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Research Note

Baylisascaris potosis larvae in mice of different strains and infectivity of tissue larvae

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Summary

Migration of *Baylisascaris potosis* larvae in different mouse strains were compared, and infectivity of the persisting larvae in mice tissues were investigated. Five strains of mice, BALB/c, C57BL/6, AKR, B10.BR, and ICR were inoculated with 1,000 *B. potosis* eggs/mouse, and necropsied at week 13 post inoculation (PI). The other uninfected ICR mice (secondary host) were inoculated with 43 larvae/mouse recovered from mice at week 13 PI with eggs, and necropsied at day 21 PI. Larvae in organs or tissues were counted at necropsy. One AKR mouse showed torticollis and circling at day 56 PI. At necropsy at week 13 PI, larvae were recovered from all mice. A mean total larvae recovered were 124.1 (n=40). Majority of larvae were found in the carcass (mean 113.9) and some in the viscera (mean 9.9). Zero to 1 larva were found in the brain or eyes of some mice. There were no differences among the mouse strains in the number of larvae, except in the viscera; more larvae were seen in BALB/c or ICR than in B10.BR mice. No larvae were found in the secondary host mice. Present study demonstrated that *B. potosis* larvae migrate well in the carcass of any strains of mice, however, the tissue larvae did not infect the secondary host. Results of our present study suggest that *B. potosis* larvae is less aggressive for the nervous tissue migration than that of *B. procyonis* larvae which is commonly known to migrate in central nervous system of mammals and birds.
Keywords: *Baylisascaris potosis*; larva migrans; mice; paratenic hosts

Introduction

The larvae of the genus *Baylisascaris* can cause extensive tissue damage in the paratenic hosts, and are considered to be causative agents of visceral, ocular or neural larva migrans in mammals, including humans (Bauer 2013; Cho *et al*., 2007; Kazacos 2016). Among the genus *Baylisascaris*, *B. procyonis* of raccoons is recognized as the most pathogenic species for paratenic hosts, causing serious or fatal neurological manifestations (Kazacos 2001; Sapp *et al*., 2017; Tiner 1953).

The large roundworm that infected kinkajous was previously thought to be *B. procyonis* (Kazacos *et al*., 2011; Overstreet 1970). However, genetic and morphological analysis revealed that the roundworm of kinkajous differed from *B. procyonis* (Taira *et al*., 2013a), and has been newly described as *B. potosis* (Tokiwa *et al*., 2014). Kinkajous (*Potos flavus*) belongs to the family Procyonidae and are closely related to the raccoon (*Procyon lotor*), which is the natural final host of *B. procyonis*.

Kinkajous are kept as exotic pets in some countries. In Japan in 2016, 8 out of 29 (27.6 %) imported captive kinkajous, mainly from the Republic of Guyana, were positive for *Baylisascaris* eggs in their feces (Tokiwa *et al*., 2016). The close genetic relationship be-

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tween *B. potosis* and *B. procyonis* as well as between their hosts underline the potential risk of the serious neural larva migrans in humans.

Taira *et al*. (2018) conducted an experimental infection study to compare the pathogenicity of *B. potosis* and *B. transfuga* (the roundworm of bears) larvae in mice. The authors reported that the migration ability of *B. potosis* larvae to the brain was lower than that of *B. transfuga*, and suggested that the tendency for *B. potosis* larvae to migrate to the brain and cause neurological signs is not as high as that known for *B. procyonis*.

Larval migration of ascarid nematode in the brain of mice differs depending on the mouse strain. Epe *et al*. (1994) and Hamilton *et al*. (2006) reported that the number of the dog roundworm *Toxocara canis* larvae in the brain of experimentally infected mice differed depending on the mice strains. These reports underline the significance for comparing roundworm larval migration in different strains of mice.

Baylisascaris potosis may have a wide range of paratenic hosts as it is known in the other *Baylisascaris* species. However, the roles of paratenic hosts in the life cycle of *B. potosis* have not been studied. The aims of this study were to investigate the occurrence of clinical signs in *B. potosis* infected mice, migration of the larvae in different mouse strains, and to evaluate the infectivity of any persisting larvae in the mice tissues.

Material and Methods

Parasites

Eggs of *B. potosis* were collected from feces of naturally infected kinkajous, which were imported from Guyana to Japan. Fecal eggs were collected by the flotation method using a fluid (1 L saturated NaCl with 500 g glucose, 1.28 SG) and cultured in 0.5 % formalin solution at 25°C for about 1 month for embryonation. The embryonated eggs were preserved in 0.5 % formalin solution at 10°C for up to 6 months. The eggs were washed with tap water prior to inoculation to remove the formalin.

Experimental animals

Five strains of mice, ICR (outbred), AKR (inbred), C57BL/6 (inbred), BALB/c (inbred) and B10.BR (congenic) were used in the study. Eight 5-week-old male mice of each strain were purchased from a commercial experimental animals company (Japan SLC, Inc., Shizuoka Japan). Mice were housed in plastic cages, provided with standard commercial pellets (CLEA Rodent Diet CE-2, CLEA Japan, Inc.) and water *ad libitum*. They were acclimatized for 1 week prior to the experimental infection.

