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Rabbit's Gastrointestinal Helminthiasis: Identification And Correlation with Age, Sex and Hygiene

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Abstract. The growing demand for high-protein low-fat meat has encouraged the development of potential rabbit farming. One of the obstacles in the rabbit farming is a disease. The studies of gastrointestinal helminthiasis in rabbits are limited, especially in Indonesia. The study aimed to identify and determine the prevalence of helmith infestation, and investigate the correlation between age, sex, and hygiene with the occurrence of gastrointestinal helminthiasis in small-scale rabbit farms. The survey was conducted on 476 rabbits in Banyumas District using a purposive sampling to collect fecal samples and questionnaire data. Both male and female rabbits were devided into four age groups: wean 1 (birth-1 month), wean 2 (>1-6 months), young (>6-18 months), and adult (>19 months). The feces were examined using the floating and the Whitlock methods and gastrointestinal helminthiasis were identified by observing the morphology of eggs and gastrointestinal helmiths. Data were subject to descriptive analysis and logistic regression using JASP software 0.16.3 version. The results demonstrated that 50 rabbits (10.50%) were infested with nematode and cestode worms. Nematode eggs found in rabbit feces in this study were strongyle (33.33%), Trichostrongylus (27.78%), Cittotaenia (14.81%), Passalurus (12.96%), Trichuris (7.41%), Hymenolepis (1.85%), Toxocara (1.85%) and Strongyloides (1.85%). The most prevalent egg worm in rabbits was Strongyle (33.33%) and lowest were Hymenolepis, Toxocara and Strongyloides (1.85% each). This study revealed that age and sex had no effect on the prevalence of gastrointestinal helminthiasis in rabbits (p>0.05). The frequency and technique of cage cleaning had a highly significant (p<0.01) effect on the occurence of gastrointestinal helminthiasis in rabbits, however there was no significant link with the separation of feces and urine in the cage (p>0.05).It can be inferred that the prevalence of gastrointestinal helminthiasis in rabbits is relatively low, and the management system including the hygiene of the cage are the most important factors in preventing its emergence.
Keywords: gastrointestinal helminthiasis, prevalence, helminthiasis, rabbit

Abstrak. Peningkatan permintaan daging yang tinggi protein dan rendah lemak mendorong berkembangnya peternakan kelinci. Salah satu kendala dalam budidaya kelinci adalah penyakit. Penelitian tentang helminthiasis atau kecacingan pada kelinci masih sangat jarang dilakukan, terutama di Indonesia. Tujuan penelitian ini adalah untuk mengetahui prevalensi infestasi cacing, korelasi antara umur, jenis kelamin, dan kebersihan dengan kejadian helminthiasis gastrointestinal pada peternakan kelinci berskala kecil. Survei dilakukan terhadap 476 ekor kelinci di Banyumas dengan teknik purposive sampling untuk pengambilan sampel feses dan data kuesioner. Kelinci jantan dan betina dengan kelompok umur: sapih 1 (umur lahir-1 bulan), sapih 2 (umur >1-6 bulan), muda (umur >6-18 bulan), dan dewasa (umur >19 bulan). Pemeriksaan feses dilakukan dengan metode apung dan metode Whitlock. Identifikasi telur dan cacing gastrointestinal diidentifikasi dengan pengamatan morfologi. Data dianalisis secara deskriptif dan regresi logistik menggunakan software JASP versi 0.16.3. Hasil penelitian menunjukkan 50 ekor kelinci terinfeksi cacing nematoda dan cestoda, dengan prevalensi 10,50%. Telur cacing nematoda yang terdapat pada feses kelinci pada penelitian ini adalah telur Strongyle (33,33%), *Trichostrongylus* (27,78%), *Cittotaenia* (14,81%), *Passalurus* (12,96%), *Trichuris* (7,41%), Hymenolepis (1,85%), Toxocara (1,85%) dan Strongyloides (1,85%). Prevalensi telur cacing pada kelinci tertinggi adalah Strongyle sebanyak 33,33% dan terendah adalah Hymenolepis, Toxocara dan Strongyloides, masing-masing 1,85%. Hasil penelitian menunjukkan bahwa umur dan jenis kelamin tidak berpengaruh signifikan terhadap kejadian helminthiasis gastrointestinal pada kelinci (p>0,05). Frekuensi pembersihan kandang dan cara pembersihan kandang berpengaruh sangat signifikan (p<0,01) terhadap kejadian helminthiasis gastrointestinal pada kelinci namun tidak menunjukkan korelasi yang siginifikan terhadap tindakan pemisahan feses dan urin dalam kandang (p>0,05). Dapat disimpulkan bahwa prevalensi helminthiasis gastrointestinal pada kelinci tergolong rendah dan sistem pemeliharaan serta kebersihan kandang merupakan faktor utama untuk mencegah terjadinya infestasi tersebut.

