After washing 3× with PBS/T, TcES-specific IgG was detected using peroxidase-conjugated goat anti-mouse IgG (ab6823, Abcam, Great Britain) diluted at 1:10,000 in PBS/T/BSA (100 µl, 1 h, RT), washed and visualized by 0.04% o-phenylenediamine dihydrochloride with 0.012% H<sub>2</sub>O<sub>2</sub> in citrate buffer. The reaction was stopped after 20 min by adding 100 µl 2M H<sub>2</sub>SO<sub>4</sub>. Optical density (OD) was measured at 490 nm (Dynatech MRX II) and analyzed in GraphPad Prism (version 8) using one-way ANOVA. The cut-off was double the mean OD of uninfected mice (G/0) [37].

### Examination of Animals and Evaluation of Motor/Balance Skills

The animals were examined continuously at defined intervals (Table 1) from the day of initial infection until the end of the experiment, i.e., week 97 p.i. At each defined time interval one mouse was killed and material was collected for microscopic and serological studies; the exception was the situation in the 65th week p.i., when two animals were killed and examined.

The weights of all mice in each group were measured in 2-week intervals using laboratory scales. Normalized percentage body weight gain was used to adjust for differences in mean weight at the start of the experiment and maximum mean weight gain between groups. Two-way analysis of variance (ANOVA) with Tukey's multiple comparison test was used for statistical analysis.

Assessment of general appearance, activity, and motor/balance abilities, as well as testing on motor abilities, were performed in 7-week intervals (Table 1). Animals were housed in a plastic cage (disinfected and cleaned with 96% ethyl alcohol and 0.02% acetic acid) in an environment covered with a tight-fitting cloth to limit any visual stimulation. All animals were observed and examined by the same observer throughout the experiment to minimize the influence of subjective observation by multiple examiners.

Animals were observed for 10 min and scored for the following external parameters: (1) overt cachexia [38]; (2) disheveled hair [30, 39]; (3) abnormal posture (kyphosis, reduced pelvic elevation) [30, 39, 40]; (4) reduced general activity [30, 41, 42]; (5) stereotyped movements [30, 31, 39]; (6) stomping; (7) tumbling movements [30, 41]; (8) impaired balance [30]; (9) limb ataxia [30] or partial/

**Table 1** Summary of experiments on BALB/c mice infected by 100 (G/100) or 10 (G/10) *Toxocara canis* larvae and controls (G/0): weighing, observation of general appearance and activity, time course of motor tests and time of necropsies

Experimental group/infection dose	No. Mice	Monitored parameters		Time of necropsies (WPI)	
		Total time covered in weeks post infection (WPI) / monitoring intervals in weeks		General appearance and activity observation	
		Weighing (g)	Motor testing	Turn-back ability	Beam test
G/100	7	97 / 2	97 / 7	97 / 7	19 53 65* 78 91 97
G/10	4	76 / 2	76 / 7	76 / 7	26 54 68 76 --- ---
G/0	3	67 / 2	67 / 7	67 / 7	19 47 67 --- --- ---

\* two mice necropsied on this week post infection

complete paralysis [38, 43]; and other abnormal findings [30, 32, 42] when recorded.

Two tests, slightly modified in our study, were used to assess the motor activity of infected animals:

- 1) Turn-back test [44]: Each mouse was placed in the supine position by tail manipulation, then released, and its behavior was observed to test the righting reflex. Resumption of the natural body position by curling up, spasmodic return to the side, or not turning at all was classified as an inability to return to the original position. The trials were repeated five times in succession and if these failures occurred on three or more trials, the resulting situation was scored as a failure of the righting reflex.
- 2) The beam test originally described by Deacon [45] was modified as follows: the two static horizontal beams used, placed on a test table, were— (i) a thin metal beam with a diameter of 0.95 cm (originally 0.6 cm) and a test length of 46 cm (originally 38 cm), placed 13 cm above the surface (originally 49 cm) and (ii) a thick, serrated beam with a diameter of 1.9 cm (originally 1.5 and 2.2 cm) and testing length of 30 cm (originally 60 cm) with paper barriers at the ends to prevent the mouse from the escaping, placed 33 cm (originally 60 cm) above the surface. Testing on a thin beam monitored the animals' ability to hold onto the beam for three consecutive 30-second trials; by repeating the trials, it was possible to rule out situations where the mice slipped off the beam due to the metal surface. Motor impairment was assessed as the mouse being unable to grasp the beam correctly with its forelimbs or being unable to

hold onto the beam for more than 10 s. When tested on the thick beam, the animals' ability to maintain stability, walk without balance, and without slipping their paws was monitored within a 30-second trial. If the mice were unable to do this, their behavior was classified as a motor disorder.

## Larva Recovery Study and Histology Assessment

Mice were euthanized by cervical dislocation [46] and necropsied at the defined intervals p.i. or at the onset of severe signs of the infection to avoid suffering (Table 1).

The liver, spleen, lungs, and brain were examined microscopically for the presence of larvae, which were also assessed for viability (Nikon Eclipse E200LED MV R, Nikon, Japan); except the brain, the other organs were weighed [19]. Larval recovery was processed from 10 tissue samples of approximately 1 mm<sup>3</sup> from the liver, spleen, and lungs. The selected tissue was compressed between two microscope slides to detect larvae (quantitative compressed biopsy technique, QCBT) and to estimate the burden for the whole organ [23, 47, 48].

One of the sagittally separated brain halves was divided into cerebrum with medulla oblongata and cerebellum [49] and also examined by QCBT for the presence and number of the larvae [19, 20]. The remaining brain hemispheres were fixed in Bouin's solution (HT101128, Sigma-Aldrich, Germany), and further histological sections stained with hematoxylin-eosin (H/E) were prepared using Gill's hematoxylin solution (HTG-A10,00 BaSo, Taiwan) and eosin Y (E4382, Sigma-Aldrich, Germany). The location and extent of tissue damage were observed microscopically and compared with uninfected tissues.

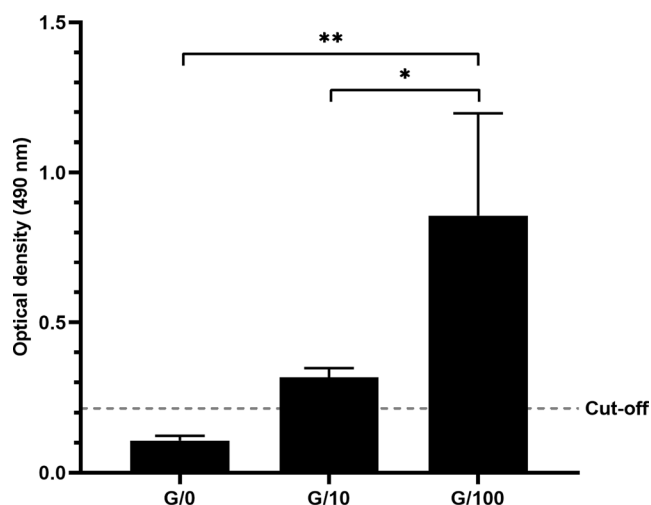
## Results

### Antibody Response

The specific antibody response was recorded in all infected mice. The intensity of the anti-TcES IgG antibody immune response in the blood of all infected mice correlated with the infection dose, i.e., the higher the dose, the more intense the immune response (Fig. 1). Statistically significant differences were found between G/10 and G/100 ( $p < 0.05$ ) and between G/0 and G/100 ( $p < 0.01$ ).

