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Aspiculuris tetraptera Infection in Mice: Parasite degree and Differential Leukocyte

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Abstract

Aspiculuris tetraptera is one of helminth parasite commonly found in mice. The response of the mice to parasite infection varies depending on various factors. Immune system is one of the factors that affect the occurrence of parasite infection in the host. The purpose of this study were to observe the parasitemia level and the immune respons of mice that infected with Aspiculuris before and after challenge infection. Mice were infected with Aspiculuris twice to see the development of parasite and the immune respon. Feces and blood samples were collected from the mice. Feces samples were examined by floating method to find the parasite egg, whereas blood samples were examined by blood smears to count the differential leukocytes. The result showed that Aspiculuris tetraptera eggs appear in the feces on the 20th day after infected as many 200 eggs/gram and increased on the 26th day as much 300 eggs/gram of feces. The peaks of eosinophils were on the 3rd and 6th days at 2.8% and 3.1% respectively. The level of Neutrophils and monocytes showed fluctuations but the trend decrease from day to day. Lymphocyte levels also showed fluctuations but tended to increase from the first day to the 26th day.

Keywords: Aspiculuris, Endoparasite, Mice, Leukocytes

Introduction

Aspiculuris tetraptera is a parasitic worm commonly found in mice which is a type of nematode worm. These worms are very small and can be found in the digestive tract of mice. These worms are also commonly known as pinworms because of their small size. *Acpiculuris* is one of the main oxyurid found in the mice. Infections with pinworms generally are considered to be non or mildly pathogenic in animals with normal immune systems (Taffs, 1976). Pinworm infection is very common and contagious in laboratory mice. This Pinworm has direct life cycle and require around 23–25 days to complete their life cycle. Adult females live in the large intestine of the

host. They can survive from 45 to 50 days to lay their eggs. The adult female helminth migrate to the end of the large intestine and lay their eggs from proximal intestine. The worms which are expelled with the feces take about six days to become infective eggs (Phillipson 1973).

A. tetraptera has a direct life cycle in which the eggs produced are excreted in the feces and are not found in the perianal area of the host. An unembryonated *A. tetraptera* eggs develop into infective eggs in 5 to 8 days at 24°C-27°C and can survive around weeks in the environment (Chan, 1955; Wescott, 1982). Eggs that enter the body hatch and come out as larvae. The larvae then go to the middle colon and enter and inhabit the crypts for 4-5 days. After that, the larvae then go to the proximal colon approximately 3 weeks after infection. Infection occur when infective eggs ingest by host. Adult helminth live in the lumen and did not attack the mucosa of the intestine.

Infection is generally more common in older mice. The peak of infection usually occurs 5-6 weeks after the initial infection. Infection is usually subclinical, light to moderate loads do not show clinical disease but heavy infection can show clinical signs. The eggs are resistant to dryness and lots of disinfectants but it's susceptible to high temperatures. *Aspiculuris tetraptera* can be diagnosed by detected of egg in the feces, or adult worms that found in the large intestine. One of the characteristics of Aspiculuris is that it can be eradicated by the immune system and it's also resistant to reinfection (Wescott, 1982)

Meterials and Methods

Material

Some of the tools and materials used in this research are microscope, glass object, beaker, culture tube, flotating solution and mice. This study used two groups of mice. including; group (A) infected with Aspiculuris, and control group (K) which was not treated. The stage of infection was cunducted on the first day and the second infection is counducted two weeks after the first infection with an infection dose of 2 times the initial infection. Observations were made for a total of 28 days to see the level of parasite from Aspiculuris infection by looking at the presence of worm eggs in the feces of mice.