Kata kunci: helminthiasis gastrointestinal, prevalensi, helminthiasis, kelinci

Introduction

Rabbits are livestock with potential as the alternative source of animal protein which surpasses that of other livestock, in addition to popular pet animals. Rabbit meat is healthier than other meats because it is high in protein and low in fat. Rabbit meat is rich in calcium (21.4 mg/100 g) and phosphorus (347 mg/100 g) than other types of meat and lower in fat (9.2 g/100 g) and cholesterol (56.4 mg/100 g) (Nistor et al., 2013). The physical and chemical characteristics of rabbit meat are influenced by the type of clump, feed, rabbit's activity, sex, and pre-slaughter processing (Brahmantiyo et al., 2014). Indonesia is one of the prospective rabbit breeding countries. The moist tropical climate with fairly high rainfall, 1.001 mbs of air pressure, and temperatures between 21.4-30.9°C are suitable environment for rabbits. However, there remain obstacles to rabbit breeding in Indonesia such as breeding factors, weather factors, and most importantly, the health factor. The prevalence of parasitic diseases in rabbit breeding persists.

Helminthiasis is a parasitic worm-borne disease. Parasites in the digestive tract are widespread parasites. In the digestive tract, helminthic parasitocynosis is more common, with the presence of infection with more than one type of parasite in the animal rather than monoinfection (Kruchynenko et al., 2021). Helminthiasis can cause declining production, weight loss, decreased appetite, diarrhea, weakness, and death (Ola-Fadunsin et al., 2018). Some species of worms found in rabbits include *Gongylonema neoplasticum, Paraspidodera uncinata, Passalurus ambiguus, Strongyloides papillosus (Strongylus papillosus), Trichostrongylus retortaeformis, Vianella fariai (Viannaia fariai)* and *Longistriata perfida, Sylvilagus brepsicus multicepsy (Multicylicus multicepsic), Trichostrongylus retortaeformis, Serialis (Multiceps serialis = Coenurus serialis), Taenia pisiformis, Taenia macrocystis (Hydatigena macrocystis)* and Taenia variations (Pinto et al., 2004). Previous studies have suggested mass drug treatment could contribute to the control of soil-borne helminthiasis as a practical short-term measure. But, the role of government in improving public health, education and infrastructure important to reduce the prevalence of these infections (Bóia et al., 2006).

The prevalence of worm infestation is more significant in gestating and lactating female rabbits than in unmated rabbits. *Trichostrongylus retortaeformis* infestation is more severe in female than male rabbits, significantly lower in both active and idle males than gestating and lactating females, and higher among old rabbits than the younger ones. Meanwhile, there are no substantial differences between the sexes in terms of parasitic worm prevalence (Molina et al., 1999). Our previous study reported that the high and low prevalence of helminthiasis infestation may be attributed to some factors including geographic locations (altitude and maintenance environments) and potentially, sex (Indrasanti et al., 2022).

Hygiene of cages and livestock facilities is one of the things that is very influential in the spread of disease. The intensity of cleaning and the level of hygiene of the cage is very important for breeders to pay attention to. Inadequate biosecurity management of cages, stress and nutritional deficiencies are a few of the causes of parasite infestation. One of the most parasite infestations is gastrointestinal worms. These worms threaten nearly all livestock (Widayati et al., 2013). To the best of our knowledge, research on helminthiasis on rabbits is still scarce compared to coccidiosis. In order to produce safe, healthy and halal meat for the community, the present study makes a substantial contribution to the control of helminthiasis among rabbits. The purpose of this investigation was to discover the types of worms that infect rabbits, the prevalence (infection rate), and the relationship between helminthiasis occurence and age, sex, cage hygiene, and type of cage flooring. This research is expected to be able to contribute to the maintenance of rabbits in the community regarding the importance of hygiene of the cage and environment in decrease the occurrence of gastrointestinal parasitism in rabbits.

Materials and Methods

Research Approval

The survey research was approved by Research and Community Service Institute University of Jenderal Soedirman, Purwokerto No P/833/UN23/14/PN/2019.

Determining the Research Location

This research employed an analytical survey with purposive sampling to rabbit population. The specific locations of the survey were districts having high densities of rabbits. The total number of samples in this study was determined by Slovin's formula, n = N / 1 + Ne2, where n is the minimum sample number, N is the population, and e is the error margin assumed at 5% (Tejada & Punzalan, 2012). Accordingly, 476 samples were employed. The infestation rates or prevalence was calculated using the formula below:

$$\frac{The\ number\ of\ infested\ livestock}{The\ number\ of\ livestock\ samples} x100\%$$

(Lawal et al., 2016)