### General Condition and Motor and Balance Skills

All mice showed a stable weight gain: G/0 to 53 weeks of the experiment, G/10 to 47 weeks p.i., and G/100 to 44



**Fig. 1** Levels of *Toxocara canis* excretory-secretory antigen-specific IgG antibodies in sera of mice infected by 10 (G/10) and 100 (G/100) larvae compared with controls (G/0) on week 7 post infection. Significant differences among groups are indicated by asterisks (\* $p < 0.05$ , \*\* $p < 0.01$ ). Cut-off is stated as double the mean OD of G/0

weeks p.i. From these time points, weight gain was stagnant or slightly decreased in all groups. The percentage gains of body weights during the experiment are shown in Fig. 2. The differences were significant between G/0 and G/100, and between G/10 and G/100 from week 3 p.i. One mouse from G/100 showed a massive loss of 11.6 g at week 53 p.i. In the infected mice, the weight loss is correlated with changes in general appearance, activity, and motor/balance disturbances.

Of all infected animals, seven mice showed changes in general appearance and activity (6 of G/100, 1 of G/10), 10 mice showed subtle motor and balance impairments (7 of G/100, 3 of G/10), and 5 mice developed obvious motor and balance impairments (4 of G/100 and 1 of G/10) (Table 2). Only one mouse from G/10 appeared to have no changes or impairments at the time of necropsy at week 26 p.i.

The changes in general appearance and activity, obvious motor, and balance disturbances were detected at week 19 p.i. in G/100 and started to develop in more animals from week 53 p.i. In G/10, these changes were only occasionally recorded from week 74 p.i. onwards (Fig. 3). However, the subtle motor and balance impairments (Fig. 3) were also

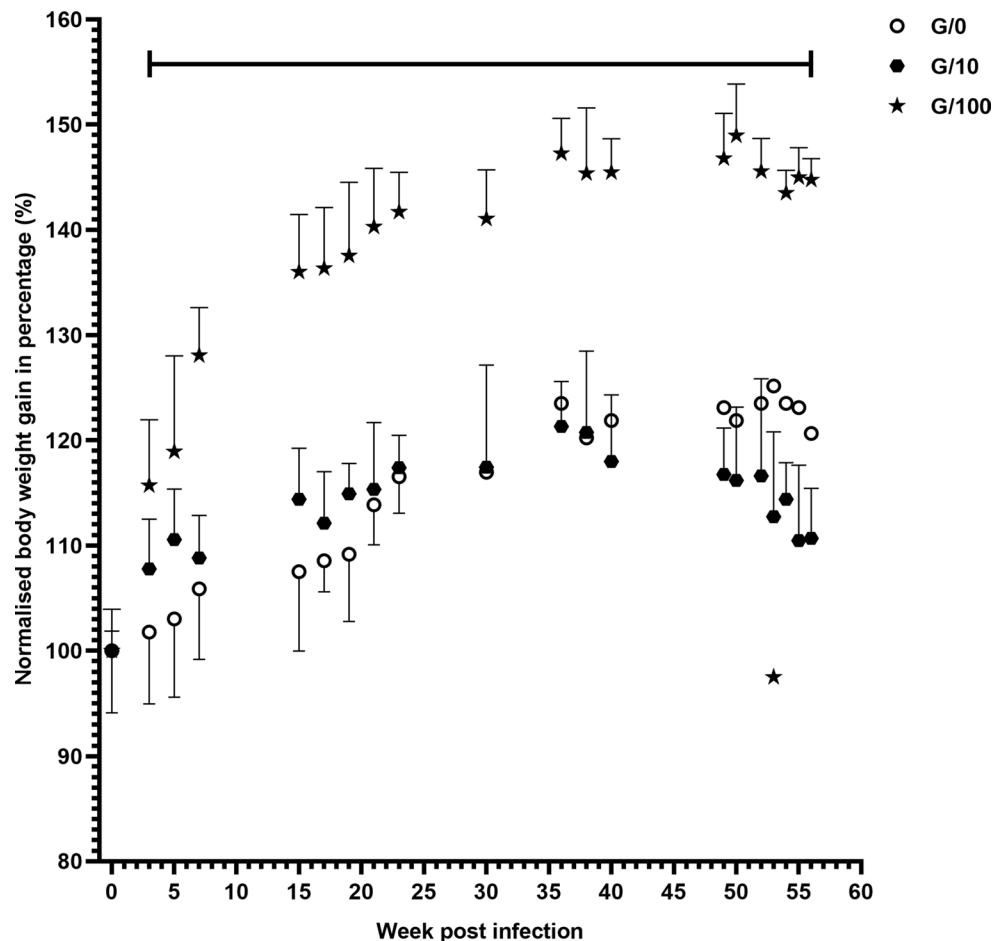
observed with earlier onset in both groups (weeks 4 and 36 p.i. in G/10 and G/100, respectively).

The changes in general appearance and activity and the impairment on the thin beam were observed in one control mouse, with the first detection at weeks 62 and 64 of the experiment. In this animal, necropsy revealed pathological changes of non-infection origin, namely lung neoplasms, which were probably the cause of the deterioration in body condition. In view of this, this mouse was not excluded from the experiment.

### Larvae Recovery and Brain Pathology

Microscopically, larvae were found at defined intervals in the CNS (Table 3), but not in the liver, lung, or spleen. They were found in all examined hemispheres of mice from G/100 (3.0 – 15.0% of ID, 19–97 weeks p.i.) and in 2 out of 4 mice from G/10 (10% and 40% of ID on week 54 p.i. and 76 p.i., respectively). QCBT showed viable intact larvae. Histology revealed at least one of the following brain pathological patterns in all mice at the G/100: necrosis, foamy (or gitter) cells, hemorrhage, and infiltration. In G/10, striking necrosis and hemorrhages were observed in 3 out of 4 infected

**Fig. 2** Mean body weight gain in percentage  $\pm$  standard deviation of mice infected by 10 (G/10) and 100 (G/100) *Toxocara canis* larvae compared with controls (G/0). The differences were significant between G/0 and G/100, and between G/10 and G/100 from week 3 p.i. to week 53 p.i. (indicated by the line)





**Table 2** The development of changes in general appearance, activity, motor, and balance impairments in mice infected by 100 (G/100) and 10 (G/10) *Toxocara canis* larvae compared with controls (G/0)

Experimental groups/infection dose	Changes in general appearance and activity					Motor and balance impairments					Number of mice in the group presenting the disorders		
	Overt cachexia	Disheveled hair	Abnormal (kyphotic) posture	Reduced overall activity	Stereotyped movements	Stomping	Subtle impairments detected by testing			Obvious motor and balance disorders			
							On thick beam	On thin beam	Turn-back failure	Tumbling movements		Impaired balance	Limb ataxia
G/100	3	5	2	1	2	3	5	3	2	4	2	2	7/7
G/10	0	1	0	1	0	0	3	2	0	1	0	0	3/4
G/0	1	1	1	0	0	0	0	1	0	0	0	0	1/3

mice (Table 3; Fig. 4). Pathologies were observed in several brain regions: corpus callosum, cerebellum, medulla oblongata and pons varolli, midbrain, caudate putamen, an optic tract in the hypothalamic area, cerebral cortex, hypothalamus, and ventral striatum. Larva in the anterior olfactory nucleus without any surrounding pathology was recorded only in mouse from G/100 necropsied at week 78 p.i.

