https://scilett-fsg.uitm.edu.my/
Vol. 17(1) JANUARY 2023

ISSN: 1675-7785
eISSN: 2682-8626

DOI: 10.24191/sl.v17i1.19874

Gastrointestinal Infection of Rabbits in Ranau Rabbit Farm, Ranau, Sabah Malaysia

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Accepted: 25 December 2022; Published: 21 January 2023

ABSTRACT

A study on gastrointestinal parasite infection in rabbits (*Oryctolagus cuniculus*) was conducted at Ranau Rabbit Farm, Ranau, Sabah, Malaysia. Fifty fresh faeces samples from rabbits were collected at the study site, located on a hill with a view of Mount Kinabalu at Kampung Kigiok, Ranau, Sabah, Malaysia. This area was chosen as the study since it has over 100 varieties of rabbits ranging in age. The species of parasites that infect the rabbits, and the intensity and prevalence of parasitic intestinal infection in rabbits, were analysed and calculated. The samples were analysed using direct smear, sedimentation, and floatation. Six gastrointestinal parasites were observed and identified, i.e., two protozoan parasites (*Coccidia* sp. and *Toxoplasma* sp.), two cestodes (*Diphyllobothrium* sp. and *Taenia* sp.), one nematode (*Ascaris* sp.), and one trematode (*Fasciola* sp.). The most prevalent parasite was *Coccidia* sp. (97.56% in adults [n = 40/41] and 88.89% in juveniles [n=8/9]), followed by *Diphyllobothrium* sp. (95.12% in adults [n = 39/41] and 77.78% in juveniles [n = 7/9]), *Taenia* sp. (92.68% in adults [n = 38/41] and 77.78% in juveniles [n = 7/9]), *Ascaris* sp. (58.54% in adults [n = 24/41] and 55.56% in juveniles [n = 5/9]), *Toxoplasma* sp. (97.56% in adults [n = 27/41] and 77.78% in juveniles [n = 4/9]) and *Fasciola* sp. (29.27% in adults [n = 12/41] and 33.33% in juveniles [n = 3/9]). No significant difference was detected between adults and juveniles regarding the intensity of infection. For the intensity, the degree of infection is <500 and is considered a mild infection and does not cause harm to rabbits and visitors. Meanwhile, the total prevalence rate for all individuals was 98%. The prevalence rate for adults was 97.56% and for juveniles was 88.87%. No significant difference was observed between the adults and juveniles in the prevalence rate. Since the prevalence is high, it can be concluded that all rabbits from this study site could easily be affected by gastrointestinal parasites because they did not show any symptoms even though they are experiencing GI. In conclusion, all 50 rabbits in Ranau Rabbit Farm were infected with all six parasites (*Coccidia* sp., *Diphyllobothrium* sp., *Taenia* sp., *Ascaris* sp., *Toxoplasma* sp., and *Fasciola* sp.). *Coccidia* sp. is more prominent and zoonotic than the other species. Therefore, it might lead to higher severity for the rabbits and visitors if the infection is high.

Keywords: *Rabbit, parasite infection, intensity, prevalence, Ranau*

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Vol. 17(1) JANUARY 2023

ISSN: 1675-7785
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INTRODUCTION

Rabbits, also known as bunnies or bunny rabbits, are small mammals belonging to Lagomorpha and the family Leporidae. *Oryctolagus cuniculus* encompasses the European rabbit species and its descendants, including all 305 domestic rabbit breeds worldwide. There are 13 types of wild rabbits in the Sylvilagus genus, including seven cottontail species [1]. The European rabbit is a wild hunt species and domesticated livestock and pet introduced to every continent except Antarctica. This study focuses on parasites that may cause gastrointestinal (GI) infections in rabbits. The overall infection with parasites, particularly pinworms and protozoa, may be demonstrated by prior research investigations undertaken in Egypt, Tabanan in Bali, and Dungun and Selangor in Malaysia. This scenario could be linked to coprophagy, which occurs in Lagomorpha order mammals, possibly linked to the rabbits at Ranau Rabbit Farm. The findings of this study show that coccidiosis has been a persistent problem in the rabbitry study over the years.