Design of experiment

Eggs inoculation study: Each mouse was inoculated with 1,000

Table 1. Number of larvae recovered from mice necropsied at 13 weeks post inoculation with1,000 *Baylisascaris potosis* eggs.

Mouse		**Mean number of larvae recovered (±SD)**				
Strain	n	Carcass	Viscera*	Brain	Eyes	Total
BALB/C (inbred)	8	171.8 (±119.5)	18.6[a] (±15.3)	0.3 (±0.5)	0	190.6 (±127.6)
C57BL/6 (inbred)	8	111.6 (±58.3)	4.8 (±2.7)	0	0	116.4 (±56.7)
AKR (inbred)	8	87.8 (±57.8)	8.3 (±4.6)	0.3 (±0.5)	0.1 (±0.4)	96.4 (±57.1)
B10.BR (congenic)	8	75.4 (±23.9)	2.6[b] (±2.8)	0.4 (±0.5)	0	78.4 (±25.4)
ICR (outbred)	8	123.1 (±53.3)	15.4[a] (±11.5)	0	0.4 (±0.5)	138.9 (±60.7)
Mean		**113.9**	**9.9**	**0.2**	**0.1**	**124.1**

a,b; Number of larvae recovered was significantly different between a and b ($P<0.05$, Steel-Dwass test).

(1028.1 ± 62.5 (95 %CI)) embryonated *B. potosis* eggs by stomach tube attached to a 1-ml syringe. Mice were monitored daily for clinical signs during routine animal care. In particular, they were carefully observed for the onsets of neurological signs such as rolling, circling or torticollis. The animals were anaesthetized with isoflurane, and then euthanized at week 13 post inoculation (PI), and the larvae in the mice were counted after using a pepsin digestion method.

Larval inoculation study: Forty three 13-week-old motile *B. potosis* larvae/mouse recovered from the carcasses of previously infected mice were inoculated into 6 other uninfected ICR mice as a secondary paratenic host. Mice were monitored daily for clinical signs during routine animal care, and were euthanized and necropsied at day 21 PI, followed by larval count of mouse tissues.

Recovery of larvae

Eight mice of each mouse strain (ICR, AKR, C57BL/6, BALB/c and B10.BR) at week 13 PI with embryonated eggs and 6 ICR mice inoculated with larvae were subjected to a pepsin digestion for larval recovery. The carcass (without skin, tail, tips of limbs and tips of nose) and the viscera (without stomach and intestines) were separated individually, and digested for larval counts.

The digestion method used was according to that of Taira *et al*. (2011). Briefly, each organ was minced and digested in an HCl-pepsin solution at 37°C for 2 h under constant stirring. The ratio of tissue (g) to digestive fluid (ml) was approximately 1:10. Following digestion, the fluid were settled for 1 h at 37°C for sedimentation of larvae. Then, the sediment containing the larvae was filtered through a tea sieve into a centrifugal tube with 37°C saline, and allowed to settle for 1 h. The number of larvae in the sediment was counted under a light microscope within 24 hours after digestion.

The brain, the spinal cord and the eyes were removed individually and pressed between two slide-glasses to count the larvae under a light microscope. For the mice that showed neurological signs such as circling, their semicircular canals were also pressed between slide-glasses to detect the presence of any larvae.

Statistics

The difference in the number of larvae in the carcass, viscera, brain, and eyes among the mice strains were analyzed for statistical significances using the Steel-Dwass test, a non-parametric multiple comparison. The difference was assessed with significance level of 5 % (P<0.05) using a statistical program Statcel 4 (OMS publ. Tokyo Japan).

Ethical Approval and/or Informed Consent

This study was approved by the Institutional Animal Care and Use Committee of Azabu University with the reference number 130207-3, and the animals were treated according to the rules and regulations.

Results

Clinical signs

One AKR mouse inoculated with 1,000 *B. potosis* eggs showed torticollis and circling (running in a circle) at day 56 PI. This mouse was euthanized, and the brain, spinal cord and semicircular canals were pressed between slide-glasses to search for larvae, but no larvae were detected. The other mice inoculated with eggs did not show any neurological nor other clinical signs, and survived until necropsy at week 13 PI. No mice inoculated with the 13-week-old larvae which were recovered from the egg-inoculated mice showed any neurological or other clinical signs.