The success rate of brain invasion was 100% for G/100 mice and 50% for G/10 mice. While we did not observe a trend towards accumulation of larvae in the brain during infection of G/100 mice, there was an increase in the number of parasites in the G/10 group from week 76 p.i. The number of larvae detected in the brain of G/100 did not correlate with any observed changes in general appearance and activity, either subtle or obvious motor and balance impairments. However, the number of larvae detected in the brains of G/10 did correlate with the development of these changes.

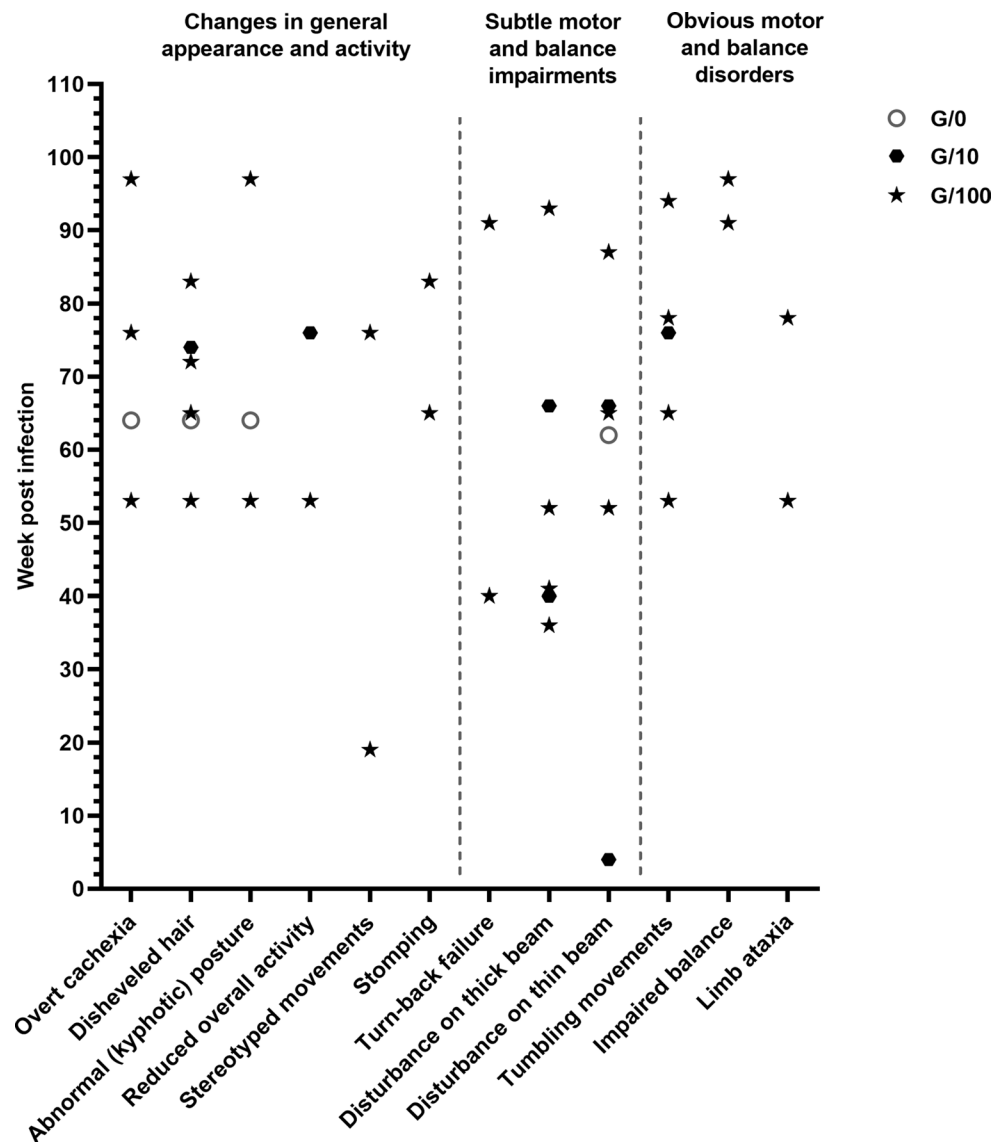
## Discussion

The present study aimed to comprehensively investigate the consequences of any neurological and behavioral changes of BALB/c mice with a long-term infection due to low and moderate numbers of *T. canis* larval invasion of the brain. The strain of mice was used for its susceptibility to *Toxocara* infections, which makes it convenient to compare our results with other studies [3, 14, 50–52].

The success of oral infection in mice was demonstrated by the detection of a specific immune response against *T. canis* larval antigens. In agreement with other studies [24, 28], we confirmed that the intensity of the antibody response correlated with the dose of infection. In our study, it was highest in animals infected with 100 larvae (Fig. 1). The difference between the G/0 and G/10 was insignificant.

The weight of the mice increased continuously from the beginning of our experiment (Fig. 2). A slight decrease in weight began to occur in G/100 at 44 weeks p.i., which corresponded to the approximate interval when animals started to show changes in general appearance, activity, and motor/balance disturbances, i.e., 36–41 weeks p.i. The rapid weight loss occurred at week 53 p.i. in one mouse of G/100. This animal also developed multiple changes in general appearance and activity, and both subtle and obvious motor and balance impairments; this mouse was sacrificed immediately to prevent suffering. According to Miller et al. [53], aging should be considered as a possible factor that could affect the weight of mice in a long-term experiment. Therefore, weight loss in our experiment was also considered as an indicator of onset physical deterioration.

**Fig. 3** The time development of changes in general appearance and activity, and motor and balance impairments in mice infected by 10 (G/10) and 100 (G/100) *Toxocara canis* larvae compared with controls (G/0)



The first observed change in general appearance and activity was stereotyped movement at week 19 p.i., followed by the rest of the changes at week 53 p.i. in mice of G/100. This time point was earlier than in animals infected with a tenfold lower dose of larvae (G/10), where the first changes were observed at 74 weeks p.i. Janecek et al. [30] also observed stereotypical circling in 2,000 *T. canis* eggs-infected mice at day 27 p.i., which corresponds to the higher ID they used. Furthermore, their mice showed another similar general change in condition, namely lowered pelvic position, at day 34 p.i., which is an earlier onset of abnormal posture compared to week 53 p.i. in G/100 in our study. Looking at experiments with other parasites and the consequences of the infection, stereotypical circling and ruffled coats in mice have also been reported in *Toxoplasma gondii* infections [39].