Most parasites, such as nematodes (pinworms), dwell in the rabbit’s intestinal tract and cause GI infections. Moreover, according to Tanjung and Ranguti [2], rabbits are also frequently attacked by ectoparasites (e.g., mites and fleas) and endoparasites (e.g., nematodes, trematodes, cestodes, and protozoa). Rabbit endoparasites, including worms and protozoa, are typically stomach or digestive tract parasites that generally affect the organ. GI infections are viral, bacterial, or parasitic diseases that result in gastroenteritis or inflammation of the stomach and small intestine. GI stasis syndrome is a common veterinary symptom of an ailing rabbit. The causes of this illness are typically multifaceted, and rabbits are prone to infection if they consume an unsuitable diet.

The most frequent helminth parasites detected in endothermal laboratory animals like rabbits are nematodes, cestodes, and trematodes. Pinworms of the Oxyuridae family are the most common helminth parasites infecting laboratory animals in the medical and veterinary fields. The helminths are gastrointestinal nematodes with a natural life cycle that resides in the host's cecum and colon, infecting domestic and wild rabbits, hares, and rodents worldwide. This parasite generally lives and grows in foods and drinks, contaminating them. Drinking contaminated water or consuming contaminated food will cause GI infections in rabbits.

Some protozoa also cause GI infections. Protozoa, such as *Eimeria* sp., are parasites commonly seen in rabbits. Some affected rabbits may exhibit an enlarged belly due to the enlarged liver and gall bladder. *Eimeria* sp. can dwell in the GI tract for 16 to 23 days, and the infection can have a chronic course that lasts many weeks or an acute illness that lasts about ten days [3]. Furthermore, the rabbit will become unconscious and have diarrhoea. The transmission route is similar to the helminths, i.e., it affects rabbits when they consume contaminated foods and drinks containing *Eimeria* sp. eggs. This was revealed in a study conducted in the Bedugul district of Tabanan, Bali, affecting ten rabbits. White nodules with a diameter of 1 to 5 mm were observed

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Vol. 17(1) JANUARY 2023

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on the surface of the liver. Furthermore, the bile duct epithelial cells showed hypertrophy [4], indicating the pathogenicity of *Eimeria* sp. The identification of parasites, intensity, and prevalence of intestinal parasites based on age was analysed and calculated.

EXPERIMENTAL

Study Site

This study was conducted at Ranau Rabbit Farm in Ranau, Sabah, Malaysia. The farm is situated on a hill in Kampung Kigiok. The study site was chosen because it has a population of more than 100 rabbits. The rabbits vary in age, colour, size, and sexes. The stool samples were collected from the chosen 50 rabbits.

Direct Faeces Smear

A drop of saline was placed on a microscopic slide labelled with the rabbit number. A small match head of the rabbit's stool specimen was picked up with a spatula and mixed with a drop of saline to form a suspension. A drop of iodin was introduced to the slide. The suspension was covered with a coverslip. The slide was examined under a light microscope with a 10' objective. A higher magnification (40' objective) was used to observe the eggs of the suspected parasites [5]. The steps were repeated for the other 49 faeces specimens, and all samples were replicated twice.

The Formalin–Ether Sedimentation

The faecal specimen, about the size of a walnut, was mixed in 1 mL of saline solution. The emulsion was filtered through a fine mesh gauze into a 1.5 mL Eppendorf tube. The suspension was centrifuged using a refrigerated centrifuge at about 200 rpm for 10 min. The fresh specimen suspension should yield 0.75 mL of sediment and 0.5 mL of formalinised faeces. The supernatant was decanted, and the sediment was washed with 1 mL of saline solution. Then, the suspension was centrifuged again, and the washing step was repeated until the supernatant was clear. After the last wash, the supernatant was decanted, and 1 mL of 10% formalin was added to the sediment. The sediment and 10% formalin were mixed and left standing for 5 min to allow fixation.

Next, 0.5 mL ethyl acetate was added and shaken vigorously. The suspension was centrifuged at 450' *g* for 10 min. Four layers will be observed in the suspension, i.e., the top layer of ethyl acetate, a plug of debris, a layer of formalin, and sediment. The plug of debris was freed from the side of the tube by ringing with a small spoon. The top three layers were carefully decanted. Lastly, using a pipette, the remaining sediment was mixed with the remaining fluid, and

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one drop each of saline solution and iodine was transferred onto a glass slide. A coverslip was put on top of the suspension, and the slide was microscopically examined for parasitic forms at 10', 400', and 1000' magnification [6].

The Faeces Floatation Method

About 1 g of faeces was ground, mixed with 15 mL Sheater's (sucrose) solution in the centrifuge tube, and vortexed. Then, the mixture was filtered through fine gauze into a small beaker. The solution was transferred using a dropper to a clean McMaster slide and examined under the microscope at 100', 400', and 1000' [7]. The faeces floatation was performed by floatation using Sheater's solution with a specific gravity (SG) of 1.27 (454 g sugar in 355 mL water) for parasite eggs.