Number of larvae recovered

The number of larvae recovered from mice necropsied at week 13 PI that were inoculated with 1,000 *B. ptosis* eggs is presented in Table 1. Larvae were recovered from all mice inoculated with the eggs, and a mean total number of larvae was 124.1 (n=40). Majority of the larvae were found from the carcass (mean 113.9 larvae, n=40) and some from the viscera (mean 9.9 larvae, n=40). One larva was each found from the brain of 6 mice (total 6 larvae), and 1 larva each was found from the eye of 4 mice (total 4 larvae). No larvae were found from the spinal cord. No statistical differences in the total number of larvae recovered, the number of larvae in the carcass, in the brain and in the eyes were seen among the mouse strains. However, the number of larvae in the viscera was significantly different among the mouse strain (P<0.05); higher number of larvae were recovered from BALB/c or ICR mice than B10.BR mice.

No larvae were found from the ICR mice inoculated with the 13-week-old larvae, which were recovered from the egg-inoculated mice.

Discussion

Serious neurological signs as manifested by torticollis and circling were observed in one AKR mouse inoculated with 1,000 *B. potosis* eggs. Although no larvae were detected from the brain, spinal cord or semicircular canals of this mouse, we speculated that only a few larvae migrated in the nervous system causing the neurological disorder in this mouse, since the other mice had a maximum of only 1 larvae in the brain. Since the mouse showed neurological disorder, it may present a potential risk of a clinical larva migrans by migration of *B. potosis* larvae in humans.

Taira *et al*. (2018) conducted an experimental infection study to compare the pathogenicity of *B. potosis* and *B. transfuga* larvae in mice. The rate of onset of neurological signs of *B. potosis* infected mice and the migration pattern of *B. potosis* larvae in the mice were almost in accordance with the present study. Epe *et al*. (1994) reported different migration behavior of *Toxocara canis* larvae in different mouse strains. In that study, higher number of larvae in the brain were observed for BALB/c mice as compared to

other strains. In contrast, no relationship between the mouse strain and the tendency of the larval migration to the central nervous system was seen in our present study.

Baylisascaris procyonis is a nematode of raccoons, and is considered to be the most common cause of clinical larva migrans in paratenic hosts including humans (Bauer 2013). The larvae migrate aggressively to somatic tissues, and invade the central nervous system (Gavin *et al*., 2005). Consequently, the infected animals died after presenting severe neurological disorders (Sorvillo *et al*., 2002). In an experimental infection study of *B. procyonis* in mice, Miyashita (1993) reported that 100 % of the mice inoculated with 100 to 2,000 embryonated eggs showed neurological signs, and died within day 32 PI. Sheppard and Kazacos (1997) described that mice inoculated with 50 to 500 eggs died within around day 20 PI, and the average number of larvae recovered from the brain was 1.3, 13 and 24.4 in mice inoculated with 50, 250 and 500 eggs, respectively. In the present study, no mice showed any clinical sign or death, with the exception of one AKR mouse that presented neurological signs. The mean number of larvae recovered from the brain of mice inoculated with 1,000 eggs/mouse in the present study was 0.2 (n=40). In terms of the onset of neurological signs and the larval preference to migrate to the brain in the mice, the results of the present study supported the notion that *B. potosis* larvae is less cranio-trophic than *B. procyonis* larvae.

Bayliascaris columnaris, the roundworm of skunks, is also genetically closely related to *B. potosis* and *B. procyonis* (Taira *et al*., 2013a; Tokiwa *et al*., 2014; Choi *et al*., 2017). Tiner (1953) reported that 64 % (7 /11) of mice that ingested 200 eggs of *B. columnaris* showed neurological symptoms or death at days 14 to 30 PI, and 1 to 9 larvae/mouse were recovered from the brain at days 43 to 46 PI. Thus, we have demonstrated that *B. potosis* larvae showed lower tendency to migrate to the brain than that of *B. columnaris* larvae.

No larvae were recovered from the mice inoculated with the 43 larvae that were obtained from the carcasses of the embryonated egg-infected mice at week 13 PI. This indicates that migration of larvae to the visceral organs or muscles in secondary paratenic hosts may not occur. On the contrary, *Toxocara cati,* the roundworm of cats, showed a high tissue migration ability in the secondary paratenic hosts such as mice, rats and chickens (Taira *et al*., 2011; Taira *et al*., 2013bc). *Toxocara* larvae do not grow in size in paratenic hosts, whereas *Baylisascaris* larvae grow in size in the paratenic hosts (Taira *et al*., 2018). Size of larvae possibly relate to the ability to infect the secondary paratenic host. The reason why the larvae of *B. potosis* in mice tissue did not migrate in the secondary paratenic hosts need to be further studied. Moreover, the infectivity of the *B. potosis* larvae in the paratenic hosts to the final host kinkajous need to be examined, so that the role of the paratenic hosts in the life cycle of *B. potosis* can be elucidated.

In conclusion, the present study demonstrated the larval migration of *B. potosis* in different strains of mice; many larvae were found in the carcass, some in the viscera, and a few in the brain and eyes. Mouse strains does not affect the number of larvae in any tissues, except in the viscera. The 13-week-old larvae of *B. potosis* recovered from mouse tissues could not infect other mice as a secondary paratenic host.

Conflict of Interest

The authors have no conflicts of interest directly relevant to the content of this article.

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