The modified tests of Fox [44] and Deacon [45] were used to detect the subtle motor and balance changes. To test the righting reflex, we used the method of flipping the mice onto their backs by their tails, because it is easier to manipulate the animals, in contrast to authors who flipped mice onto their backs using a V-shaped bed [54] or used the backward rotation test [55–57]. Failure to turn-back was only observed in G/100 at weeks 40 and 91 p.i. The mice had difficulty in regaining the position of the four limbs and were spastic. These manifestations were similar to those described by Janecek et al. [30] in animals infected with a significantly higher numbers of parasites, i.e., 2,000 larvae. Thus, our results show that neurological problems can occur even with milder infections.

The fine motor and balance skills of the animals were also assessed using a modified beam test [45], which was performed using a lower bar position above the surface.

**Table 3** The number of larvae recovered and pathological findings in hematoxylin/eosin-stained brain sections of mice infected by 100 (G/100) and 10 (G/10) *Toxocara canis* larvae. Parentheses in pathological findings indicate the location and number of pathological changes

Group	G/100				G/10				
	19 (n = 1)	53 (n = 1)	65 (n = 2)	78 (n = 1)	91 (n = 1)	97 (n = 1)	54 (n = 1)	68 (n = 1)	76 (n = 1)
Week post-infection (Number of mice)	9	2	4 ± 1.41	4	10	6	1	0	4
Larval recovery (Number of larvae in brain hemisphere)	6	4	0	1	1	1	0	0	0
Total	15	6	4 ± 1.41	5	11	7	1	0	4
Number of larvae in section	No	No	No	1	No	No	No	No	No
Necrosis	Yes (Cc 3, Vs 1, Mb 1, Crbl 1)	Yes (Cc 1)	Yes (Crbl 1; Cc 2)	Yes (Cc 3)	Yes (CauPut 1, Cc 1, Ht 1)	Yes (CauPut 1, Cc 1, Ht 1)	No	Yes (Cc 2)	Yes (Cc 1, CauPut 2)
Pathological findings	Hemorrhage	No	Yes (Crbl 1; CrCt 1)	No	Yes (Med 2)	Yes (Med 1)	Yes (Med 1)	Yes (Med 1)	Yes (Med 2)
Foamy cells	Yes (Crbl 2)	Yes (Ot 1)	No	Yes (Ot 1, Crbl 1, Cc 1)	Yes (Cc 1)	No	No	No	No
Infiltration	No	No	Yes (Cc 2)	No	Yes (Cc 1)	No	No	No	No

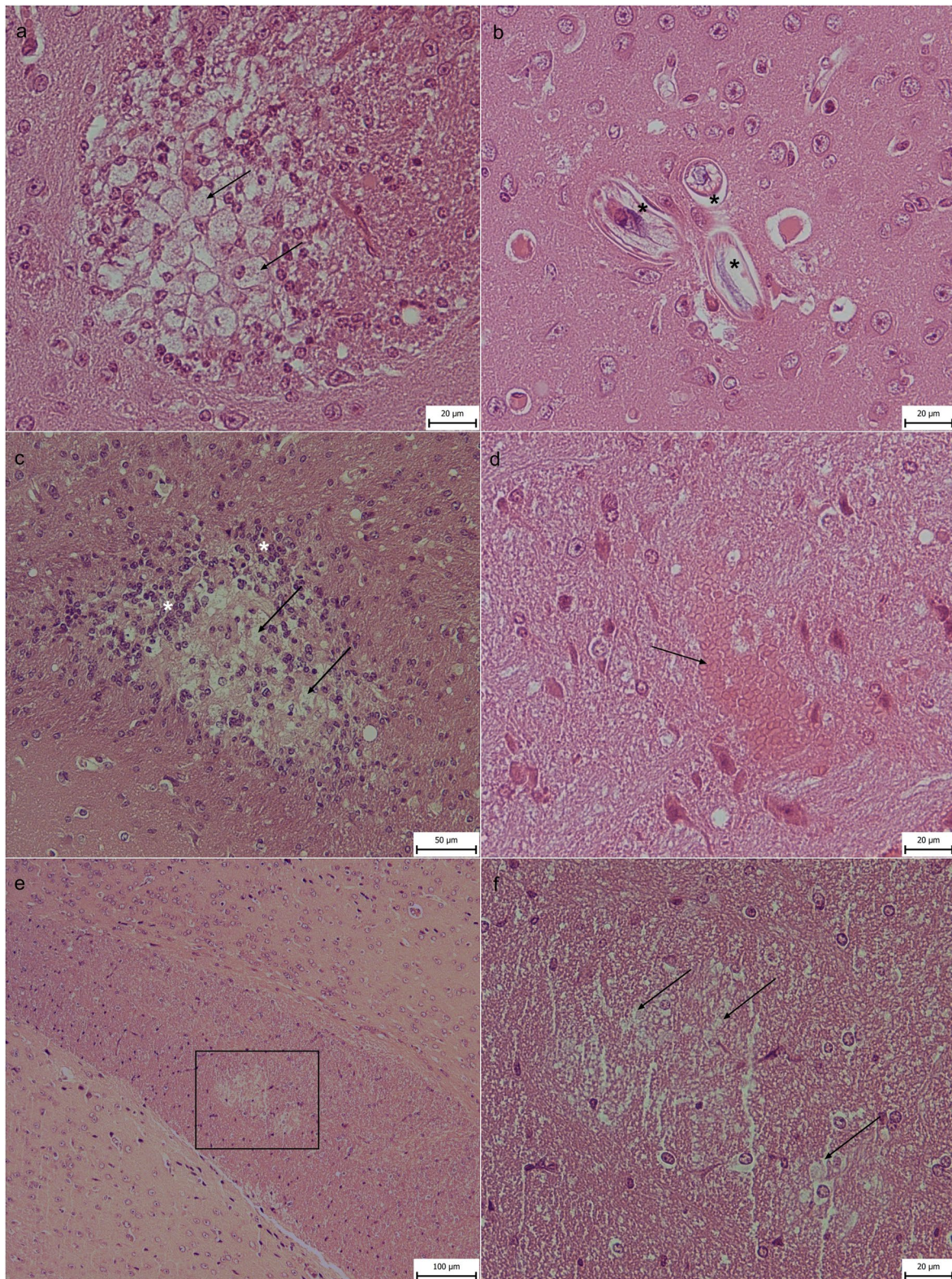
Yes, pathology detected; No, pathology/larva in section not detected; CauPut, caudate putamen; Cc, corpus callosum; CrCt, cerebral cortex; Crbl, cerebellum; Ht, hypothalamus; Mb, midbrain; Med, medulla oblongata and pons varolli; Ot, optic tract in hypothalamic area; Vs, ventral striatum

This test allowed for safer animal testing, while the beam height was sufficient to prevent the animals from deliberately jumping off the bar. The subtle motor and balance changes were first observed in one G/10 mouse at week 4 p.i.; the animal showed impairment throughout the experiment and later developed a disturbance on the thick beam at week 40 p.i. The remaining animals developed the disorder much later, from 66 weeks p.i. onwards. In comparison, the G/100 mice first developed thick beam impairment at week 36 p.i., followed by thin beam impairment from week 40 p.i. and the final development of thick beam impairment at week 93 p.i. The subtle neurological signs occurred earlier (week 4 p.i. in G/10 and week 36 p.i. in G/100) than obvious ones (on week 53 p.i. onwards). The changes in inconspicuous neurological signs (righting reflex) were also observed by Janecek et al. [30] from day 69 p.i. onwards in 2,000 ID mice. The difference in the ID used makes it difficult to compare the temporal continuity of subtle and overt neurological signs between this study and ours.