Prevalence Rate and Intensity Rate

The intensity rate was calculated using the McMaster egg counting technique, which employs a counting chamber to microscopically examine a known volume of faecal suspension (0.30 mL) [8]. As a result, 1 g is chosen so that the faecal egg count can be easily calculated by multiplying the number of eggs under the marked areas by a simple conversion factor. When a container is filled with a faeces-in-flotation-fluid suspension, much of the debris sinks, while eggs float to the surface and can be easily seen and counted. The effects of rabbit age must be controlled for and calculated to determine the prevalence and intensity rates. Equation 1 was used to calculate the differences in prevalence between rabbit groups based on age (adult and juvenile) [9].

$$\text{Prevalence: } \frac{Number\ of\ rabbits\ (age)\ affected\ by\ gastrointestinal\ infection}{Total\ rabbits\ sample}\ X\ 100 \quad (1)$$

For the intensity parameter, 2 g of faeces were weighed and mixed with 60 mL saturated NaCI in a beaker to form a homogenous solution. The beaker was left for 5 min. After filtering the mixture with fine gauze, the filtrate was collected in a new beaker. The filtrate was forcefully agitated, and a sample was taken using a pipette and put into one of the chambers of the McMaster slide. The slide should not contain any bubbles. The slide was left for at least 5 min for the parasite eggs to float to the surface. The slides should be examined within an hour. If the slides are left for too long, the fluid will evaporate, and salt crystals will form. The mixing and drawing of the sample will be repeated, and other chambers will be filled [10]. The grid lines of the McMaster slide were examined under a microscope at 100x, 400x, and 1000x magnifications. The total number of eggs under both etched sections was counted inside the grid areas, including eggs on the grid gridline instead of half of the eggs inside the grid in both chambers. The total number of eggs per gram was determined. The total number of eggs in the two chambers was multiplied by 50 to determine

https://scilett-fsg.uitm.edu.my/
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the intensity of eggs per gram. The total number of eggs for both chambers was calculated using Equation 2 [10]:

$$\text{Total egg count: } (Chamber\ 1 + chamber\ 2) \times 50 \quad (2)$$

The multiplication factor of 50 is unique to the faeces-to-water ratio (2 g) described in this procedure. This procedure will not detect less than 50 eggs/gram, which is equivalent to seeing one egg on the McMaster slide because each observed egg represents 50 eggs/gram [10].

Statistical Analyses

The intensity of parasitic intestinal infection in rabbits was calculated using the t-test. At the same time, the Mann-Whitney U test was used to determine the prevalence of gastrointestinal parasite infection between adults and juveniles.

RESULTS AND DISCUSSION

Table 1 shows six different gastrointestinal parasites were observed and identified in this study, i.e., two protozoan parasites (*Coccidia* sp. and *Toxoplasma* sp.), two cestodes (*Diphyllobothrium* sp., and *Taenia* sp.), one nematode (*Ascaris* sp.), and one trematode (*Fasciola* sp.). All faecal samples are positive for GI parasites.

Table 1: Identification of parasites

Group	**Genus**	**Present in Age**	
		Adults	**Juveniles**
Protozoan	*Coccidia* sp.	Present	Present
	Toxoplasma sp.	Present	Present
Cestodes	*Diphyllobothrium* sp.	Present	Present
	Taenia sp.	Present	Present
Nematode	*Ascaris* sp.	Present	Present
Trematode	*Fasciola* sp.	Present	Present

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Coccidia sp. is the primary protozoal parasite that commonly affects rabbits [11]. The findings showed that 23 (76.6%) rabbits were infected by the oocysts of *Eimeria* sp., with the highest percentage of intensity (73.9%). The *Eimeria* parasites that cause coccidiosis and oocysts are present in the environment and, when consumed, result in GI infection. Mohebali et al. [12] reported that *Coccidia* sp. is the most zoonotic parasite in rabbits that causes GI infection. Wild and domestic rabbits have been reported to be affected by *Toxoplasma* sp. [13]. Likewise, Nabil [14] revealed the presence of *Taenia* sp. in the intestine of rabbits. Besides protozoa, helminths also live in rabbits. The lawn gets polluted when a final infected host excretes tapeworm segments, including its eggs. Rabbits can consume the infected eggs. The tapeworm eggs develop into larvae in the rabbit intestine before migrating throughout the intestine [15].