Method

Acclimatization was conducted for all mice prior to infection. Acclimatization was conducted for 10 days by giving antibiotics on day 1 to day 5 and anti-protozoa from day 6 to day 10, plus the administration of anthelmintic drugs on day 1 and day 6. We used Aspiculuris worm eggs for Infection. Aspiculuris worm eggs were obtained by collecting adult worms from Aspiculuris. An Adult worms obtained from the intestines of mice by previous surgery. The adult worms were transferred to a NaCl solution and crushed using a nippers to remove the eggs. The eggs then transferred to a new NaCl solution to be inserted into the incubator, which the eggs can develop into an infective stage and are ready to be infected. We used flotation method to identify the parasite. Three grams of feces were added to 58 ml of flotation solution in a container, stirred until homogeneous and then filtered through a tea filter. The filtrate is put into a test tube until it forms a convex meniscus at the top of the tube. The cover glass was then placed on the top of the test tube and left for 5 minutes.

After that, the cover glass is removed from the tube and placed on an object glass to be examined under a microscope (Hansen and Perry 1994). Quantitative test was conducted using the McMaster technique which was used to count the number of eggs in the feces. The visible eggs were observed based on their shape and morphology to classify the types of eggs found. Amount 0.3 ml of the float solution put into the McMaster slide and counting under microscope. After that Differential Leukocyte Count was conducted to determine the differential Leukocyte in blood.

Results and Discussion

In the group of mice infected with Aspiculuris, helminth eggs were identified in the feces on the 20th day as many as 100 eggs/gram of feces and the number increased on the 26th day to 300 eggs/gram of feces, although helminth eggs were not found on the 23th day. According to Dole et al. (2011) *Aspiculuris tetraptera*, Aspiculuris that found in mice, has a 21-25 days prepatent period. The same thing was also stated by Anya (1955) who had found that *Aspiculus tetraptera* took 20 days for male worms and 23 days for female worms to develop into adults after being infected for the first time.

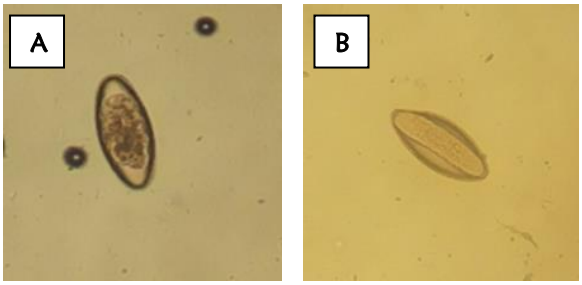

Fig. 1 Aspiculuris egg in day-20 (a) and day-26 (b) from feces samples

There was a difference form of the eggs on the 20th day and 26th day. On the 20th day, the worm eggs still showed development of the balstomers which would then develop into larval postulant as on the 26th day. The eggs will later come out with the feces to become infective stage and infect the host. After host ingest the eggs, larvae (Ll) will hatch and enter the crypts of the colon (Anya, 1966; Behnke, 1974). The larval parasites remain in close contact with the host tissues in digestive system around seven days (Behnke, 1974). The larvae (L3) then return to the lumen of the colon, and after that, they migrate to anterior colon and then develop into adults helminth. Gravid females worm will emerge on the 24th day after initial infection (Anya, 1966).

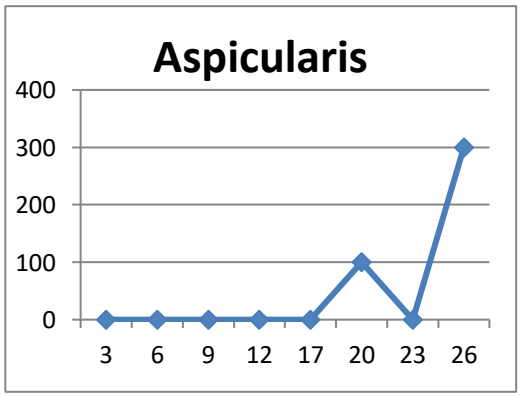

Fig. 2 Egg per gram (epg) of Aspiculuris tetraptera each day

The absence of worm eggs on day 23 indicated that the adult worms did not lay eggs consistently. Eggs in *Aspiculuris tetraptera* are not released by adult worms continuously, but intermittently (Philipson 1974). This suggests that the release of helminth eggs by adult female *A. tetraptera* may be stimulated by changes in the host's physiological state associated with host condition, immune system or other general activity.