The increasing age of the experimental animals is a factor that had to be considered for any changes observed with advancing long-term infections. The possibility that the age of the animals influenced the behavior and motor skills of the animals is impossible to exclude. Many factors can affect movement during aging, such as bone and joint disability, loss of muscle mass, poor vision causing reduced activity, and CNS impairment and neurodegenerative changes due to old age [58]. Age-associated osteoporosis is known in BALB/c strain [58]. However, our experiment demonstrated the same symptoms in the G/10 and G/100 groups with earlier symptom onset in the higher infectious dose mice. In the experiments aimed at long-term infection observation, selecting experimental animals with longer average lifespans would be advisable. For example, using experimental rats could prolong the time of experimental infection [59]. Still, comparing it to other studies would require more work. Using different murine strains or applying caloric restriction to prolong murine lifespan was not considered an option, as the time of experiments is too long to be covered by the different murine strains and diets [59].

Viable parasite larvae were only detected in the brains of mice (Table 3). Our results differ from the literature, which shows that even in mild infections, larvae can be detected in the organs through which they migrate. For example, Hanh et al. [8] detected *T. canis* larvae in the lungs of mice infected with 10 eggs/mouse and 100 eggs/mouse one-week p.i. In mice with an initial infection dose of 200 *T. canis* eggs, Kayes and Oaks [60] found 3.2–12.3% larvae in the liver and 0.1–4.9% parasites in the heart/lungs at weeks 1 to 8 p.i. In contrast to these authors, who detected parasites in the liver and lungs at week 8 p.i. (i.e., shortly after infection), we only started to examine these organs from week





**Fig. 4** The brain pathologies in hematoxylin/eosin-stained sections of mice infected by 100 (**a-c**) and 10 (**d-f**) *Toxocara canis* larvae: **a** - foamy cells (arrows) in optic tract in the hypothalamus area on week 53 p.i.; **b** - the larva (asterisks) without any tissue change around in the anterior olfactory nucleus on week 78 p.i.; **c** - necrosis with foamy

cells (arrows) and infiltration of monocytes (white asterisks) in the corpus callosum on week 91 p.i.; **d** - hemorrhage (arrow) in the medulla oblongata on week 68 p.i.; **e** and **f** - necrosis (rectangle, arrows) in otherwise intact tissue in the corpus callosum on week 68 p.i., **f** presenting magnified detail of necrosis marked by rectangle in **e**



19 p.i., i.e., when the larvae had already left these organs. The visceral organs of G/10 animals showed no visible macroscopic changes, granulomas, hepatomegaly, or splenomegaly. In contrast, spleen color changes were detected in G/100 at 53 and 65 weeks p.i., but no granulomas were demonstrated in the liver. The spleen of these animals reached 2.5 cm in length at 65 weeks p.i., slightly longer compared to control uninfected animals. Given the small number of animals, this figure cannot be evaluated statistically.

Our present study found that at an earlier time after infection, the maximum recovered rate of larvae in the brains of mice inoculated with 100 larvae was 15% which was similar to the study by Skerrett and Holland [61] who detected recovery rates of 10% larvae from 100 *T. canis* eggs/mouse ID on days 5 and 14 p.i., however, ours was in between 8.6 and 32.5%, which was a study by Kayes and Oaks [60] who used 200 *T. canis* eggs/mouse ID during a period of 1 to 8 weeks p.i. Nevertheless, a higher rate of 40% for mice with 10 larvae inoculation was detected in the present study. Havasiová-Reiterová et al. [24] noted an average of 6.6% larval recovery from 5 *T. canis* eggs/mouse ID (from 5 examined mice) and an average recovery of 14.3% from 7 *T. canis* eggs/mouse ID (from 5 examined mice) at week 10 p.i.

Unlike these authors, we used infection-viable larvae instead of embryonated eggs and the larva has a resistant acellular cuticle, which allows them to escape the gastric acid attack, whereas the embryonated eggs if contain immature larvae making them unable to emerge from the eggs to perform the penetration function through the small intestine. The higher larval recovery rate in our experiment was also influenced by the cumulative effect of larvae over time. Similar or higher recovery rates have already been observed for low ID compared to moderate ID at some time points [24, 61]. This phenomenon is not well understood, but the immune response of the paratenic host may play a role [1, 17].

In our experiment, the selection between the right and left hemispheres of the brain for the larval recovery study was randomized. Similarly to Farjat et al. [62] and Eid et al. [19], we used QCBT for its advantage of a complex examination and quick slide preparation. And similar to Kolbeková et al. [14, 37], we detected individual larvae in different brain regions or clusters, with a higher number in the cerebrum than in the cerebellum. Like Janecek et al. [50], neither the compression technique nor histology showed a significant difference in the number of larvae between the right and left brain hemispheres. On the other hand, Good et al. [49] observed different distribution of larvae between the telencephalon and diencephalon of mice, with more larvae detected on the right side. Some authors found a higher predilection of larvae in the cerebellum

[63]; but we found a higher number of larvae in the cerebrum, which is in agreement with Kolbeková et al. [37] and Janecek et al. [50]. Based on our results, the detected larval load corresponds more to the size of the brain part than to the affinity of the larvae for selected parts. Localization [64] and type of tissue damage [20, 37, 50, 65–68] were also assessed histologically. Except for one mouse infected with 10 larvae, which showed no pathological changes in the brain and no physiological or neurological changes, all other infected animals manifested these changes to varying degrees. Histopathology revealed necrosis and cellular infiltration predominantly in the corpus callosum, foamy cells in the cerebellum, and in optic tract in the hypothalamic area; hemorrhages were recorded predominantly in the medulla oblongata and pons varolli. Our results are consistent with the pathology reported in the high-dose infection study (ID 2,000 eggs/mouse) [20], including the accumulation of foamy cells in fiber tracts, as seen in the corpus callosum of G/100. We also observed foamy cells in the optic tract of G/100 mice. This suggests that the presence of foamy cells in the optic tract may be related to larval invasion to the eyes, optic nerve damage, or ocular toxocarosis. However, the eyes of the mice were not examined in our study and therefore we cannot link possible presence of larvae in eyes of the mice to the observed motor impairments or pathologies detected in optic tract in the hypothalamic area.

Although our study was carried out on small groups of mice, our results showed that long-term low to moderate infections with *T. canis* larvae can still lead to severe neurological consequences.

## Conclusions

The present study confirmed that even mild infections of mice with *Toxocara canis* larvae can lead to neurotoxocarosis during the chronic phase of infection. Low and moderate infections could induce a specific immune response and, in addition to changes in the general appearance and activity of the animals, disturbances in motility and balance accompanied by the development of necrosis, hemorrhage, and foamy cell accumulation. Our study showed that weak infections with as few as 10 larvae may be asymptomatic initially, but may manifest neurological complications as they progress to the chronic phase. The results suggest that even low-level infections of a paratenic host, which commonly occur in the wild, can have serious health consequences.