Abdiel-Nasser et al. [16] have reported *Fasciola* sp. in rabbits, albeit with its lowest recovery. Rabbits were not exempt from GI infection, although the infection rate was very low. According to Opara [17], some of the rabbits in the Nekede zoological garden were affected by *Ascaris* sp. (21.4%). The infection of *Ascaris* sp. was low and did not cause death to the rabbits. Differences in geographical distribution and environmental factors may also influence the interaction between a parasite and a host. The favourable environment aids in spreading the disease and parasite eggs rapidly. However, inadequate anthelmintic therapy is to be blamed for the high frequency of GI parasites in the current study. The rabbits serve as an intermediary and definitive host [18].

Table 2: Intensity rate of the parasite infection in the rabbit population

Age (No of observed samples)	No infected sample	EPG/CPG	Intensity ± SE	P value
Adults (41)	41	6900	168.3 ± 17.26	0.000
Juveniles (9)	9	1250	138.9 ±18.75	0.290

According to Elmeligy et al. [19], the degree of infection of <500 is considered mild, 500–1000 moderate, and >1000 severe. From Table 2, the degree of infection of <500 is considered a mild infection. No significant difference (P = 0.454) is observed between the adults and juveniles regarding the intensity of infection. However, the infection in adults (168.3 ± 17.26) is slightly higher than the juveniles (138.9 ± 18.75) (Table 2). It indicates that the data are not normally distributed, non-parametric, and insignificant below 0.05. The findings from Papeschi et al. [20] reported that the number of eggs and oocysts in faeces correlates with the host rabbit's age and state of health.

According to Kołodziej-Sobocińska [21], the level of infection varies with host age. Studying how parasitism changes with host age might shed light on the possibility of acquired

ISSN: 1675-7785
eISSN: 2682-8626

DOI: 10.24191/sl.v17i1.19874

immunity in hosts, as well as age-dependent differences in host exposure to parasites. Kanyari et al. [22] stated that animals such as mammals with poor bodily conditions had a high intensity of infection. According to Elshahawy and Elgoniemy [23], all age groups (adults and juveniles) were equally at risk of infection, as the young and adult rabbits were equally susceptible to it. The possibility of a similar infection risk arises from the exposure of both young and adult rabbits to the same infection risk.

Table 3 shows that the most prevalent parasite is *Coccidia* sp. (97.56% in adults [n = 40/41] and 88.89% in juveniles [n = 8/9]) in both ages, followed by *Diphyllobothrium* sp. (95.12% in adults [n = 39/41] and 77.78% in juveniles [n = 7/9]), *Taenia* sp. (92.68% in adults [n = 38/41] and 77.78% in juveniles [n = 7/9]), *Ascaris* sp. (58.54% in adults [n = 24/41] and 55.56% in juveniles [n = 5/9]), *Toxoplasma* sp. (97.56% in adults [n = 27/41] and 77.78% in juveniles [n = 4/9]), and *Fasciola* sp. (29.27% in adults [n = 12/41] and 33.33% in juveniles [n = 3/9]).

Table 3: Prevalence rate of the parasite infection in the rabbit population

Age	***Diphyllobothrium* sp. (No. of Infected sample and %)**	***Taenia* sp. (No. of Infected sample and %)**	***Ascaris* sp. (No. of Infected samples and %)**	***Fasciola* sp. (No. of Infected samples and %)**	***Toxoplasma* sp. (No. of Infected sample and %)**	***Coccidia* sp. (No. of Infected sample and %)**
Adults	39 (41)	38 (41)	24 (41)	12 (41)	27 (41)	40 (41)
Juveniles	7 (9)	7 (9)	5 (9)	3 (9)	4 (9)	8 (9)

All 50 rabbits in Ranau Rabbit Farm were infected by the six parasites (*Coccidia* sp., *Diphyllobothrium* sp., *Taenia* sp., *Ascaris* sp., *Toxoplasma* sp., and *Fasciola* sp.). The *Coccidia* sp. showed a higher prevalence in adults (97.56% in adults and 88.89% in juveniles) (Figure 1). The lowest prevalence is *Fasciola* sp. (29.27% in adults and 33.33% in juveniles). The studies of Radzi [24] showed that *Coccidia* sp. is the parasite that usually infects rabbits. The study showed that *Coccidia* sp. had the highest prevalence in meat-farmed rabbits from three commercial farms in Sepang (Dengkil), Shah Alam (Sungai Buloh), and Klang, Selangor, Malaysia. McMaster's approach determined the species on faecal samples, with *Eimeria* sp. being prevalent.

