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Research Note

Experimental infection with *Baylisascaris potosis* in chickens

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Summary

The larvae of the genus *Baylisascaris* can cause larva migrans in mammals and birds. This study investigated the larval migration of *Baylisascaris potosis*, the roundworm of kinkajou (*Potos flavus*), in chickens and the associated clinical manifestations of the host. Thirty-six 3-week-old chickens divided into 6 groups were orally inoculated with 3,000 *B. potosis* eggs/chick. Each group of chicken was necropsied at days 1, 2, 3, 7, 30 and 90 PI (post inoculation), and the number of larvae in various organs were counted until day 90 PI. No clinical signs were observed in chickens during the study. Larvae were detected from the liver, lungs or breast-muscles of 13/36 (36.1%) chickens. The mean total number of larvae in the liver, lungs and breast-muscles at days 1, 2, 3, 7, 30 and 90 PI were 0.34, 0.17, 1.66, 1.01, 0.17 and 0, respectively. No larvae were found in the brain, eyes, hid-limb muscles, heart, kidneys and spleen. Although infectivity of larvae in egg-inoculated chickens was low, the present study demonstrated that *B. potosis* larvae can migrate in chickens tissues up to day 30 PI. The result suggests that chickens can serve as a paratenic host for *B. potosis* and may underline a public health importance of *B. potosis* infection as a potential foodborne disease in humans.
Keywords: *Baylisascaris potosis*; Larva migrans; Chicken; Paratenic hosts

Introduction

The larvae of the genus *Baylisascaris* can cause tissue damage in the paratenic hosts, and are considered to be causative agents of visceral, ocular or neural larva migrans in mammals and birds (Sapp *et al*. 2017; Bauer 2013; Gavin *et al*. 2005). *Baylisascaris procyonis* of raccoons are known to cause avian cerebrospinal nematodiasis leading to mortality, and the larvae had been found in central nervous system of chickens (Richardson *et al*. 1979), emus (Kazacos *et al*. 1981), partridges (Sass 1978), bobwhites quails (Reed *et al*. 1981) or Australian brush turkeys (Kazacos *et al*. 1982). The roundworm of kinkajous was previously thought to be *B. procyonis* (Kazacos *et al*. 2011; Overstreet 1970). Genetic and morphological analysis revealed that the roundworm of kinkajous differed from *B. procyonis* (Taira *et al*. 2013), and has been newly described as *B. potosis* (Tokiwa *et al*. 2014). Kinkajous (*Potos flavus*) belong to the family Procyonidae and are closely related to the raccoon (*Procyon lotor*), which is the natural final host of *B. procyonis*.

Kinkajous is kept as pets in household in some countries. In Japan in 2016, 8 out of 29 (27.6 %) imported captive kinkajous, the majority from the Republic of Guyana, were positive for *Baylisascaris* eggs in the feces (Tokiwa *et al*. 2016). The close genetic relationship between *B. potosis* and *B. procyonis* as well as between their hosts underline a potential risk of larva migrans both in humans and birds.

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Experimental study of *B. potosis* larvae in mice, rats and rabbits was revealed that the migration activity of larvae in host tissues is not as high as the larvae of *B. transfuga*, the roundworm of bears (Taira *et al*, 2018). The authors suggested that the pathogenicity of *B. potosis* larvae would be lower than that of *B. procyonis*, the roundworm of raccoons. There are no reports on the migration and the clinical sings of hosts by *B. potosis* larvae in chickens. Chickens is known to be a paratenic host of ascarid larvae such as *Toxocara canis* and *T. cati*, the round worm of dogs and cats (Taira *et al*. 2003; Taira *et al*. 2011), is able to transmit the larvae to humans through the ingestion of raw or undercooked meat, and consequently cause larva migrans syndrome in humans.
The aims of this study are to investigate *B. potosis* larval migration in chickens and the associated clinical manifestations of host.

Material and Methods

Parasites
Eggs of *B. potosis* were collected from feces of naturally infected kinkajous, which were imported from Guyana to Japan, by a sugar-salt flotation technique (Taira *et al*., 2018) 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 3 months. The eggs were washed twice with tap water prior to inoculation to remove formalin.

Experimental animals
Thirty six 3-week-old chickens (Boris-Brown breed) of both sexes and seven 5-week-old male mice (ICR strain, outbred) were used in the study. Chickens were hatched in our laboratory from fertilized eggs purchased from a commercial farm, and kept in our laboratory. Mice were purchased from a commercial supplier of experimental animals (Japan SLC, Inc., Shizuoka, Japan). All animals were kept at 25°C and provided with commercial feed (Bird food, Pet's One Japan, Inc. or CLEA Rodent Diet CE-2, CLEA Japan, Inc.) and water *ad libitum*. Animals were acclimatized for 1 week prior to the experimental infection.

Design of experiment
Chickens were orally inoculated with 3,000 (2918.3 ± 193.7 (95 % confidence interval) *B. potosis* embryonated eggs/chick by a 1 ml pipet, and 6 birds were euthanized by intracranial injection of 70 % ethanol and necropsied at days 1, 2, 3, 7, 30 and 90 post inoculation (PI), respectively, for larval counts.
Mice were inoculated with 2,000 (1941.0 ± 135.3 (95 %CI)) embryonated *B. potosis* eggs/mouse to serve as a control for the infectivity of eggs. The eggs were of the same batch as those used to inoculate the chickens. Inoculation in mice was conducted by a stomach tube attached to a 1 ml syringe. The infected mice were euthanized by giving isoflurane anesthesia followed by cervical dislocation and necropsied at days 28 or 60 PI for larval counts.
Animals were monitored daily for clinical symptoms during routine animal care. In particular, they were carefully observed for the onset of neurological signs, such as torticollis, ataxia, circling, extensor rigidity and paralysis.