**Acknowledgements** The first author would like to thank Dr. Tomáš Macháček for consultations on manuscript and Mrs. Markéta Leissová for help with the preparation and implementation of the hatching, cultivation, and long-term maintenance of *Toxocara canis* larvae used in this study.

**Author Contributions** All authors contributed to the study conception and design. N.B. conceived and designed the study. L.K. and C.K.F. supervised the study. N.B. conducted data gathering. N.B. conducted data analysis. N.B. prepared the original draft. L.K., J.N., P.H. and C.K.F. reviewed the draft. All authors approved the final form of the manuscript before submission.

**Funding** This research was supported by the 1st Faculty of Medicine, Charles University, Prague (SVV 260 636), by Cooperatio– Infection and Immunity, and Cooperatio - Biology, European Union project National Institute for Neurological Research (Program EXCELES, ID Project No. LX22NPO5107) and by Visegrad Scholarship Program, funded by the Governments of Czechia, Hungary, Poland and Slovakia through International Visegrad Fund. The presented results and conclusions are stated by the authors of the publication and do not represent the International Visegrad Fund.

**Data Availability** No datasets were generated or analysed during the current study.

## Declarations

**Ethical Approval** For ethical reasons, the number of experimental animals was kept low, although this limited the statistical power of the data. Mice were housed in an animal facility under standard conditions (commercial pellet chow and water ad libitum, 12-h light/dark cycle) in specially accredited laboratories of the Institute of Immunology and Microbiology of the First Faculty of Medicine, Charles University and the General University Hospital in Prague (accreditation numbers 70030/2013-MZE-17214 and 8615/2019-MZE-17214). The certified person (CZ 02627) kept the animals in accordance with ethical standards and Directive 2010/63/EU on protecting animals used for scientific purposes. The experimental project involving laboratory animals was approved by the Ministry of Education, Youth and Sports of the Czech Republic (approval number MSMT– 7063/2017-2).

**Competing Interests** The authors declare no competing interests.

## References

- Strube C, Heuer L, Janecek E (2013) *Toxocara* spp. infections in paratenic hosts. *Vet Parasitol* 193:375–389. <https://doi.org/10.1016/j.vetpar.2012.12.033>
- Magnaval JF, Glickman LT, Dorchie P, Morassin B (2001) Highlights of human toxocariasis. *Korean J Parasitol* 39:1–11. <https://doi.org/10.3347/kjp.2001.39.1.1>
- Bardón R, Cuéllar C, Guillén J (1994) Larval distribution of *Toxocara canis* in BALB/c mice at nine weeks and one year post-inoculation. *J Helminthol* 68:359–360. <https://doi.org/10.1017/S0022149X00001644>
- Fan CK, Liao CW, Cheng YC (2013) Factors affecting disease manifestation of toxocarosis in humans: genetics and environment. *Vet Parasitol* 193:342–352. <https://doi.org/10.1016/j.vetpar.2012.12.030>
- Macpherson CN (2013) The epidemiology and public health importance of toxocariasis: a zoonosis of global importance. *Int J Parasitol* 43:999–1008. <https://doi.org/10.1016/j.ijpara.2013.07.004>
- Chou CM, Lee YL, Liao CW, Huang YC, Fan CK (2017) Enhanced expressions of neurodegeneration-associated factors, UPS impairment, and excess A $\beta$  accumulation in the hippocampus of mice with persistent cerebral toxocariasis. *Parasites Vectors* 10:1–14. <https://doi.org/10.1186/s13071-017-2578-6>
- Pinelli E, Brandes S, Dormans J, Fonville M, Hamilton CM, van der Giessen J (2007) *Toxocara canis*: effect of inoculum size on pulmonary pathology and cytokine expression in BALB/c mice. *Exp Parasitol* 115:76–82. <https://doi.org/10.1016/j.exppara.2006.06.002>
- Hanh NTL, Lee YL, Lin CL, Chou CM, Cheng PC, Quang HH, Fan CK (2020) Evidence for asthma in the lungs of mice inoculated with different doses of *Toxocara canis*. *Am J Trop Med Hyg* 103:2305–2314. <https://doi.org/10.4269/ajtmh.20-0484>
- Deshayes S, Bonhomme J, de La Blanchardière A (2016) Neurotoxocariasis: a systematic literature review. *Infection* 44:565–574. <https://doi.org/10.1007/s15010-016-0889-8>
- Gale SD, Hedges DW (2020) Neurocognitive and neuropsychiatric effects of toxocariasis. *Adv Parasitol* 109:261–272. <https://doi.org/10.1016/bs.apar.2020.01.009>
- Finsterer J, Auer H (2007) Neurotoxocarosis. *Revista do Instituto De Medicina. Trop de São Paulo* 49:279–287. <https://doi.org/10.1590/S0036-46652007000500002>
- Fan CK, Holland CV, Loxton K, Barghouth U (2015) Cerebral toxocariasis: silent progression to neurodegenerative disorders? *Clin Microbiol Rev* 28:663–686. <https://doi.org/10.1128/cmr.00106-14>
- Çelik T, Kaplan Y, Ataş E, Öztuna D, Berilgen S (2013) *Toxocara* seroprevalence in patients with idiopathic parkinson's disease: chance association or coincidence? *Biomed Res Int* 2013:685196. <https://doi.org/10.1155/2013/685196>
- Kolbeková P, Kolářová L, Větvička D, Syrůček M (2011) Imaging of *Toxocara canis* larvae labelled by CFSE in BALB/c mice. *Parasitol Res* 108:1007–1014. <https://doi.org/10.1007/s00436-010-2145-y>
- Liao CW, Fan CK, Kao TC, Ji DD, Su KE, Lin YH, Cho WL (2008) Brain injury-associated biomarkers of TGF-beta1, S100B, GFAP, NF-L, tTG, AbetaPP, and tau were concomitantly enhanced and the UPS was impaired during acute brain injury caused by *Toxocara canis* in mice. *BMC Infectious Diseases* 8:1–15. <https://doi.org/10.1186/1471-2334-8-84>
- Dunsmore J, Thompson R, Bates I (1983) The accumulation of *Toxocara canis* larvae in the brains of mice. *Int J Parasitol* 13:517–521. [https://doi.org/10.1016/S0020-7519\(83\)80017-4](https://doi.org/10.1016/S0020-7519(83)80017-4)
- Ruiz-Manzano RA, Hernández-Cervantes R, Del Rio-Araiza VH, Palacios-Arreola MI, Nava-Castro KE, Morales-Montor J (2019) Immune response to chronic *Toxocara canis* infection in a mice model. *Parasite Immunol* 41:e12672. <https://doi.org/10.1111/pim.12672>
- Holland CV, Hamilton CM (2013) The significance of cerebral toxocariasis: a model system for exploring the link between brain involvement, behaviour and the immune response. *J Exp Biol* 216:78–83. <https://doi.org/10.1242/jeb.074120>
- Eid MM, El-Kowrany SI, Othman AA, El Gendy DI, Saied EM (2015) Immunopathological changes in the brain of immunosuppressed mice experimentally infected with *Toxocara canis*. *Korean J Parasitol* 53:51–58. <https://doi.org/10.3347/kjp.2015.53.1.51>
- Springer A, Heuer L, Janecek-Erfurth E, Beineke A, Strube C (2019) Histopathological characterization of *Toxocara canis*- and *T. cati*-induced neurotoxocarosis in the mouse model. *Parasitol Res* 118:2591–2600. <https://doi.org/10.1007/s00436-019-06395-7>
- Fan CK (2020) Pathogenesis of cerebral toxocariasis and neurodegenerative diseases. *Adv Parasitol* 109:233–259. <https://doi.org/10.1016/bs.apar.2020.01.008>
- Antolová D, Reiterová K, Stanko M, Zalesny G, Fričová J, Dvorožňáková E (2013) Small mammals: paratenic hosts for species of *Toxocara* in eastern Slovakia. *J Helminthol* 87:52–58. <https://doi.org/10.1017/S0022149X11000848>