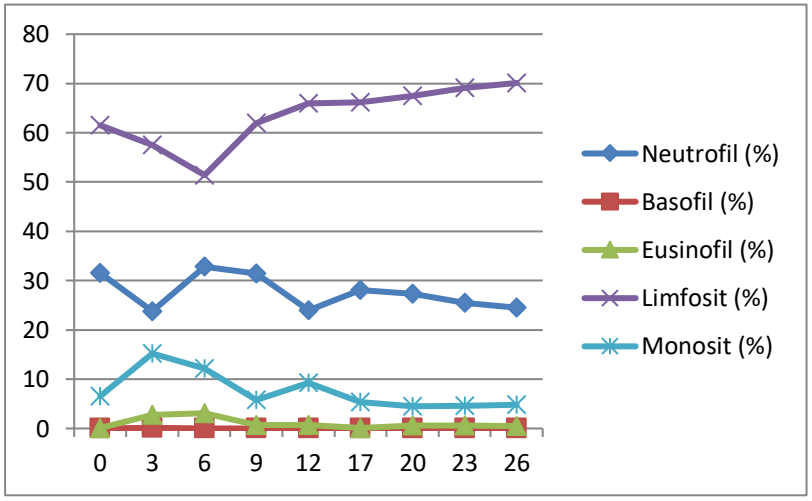

Fig. 3 Aspiculuris egg in day-20 (a) and day-26 (b) from feces samples

The graph above shows that the value of neutrophils and monocytes fluctuated from the 1st day to the 26th day, but the values of neutrophils and monocytes tended to decrease from day to day. This is presumably because neutrophils and monocytes are the body's first response from mice that act as initial immunity to fight infection, so their levels will decrease day by day. Aspiculuris eggs that are infected in mice will hatch in the intestines and cecum of the mice in approximately two hours (Anya, 1955), so it can be concluded that the immune system of mice had appeared since the first day after infection. Monocytes along with neutrophils play a role in the early stages of tissue repair due to inflammation caused by parasite worm larvae (Motran, 2018). The peak of Neutrophil were seen on the 6th and 9th days. This is thought due to the invasion of larvae into the intestinal epithelium, thereby triggering an increase in the number of neutrophils. The predominant cells in the initial response to parasitic worms are neutrophils, and the earliest histologic signs of parasitic infection involve interactions of larvae and neutrophils (Moreau, 2010). Several studies have also demonstrated the ability of neutrophils to paralyze parasite worm larvae (Dean, 1974; Bass, 1979; Esser, 2013).

Lymphocyte levels also showed fluctuations but tended to increase from the first day to the 26th day because lymphocytes are generally found in chronic infections or infections that have lasted a long time, and will only be active when the level of neutrophils as an initial response to infection has decreased. Lymphocytes play a very important role in parasitic infections. Lymphocytes will produce T-cells and interleukins that function as defense mechanisms against parasites, such as increased mucosal permeability of epithelial cells, increased smooth muscle contractility, all of which can

contribute to the expel of adult worms from the lumen (Shea, 2001; Jankovic, 2001). The value or level of eosinophils as shown in the graph has a relatively constant value. The peaks of eosinophils on the chart are on the 3rd and 6th days at 2.8 and 3.1% respectively. The normal value of eosinophils in rats is in the range of 0.9-3.8% (Loeb, 1999). So it can be assumed that the eosinophil value is at its peak. This is presumably because at that time it was the initial period of infection in which larvae formed and infected the digestive system of mice. As we know that eosinophils are a type of leukocyte that was used as an indication of infection with parasitic worms. Eosinophils play a role in secreting granules that contain parasitic toxic proteins, such as the main basic protein, eosinophil peroxidase, cationic protein, and neurotoxins that will paralyze the parasite. Eosinophils are highly involved in immunoregulation and exert a protective effect on the host during parasitic helminth infections (Motran, 2018).