Recovery of larvae
For chickens, the liver, lungs, breast muscles, hind-limb muscles, eyes, heart, kidneys, and spleen were removed individually, and digested for larval counts. For mice, the whole body without stomach, intestines, skin, tail, tips of limbs and tips of nose were digested for larval counts.
The digestion was done according to Taira *et al*., (2018). Briefly, each organ was minced and digested in an 1 % of HCl (37 %) - pepsin (1:10,000) 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 was filtered through a 42-mesh metal 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 and the eyes were removed individually and pressed between two slide-glasses to count the larvae under a light microscope.

Table 1. Number of larvae recovered from 3 week-old chickens (n=6/group) inoculated with 3,000 *Baylisascaris potosis* eggs/chick.

Days PI[1)]	Mean number of larvae recovered					Recovery (%)[3)]
	Liver	Lungs	Breast muscles	Others[2)]	Total	
1	0.17	0.17	0	0	0.34	0.011
2	0.17	0	0	0	0.17	0.006
3	0.33	1.33	0	0	1.66	0.055
7	0.17	0.67	0.17	0	1.01	0.034
30	0.17	0	0	0	0.17	0.006
90	0	0	0	0	0	0

1) Post inoculation
2) Hind-limb muscles, heart, brain, eyes, kidneys and spleen
3) Total recovered larvae / 3,000 (inoculum) x 100

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 experimental animals were kept according to the rules and regulations.

Results

Larvae in chickens

No neurological symptoms nor abnormal behaviors were observed in chickens throughout the study. Larvae were detected in the liver, lungs and breast muscles of 13/36 (36.1 %) chickens (Table 1). The mean total number of larvae in the liver, lungs and breast-muscles at days 1, 2, 3, 7, 30 and 90 PI were 0.34, 0.17, 1.66, 1.01, 0.17 and 0, respectively. No larvae were found from the hind-limb muscles, brain, eyes, heart, kidneys and spleen. The mean recovery of larvae were less than 0.055 % for all the groups of chicken sacrificed at each day.

Larvae in mice

No neurological symptoms nor abnormal behaviors were observed in the mice throughout the study. There are no statistical differences in the number of larvae between mice necropsied at days 28 and 60 PI (t=-.0205, df=4.47, n.s.: Welch's t-test) (Table 2). The mean number of larvae recovered was 328.9 (n=7), and the mean recovery of larvae was 16.4 %.

Discussion

The results of the present study suggested that the chicken can be a paratenic host for *B. potosis*, because larvae could still be found in tissues of experimentally infected chickens up to days 30 PI. The result implies a risk in public health since the chicken sashimi (raw chicken meat) is a delicacy in some regions in Japan and Korea.

Kazacos and Wirtz (1982) reported that 3-day-old chickens orally inoculated with 400 to 3,200 infective eggs/chick of *B. procyonis*, the round worm of raccoons, showed clinical signs of central nervous system (CNS) such as torticollis, ataxia, circling, extensor rigidity and paralysis. The onset of the CNS signs was at an average of day 20.4 PI, and the duration of CNS signs varied from less than 1 to 23 days. They also reported that chickens receiving higher dosages exhibited much more severe clinical signs, had higher mortality rates, and survived for a shorter duration after the onset of the signs than those receiving lower dosages. Richardson (1979) reported an outbreak of clinical case of fatal encephalitis by *B. procyonis* larvae in 1 to 7-week-old White-Leghorn chickens in Indiana, USA.

In our present study, 3-week-old chickens inoculated with 3,000 embryonated eggs of *B. potosis* neither died nor had shown any symptoms, and no larvae were recovered from the brain of chickens. Comparing the results of our study and those of Kazacos and Wirtz (1982) and Richardson (1979), it is suggested that *B. potosis* larvae is less aggressive in tissue migration in chickens than that of *B. procyonis* larvae.

We also observed that the recovery of larvae from mice was 16.4 %, while it was less than 0.06 % from chickens. This disparity might be due to the physiological difference between mammals and birds, such as difference in body temperature and immunological responses. Difference between rodent and birds in coevolutionary status with *B. potosis* may also be a factor of the disparity (Gernick 1992). It is also possible to speculate that most of embryonated egg failed to hatch in the gut of the chickens or those that hatched were not able to penetrate through the intestinal wall. Further studies are needed to elucidate the factors that led to the difference in the larval migration activity of *B. potosis* in mice and chickens.

In conclusion, the present study demonstrated that *B. potosis* larvae can infect chickens, and the result suggested that the chicken can be a paratenic host for *B. potosis*. The result may underline a public health importance of *B. potosis* infection as a potential foodborne disease in humans.

Table 2. Number of larvae recovered from mice inoculated with 2,000 *Baylisascaris potosis* eggs/mouse.

Mouse ID	Days PI[1)]	Recovered larvae	Recovery (%)[2)]
1	28	181	9.1
2		363	18.2
3		437	21.9
4	60	135	6.8
5		104	5.2
6		378	18.9
7		704	35.2
Mean (n=7)		328.9	16.4

1) Post inoculation

2) Recovered larvae / 2,000 (inoculum) x 100

Conflict of Interest

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

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