23. Hildebrand J, Zalesny G, Okulewicz A, Baszkiewicz K (2009) Preliminary studies on the zoonotic importance of rodents as a reservoir of toxocarosis from recreation grounds in Wrocław (Poland). *Helminthologia* 46:80–84. <https://doi.org/10.2478/s11687-009-0016-9>
24. Havasióvá-Reiterová K, Tomašovicová O, Dubinský P (1995) Effect of various doses of infective *Toxocara canis* and *Toxocara cati* eggs on the humoral response and distribution of larvae in mice. *Parasitol Res* 81:13–17. <https://doi.org/10.1007/BF00932411>
25. Cox D, Holland C (2001) Relationship between three intensity levels of *Toxocara canis* larvae in the brain and effects on exploration, anxiety, learning and memory in the murine host. *J Helminthol* 75:33–41. <https://doi.org/10.1079/JOH200028>
26. Zaia MG, Oliveira SRPd C, CAd, Soares EG, Afonso A, Monnazzi LGS, Peitl Filho O, Faccioli LH, Anibal FdF (2015) *Toxocara canis* and the allergic process. *Memórias do Instituto Oswaldo Cruz* 110:726–731. <https://doi.org/10.1590/0074-02760150051>
27. Zhang X, Yang Y, Zheng Y, Hu Y, Rao Y, Li J, Zhao P, Li J (2022) The value of the antibody detection in the diagnosis of ocular toxocarosis and the aqueous cytokine profile associated with the condition. *Front Med* 9:838800. <https://doi.org/10.3389/fmed.2022.838800>
28. Kayes S, Omholt P, Grieve R (1985) Immune responses of CBA/J mice to graded infections with *Toxocara canis*. *Infect Immun* 48:697–703. <https://doi.org/10.1128/iai.48.3.697-703.1985>
29. Fenoy S, Rodero M, Pons E, Aguila C, Cuéllar C (2008) Follow-up of antibody avidity in BALB/c mice infected with *Toxocara canis*. *Parasitology* 135:725–733. <https://doi.org/10.1017/S0031182008004368>
30. Janecek E, Waindok P, Bankstahl M, Strube C (2017) Abnormal neurobehaviour and impaired memory function as a consequence of *Toxocara canis*-as well as *Toxocara cati*-induced neurotoxocarosis. *PLoS Negl Trop Dis* 11:e0005594. <https://doi.org/10.1371/journal.pntd.0005594>
31. Heuer L, Beyerbach M, Lühder F, Beineke A, Strube C (2015) Neurotoxocarosis alters myelin protein gene transcription and expression. *Parasitol Res* 114:2175–2186. <https://doi.org/10.1007/s00436-015-4407-1>
32. Hamilton CM, Stafford P, Pinelli E, Holland C (2006) A murine model for cerebral toxocarosis: characterisation of host susceptibility and behaviour. *Parasitology* 132:791–801. <https://doi.org/10.1017/S0031182006009887>
33. Akao N, Tomoda M, Hayashi E, Suzuki R, Shimizu-Suganuma M, Shichinohe K, Fujita K (2003) Cerebellar ataxia due to *Toxocara* infection in Mongolian gerbils, *Meriones unguiculatus*. *Vet Parasitol* 113:229–237. [https://doi.org/10.1016/S0304-4017\(03\)00079-7](https://doi.org/10.1016/S0304-4017(03)00079-7)
34. Fan CK, Lan HS, Hung CC, Chung WC, Liao CW, Du WY, Su KE (2004) Seroepidemiology of *Toxocara canis* infection among mountain aboriginal adults in Taiwan. *Am J Trop Med Hyg* 71:216–221. <https://doi.org/10.4269/ajtmh.2004.71.216>
35. Novák J, Panská L, Macháček T, Kolářová L, Horák P (2017) Humoral response of mice infected with *Toxocara canis* following different infection schemes. *Acta Parasitol* 62:823–835. <https://doi.org/10.1515/ap-2017-0099>
36. De Savigny D (1975) In vitro maintenance of *Toxocara canis* larvae and a simple method for the production of *Toxocara* ES antigen for use in serodiagnostic tests for visceral larva migrans. *J Parasitol* 61:781–782. <https://doi.org/10.2307/3279492>
37. Kolbeková P, Větvicka D, Svoboda J, Skirnisson K, Leissová M, Syrůček M, Marečková H, Kolářová L (2011b) *Toxocara canis* larvae re-infecting BALB/c mice exhibit accelerated speed of migration to the host CNS. *Parasitol Res* 109:1267–1278. <https://doi.org/10.1007/s00436-011-2371-y>
38. Fok É, Kassai T (1998) *Toxocara canis* infection in the paratenic host: a study on the chemosusceptibility of the somatic larvae in mice. *Vet Parasitol* 74:243–259. [https://doi.org/10.1016/S0304-4017\(97\)00086-1](https://doi.org/10.1016/S0304-4017(97)00086-1)
39. Hermes G, Ajioka JW, Kelly KA, Mui E, Roberts F, Kasza K, Mayr T, Kirisits MJ, Wollmann R, Ferguson DJ (2008) Neurological and behavioral abnormalities, ventricular dilatation, altered cellular functions, inflammation, and neuronal injury in brains of mice due to common, persistent, parasitic infection. *J Neuroinflamm* 5:1–37. <https://doi.org/10.1186/1742-2094-5-48>
40. Al-Hassnawi ATS, Al-Quraishi MA (2014) The effect of experimental *Toxocara canis* infection on behavior manipulating in albino rats. *J Babylon Univ Pure Appl Sci* 22:2389–2395
41. Strube C, Waindok P, Raulf MK, Springer A (2020) *Toxocara*-induced neural larva migrans (neurotoxocarosis) in rodent model hosts. *Adv Parasitol* 109:189–218. <https://doi.org/10.1016/bs.apar.2020.01.006>
42. Cox D, Holland C (2001) Influence of mouse strain, infective dose and larval burden in the brain on activity in *Toxocara*-infected mice. *J Helminthol* 75:23–32. <https://doi.org/10.1079/JOH200027>
43. Janecek E, Wilk E, Schughart K, Geffers R, Strube C (2015) Microarray gene expression analysis reveals major differences between *Toxocara canis* and *Toxocara cati* neurotoxocarosis and involvement of *T. canis* in lipid biosynthetic processes. *Int J Parasitol* 45:495–503. <https://doi.org/10.1016/j.ijpara.2015.02.009>
44. Fox W (1965) Reflex-Ontogeny and behavioural development of the mouse. *Anim Behav* 13:234–241. [https://doi.org/10.1016/0003-3472\(65\)90041-2](https://doi.org/10.1016/0003-3472(65)90041-2)
45. Deacon RM (2013) Measuring motor coordination in mice. *J Visualized Experiments* 2013(e2609). <https://doi.org/10.3791/2609>
46. Carbone L, Carbone ET, Yi EM, Bauer DB, Lindstrom KA, Parker JM, Austin JA, Seo Y, Gandhi AD, Wilkerson JD (2012) Assessing cervical dislocation as a humane euthanasia method in mice. *J Am Assoc Lab Anim Sci* 51:352–356
47. Poggensee G, Sahebali S, Van Marck E, Swai B, Krantz I, Feldmeier H (2001) Diagnosis of genital cervical schistosomiasis: comparison of cytological, histopathological and parasitological examination. *Am J Trop Med Hyg* 65:233–236. <https://doi.org/10.4269/ajtmh.2001.65.233>
48. Taira K, Saeed I, Lind P, Murrell K, Kapel C (2003) Population dynamics of *Toxocara canis* in pigs receiving a single or multiple infection. *Parasitology* 127:593–602. <https://doi.org/10.1017/S0031182003004074>
49. Good B, Holland C, Stafford P (2001) The influence of inoculum size and time post-infection on the number and position of *Toxocara canis* larvae recovered from the brains of outbred CD1 mice. *J Helminthol* 75:175–181. <https://doi.org/10.1079/JOH200178>
50. Janecek E, Beineke A, Schnieder T, Strube C (2014) Neurotoxocarosis: marked preference of *Toxocara canis* for the cerebrum and *T. cati* for the cerebellum in the paratenic model host mouse. *Parasites Vectors* 7:1–13. <https://doi.org/10.1186/1756-3305-7-194>
51. Schoenardie ER, Scaini CJ, Avila LFdCd, Sperotto RL, Borsuk S, Felicetti CDP, Pepe M, Berne MEA (2014) Determination of IgG avidity in BALB/c mice experimentally infected with *Toxocara canis*. *Revista Brasileira De Parasitol Veterinária* 23:403–406. <https://doi.org/10.1590/S1984-29612014060>
52. de Souza Aguiar P, Furtado RD, de Avila LFC, de Lima Telmo P, Martins LHR, Berne MEA, da Silva PEA, Scaini CJ (2015) Transmammary infection in BALB/c mice with chronic toxocarosis. *Parasitol Int* 64:145–147. <https://doi.org/10.1016/j.parint.2014.04.010>
53. Miller RA, Harper JM, Galecki A, Burke DT (2002) Big mice die young: early life body weight predicts longevity in