Conclusion

Aspiculuris tetraptera eggs were identified in the feces on the 20th day and the number increased on the 26th day as much 300 eggs/gram of feces. We found that the adult worms did not lay eggs consistently every day. The level of Neutrophils and monocytes showed fluctuations but tended to decrease from day to day. Lymphocyte levels also showed fluctuations but tended to increase from the first day to the 26th day. The value or level of eosinophils has a relatively constant value. The peaks of eosinophils were on the 3rd and 6th days at 2.8% and 3.1% as the main protection of immune system against parasite.

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Reference

Anya A. 0. 1966. Studies on the biology of some oxyuroid nematodes. I. Factors in the development of eggs of Aspiculuris tetraptera Schultz. JournalofHelminthology 40: 253- 260.

Bass, D. A., & Szejda, P. (1979). Eosinophils versus neutrophils in host defense: Killing of newborn larvae of Trichinella spiralis by human granulocytes in vitro. The Journal of clinical investigation, 64(5), 1415-1422.

Behnke J. M. 1974. The distribution of larval Aspiculuris tetruptera Schultz during a primary infection in Mus musculus. Rattus norvegicus and Apodemus sylvaticus. Parasitology 69: 391402. Behnke J. M. 1975. Imm

Chan, K. F. (1955). The distribution of larval stages of Aspiculuris tetraptera in the intestine of mice. The Journal of Parasitology, 41(5), 529-532.

Dean, D. A., Wistar, R., & Murrell, K. D. (1974). Combined in vitro effects of rat antibody and neutrophilic leukocytes on schistosomula of Schistosoma mansoni. The American journal of tropical medicine and hygiene, 23(3), 420-428.

Dole, V.S., Zaias, J., Kyricopoulos-Cleasby, D.M., Banu, L.A., Waterman, L.L., Sanders, K. and Henderson, K.S. (2011) Comparison of traditional and PCR methods during screening for and confirmation of Aspiculuris tetraptera in a mouse facility.

JAALAS 50: 904-909

Esser-von Bieren, J., Mosconi, I., Guiet, R., Piersgilli, A., Volpe, B., Chen, F., ... & Harris, N. L. (2013). Antibodies trap tissue migrating helminth larvae and prevent tissue damage by driving IL-4Rα-independent alternative differentiation of macrophages. PLoS pathogens, 9(11), e1003771.

Hansen J and Perry B. 1994. The epidemiology, diagnosis and control of helminth parasites of ruminants. A handbook. Nairobi (KY) : International Laboratory for Research on Animal Diseases

Jankovic, D., Liu, Z., & Gause, W. C. (2001). Th1-and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. Trends in immunology, 22(8), 450-457.

Loeb W, Quimby F (1999). The clinical chemistry of laboratory animals. Taylor and Francis. Philadelphia

Motran, C. C., Silvane, L., Chiapello, L. S., Theumer, M. G., Ambrosio, L. F., Volpini, X., ... & Cervi, L. (2018). Helminth infections: recognition and modulation of the immune response by innate immune cells. Frontiers in immunology, 9, 664.

Phillipson, R. (1974). Intermittent egg release by Aspiculuris tetraptera in mice. Parasitology, 69(2), 207-213.

Phillipson, R. F. 1974. Intermittent egg release by Aspiculuris tetraptera in mice. Parasitology 69:207-213.

Shea-Donohue, T., Sullivan, C., Finkelman, F. D., Madden, K. B., Morris, S. C., Goldhill, J., ... & Urban, J. F. (2001). The role of IL-4 in Heligmosomoides polygyrus-induced alterations in murine intestinal epithelial cell function. The Journal of Immunology, 167(4), 2234-2239.

Taffs, L. F. 1976. Pinworm infections in laboratory rodents: a review. Lab. Anim. 10:1-13.

Wescott, R. B. (1982). Helminths. In Diseases (pp. 373-384). Academic Press.