ISSN: 1675-7785
eISSN: 2682-8626

DOI: 10.24191/sl.v17i1.19874

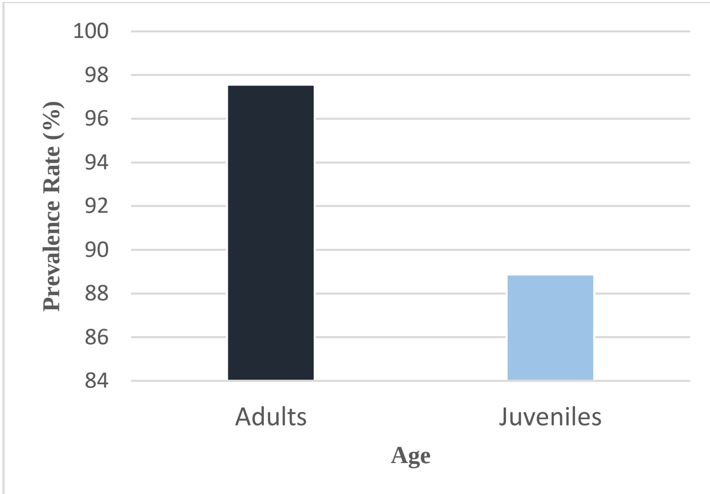

Figure 1: Prevalence rate of the parasite infection in rabbit population graph

The total prevalence rate for all individuals is 98%. The prevalence rate for adults is 97.56% and for juveniles is 88.87%. No significant difference (P = 0.317) is noted between adults and juveniles regarding the prevalence rate. According to Thomas et al. [25], older individuals may stop acquiring parasites due to dietary changes or behavioural patterns, their use of the habitat, or the development of their immunity. In contrast, Pakandl et al. [26] and Szkucik et al. [15] contradict the study by Thomas et al. [25], whereby Pakandl et al. [26] stated that the effect of age had increased the prevalence of coccidiosis in juvenile and adult rabbits due to lower resistance or less immunity to coccidian infection. Szkucik et al. [15] stated that the *Coccidian* sp. parasite mostly showed the highest prevalence regardless of age. Similarly, a study in Dungun, Malaysia, by Ong and Nazira [27] also revealed that coccidiosis is a parasitic infection that causes hepatic coccidiosis in rabbits of both ages. Additionally, according to Hess and Axelson [28], rabbits with GI issues will not exhibit any symptoms or clinical signs. They transmit the protozoan to other rabbits by discharging infected faeces. The symptoms include irregular or intermittent bloody diarrhoea or a watery, mucousy, rough-haired coat (stopping and starting again).

CONCLUSION

Faecal samples from 50 rabbits were collected from Ranau Rabbit Farm. Six different gastrointestinal parasites were observed and identified, with *Coccidia* sp. being the most prominent and zoonotic species than the others. The infection might be highly severe to the rabbits and visitors if the infection rate is high. The most prevalent parasite was *Coccidia* sp., and the lowest prevalent parasite was *Fasciola* sp. No significant difference was noted between the adults and

https://scilett-fsg.uitm.edu.my/
Vol. 17(1) JANUARY 2023

ISSN: 1675-7785
eISSN: 2682-8626

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juveniles regarding the intensity of infection. For the intensity of infection, the degree of infection of <500 is considered a mild infection and does not cause harm to rabbits and visitors. The total prevalence rate for all individuals was 98%. The prevalence rate for adults was 97.56% and for juveniles was 88.87%. No significant difference was observed between adults and juveniles concerning the prevalence rate. Since the prevalence rate of infection is high, it can be concluded that all rabbits from this study site could be easily infected by GI parasites, although they did not show any symptoms.

ACKNOWLEDGMENTS

The authors would like to thank the staff of Ranau Rabbit Farm for their cooperation and "Kompleks Makmal dan Agroteknologi" (KOMSAT) of Universiti Teknologi Mara (UiTM) for providing the facilities for this study. The author would also like to thank the Sabah Biodiversity Centre (SaBC) for the research permit (JKM/MBS.1000-2/13JLD.2 (3)).

AUTHOR'S CONTRIBUTION

JAP carried out the research and wrote and revised the articles. SSAM conceptualised the central research idea and provided a theoretical framework. At the same time, SSAM supervised the research progress, anchored the review and revisions and approved the article submission.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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ISSN: 1675-7785
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