- genetically heterogeneous mice. *Aging Cell* 1:22–29. <https://doi.org/10.1046/j.1474-9728.2002.00006.x>
54. Correa M, Sanchis-Segura C, Aragon C (2001) Influence of brain catalase on ethanol-induced loss of righting reflex in mice. *Drug Alcohol Depend* 65:9–15. [https://doi.org/10.1016/S0376-8716\(01\)00142-9](https://doi.org/10.1016/S0376-8716(01)00142-9)
  55. O’Leary T, Robertson A, Chipman P, Rafuse V, Brown R (2018) Motor function deficits in the 12 month-old female 5xFAD mouse model of Alzheimer’s disease. *Behav Brain Res* 337:256–263. <https://doi.org/10.1016/j.bbr.2017.09.009>
  56. Kim JH, Lee YW, Park YM, Park KA, Park SH, Lee WT, Lee JE (2011) Agmatine-reduced collagen scar area accompanied with surface righting reflex recovery after complete transection spinal cord injury. *Spine* 36:2130–2138. <https://doi.org/10.1097/BRS.0b013e318205e3f7>
  57. Gao S, Calderon DP (2020) Robust alternative to the righting reflex to assess arousal in rodents. *Sci Rep* 10:20280. <https://doi.org/10.1038/s41598-020-77162-3>
  58. Harkema L, Youssef SA, de Bruin A (2016) Pathology of mouse models of accelerated aging. *Vet Pathol* 53:366–389. <https://doi.org/10.1177/0300985815625169>
  59. Vanhooren V, Libert C (2013) The mouse as a model organism in aging research: usefulness, pitfalls and possibilities. *Ageing Res Rev* 12:8–21. <https://doi.org/10.1016/j.arr.2012.03.010>
  60. Kayes SG, Oaks JA (1976) Effect of inoculum size and length of infection on the distribution of *Toxocara canis* larvae in the mouse. *Am J Trop Med Hyg* 25:573–580. <https://doi.org/10.4269/ajtmh.1976.25.573>
  61. Skerrett H, Holland C (1997) Variation in the larval recovery of *Toxocara canis* from the murine brain: implications for behavioural studies. *J Helminthol* 71:253–256. <https://doi.org/10.1017/S0022149X0001600X>
  62. Farjat JAB, Minvielle MC, Pezzani BC, Niedfeld G (1995) Relationship between parasitological inoculum and immunological parameters in experimental toxocariasis. *Zentralblatt für Bakteriologie* 282:465–473. [https://doi.org/10.1016/S0934-8840\(11\)80720-6](https://doi.org/10.1016/S0934-8840(11)80720-6)
  63. Burren C (1971) The distribution of *Toxocara* larvae in the central nervous system of the mouse. *Trans R Soc Trop Med Hyg* 65:450–453. [https://doi.org/10.1016/0035-9203\(71\)90155-6](https://doi.org/10.1016/0035-9203(71)90155-6)
  64. Heintz N (2004) Gene Expression Nervous System Atlas (GENSAT) <http://www.gensat.org/>. 28.6.2004
  65. Wong K, Armstrong RC, Gyure KA, Morrison AL, Rodriguez D, Matalon R, Johnson AB, Wollmann R, Gilbert E, Le TQ (2000) Foamy cells with oligodendroglial phenotype in childhood ataxia with diffuse central nervous system hypomyelination syndrome. *Acta Neuropathol* 100:635–646. <https://doi.org/10.1007/s004010000234>
  66. Resende NM, Gazzinelli-Guimarães PH, Barbosa FS, Oliveira LM, Nogueira DS, Gazzinelli-Guimarães AC, Gonçalves MTP, Amorim CC, Oliveira FM, Caliani MV (2015) New insights into the immunopathology of early *Toxocara canis* infection in mice. *Parasites Vectors* 8:1–11. <https://doi.org/10.1186/s13071-015-0962-7>
  67. Topal A, Alak G, Altun S, Erol HS, Atamanalp M (2017) Evaluation of 8-hydroxy-2-deoxyguanosine and NFκB activation, oxidative stress response, acetylcholinesterase activity, and histopathological changes in rainbow trout brain exposed to linuron. *Environ Toxicol Pharmacol* 49:14–20. <https://doi.org/10.1016/j.etap.2016.11.009>
  68. Kühl B, Beyerbach M, Baumgärtner W, Gerhauser I (2022) Characterization of microglia/macrophage phenotypes in the spinal cord following intervertebral disc herniation. *Front Veterinary Sci* 9:942967. <https://doi.org/10.3389/fvets.2022.942967>

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