Data Collection and Rabbit Worms Identification

The study was conducted on rabbit farms in both the highlands (close to Mount Slamet) and lowlands of Banyumas Regency, representing rabbits from different landscapes. A total of 476 rabbits were obtained from five subdistricts in Banyumas (Sumbang, Kalibagor, Pekuncen, Cilongok, and Baturraden). Rabbit feces were taken from rabbits with variations in type, sex, body weight, and age. In addition to sex, the observed variables included four age groups: wean 1 (birth-1 month), wean 2 (>1-6 months), young (>6-18 months), and adult (>19 months). Fresh feces were collected in the afternoon before breeders cleaned the cage, then 5-10 grams of feces were sampled and stored at 4°C until examination to determine the prevalence of infection and identify the types and number of worm eggs using the Whitlock method (Taira et al., 2003; Khurana and Sethi, 2017) and Mc Master's counting chamber (Hawksley & Son Ltd). The floating method was carried out according to Prawestry, et al. (2021). ± 3 grams of collected feces is put into a mortar, mixed with 15 ml of saturated sugar solution and then stirred until homogeneous. Faeces and saturated sugar that have been homogenized are filtered twice using a sieve, the filtered results are put into a becker glass. A cover glass was placed on the surface of the solution, and left for 5 minutes to egg can float and stick to the cover glass. Cover glass lifted and placed on a glass object. The preparations were examined using a microscope with a magnification of 10 x 40, the examination results were photographed for qualitative analysis based on books and literature. Quantitative examination or counting the number of eggs uses a modified method Mc Master, where ± 3 grams of collected feces was put into a mortar, mixed with 15 ml of saturated sugar solution, and stirred until homogeneous. Then, the solution was filtered twice using a sieve, and the yield was put into a beaker glass, covered with a glass lid, and let sit for 5 minutes so the egg could float and stick to the glass lid. After that, the lid was removed, placed on a glass object, and examined using a microscope with a 10 × 40 magnification. The results were photographed for qualitative analysis based on books and literature. Quantitative examination counted the number of eggs using the modified McMaster method. Approximately 3 grams of feces from each rabbit were dissolved in 15 ml of saturated sugar until homogeneous and the feces became soft. The homogenized mixture of feces and saturated sugar was filtered twice using a sieve, then the yield was put into a beaker glass. The filtered solution was stirred, then placed in the McMaster counting chamber using a pipette, and let sit for 5 mins. After that, the solution was observed under a microscope with a 10×10 magnification. The number of eggs was calculated in EPG units (eggs per gram) by multiplying the total worm eggs found in two counting rooms by 50 (n x 50). Worm eggs were identified based on a previous method on the morphology of worms and worm eggs found in rabbit feces (Taira et al., 2003; Pinto et al., 2004; Zajac and Conboy, 2012). Feces examinations and egg worm counting were carried out at the Animal Health Laboratory, using a microscope with a magnification of x400 and x1000 (Leica DM500). The cleanliness parameter was identified based on the frequency of cage cleaning, the methods for cleaning the cage, and the separation of feces and urine. Based on our observation, the cages were cleaned 1–5 times per day. The method of cleaning the cage included cleaning with a napkin (1), dousing with water (2), dousing with water and detergent (3), brushing and dousing with water and detergent (4), and cleaning with disinfectant (5).

Data Analysis

We obtained data of the types of worm eggs and worms found in rabbit feces samples and the prevalence of helminthiasis. We conducted a qualitative descriptive analysis to evaluate the prevalence and identification of worm infestation, and ran a chi-square (X^2) formula to determine the correlation between age and sex and gastrointestinal worm infestation.

Results and Discussion

Identification of Rabbit's Worm

The majority of rabbit farmers in Indonesia raise their rabbits under intensive management (with cages) and traditional systems. They utilize rabbit hutches with slate floors to keep them clean because rabbit feces will fall directly into the feces and urine container. In addition, ventilation provided by the slate floor facilitates adequate air movement. Upon examining 476 samples of rabbit feces, we found that 50 rabbits were single- or multiple-worm-infested, indicating a low prevalence of helminthiasis infestation (0.50%). The types of worm eggs found in rabbits' feces were Strongyle, Trichostrongylus, Cittotaenia, Passalurus, Trichuris, Toxocara, Hymenolepis, and Strongyloides. Some rabbits were infested with two classes of worms, nematodes and cestodes, as shown in Table 1 and Figure 1. Domestic rabbits appear to have fewer worm infestations than wild rabbits (Fernández-Alvarez et al. 2013).

Table 1. Percentage of worm species in rabbits

Species	Number of infested rabbits	Types of worm (%)	Class
Strongyle eggs	18	33.33	Nematode
Trichostrongylus	15	27.78	Nematode
Cittotaenia	8	14.81	Cestode
Passalurus	7	12.96	Nematode
Trichuris	4	7.41	Nematode
Hymenolepis	1	1.85	Cestode
Toxocara	1	1.85	Nematode
Strongyloides	1	1.85	Nematode

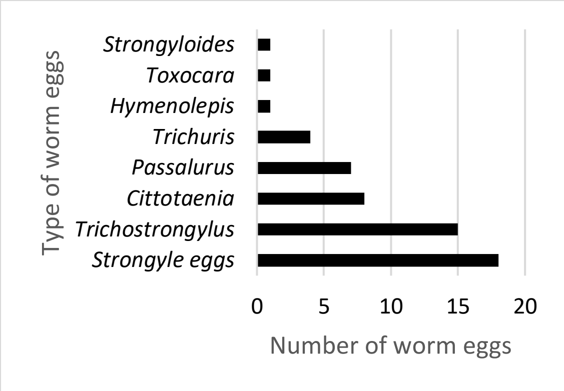

Figure 1. Amount of egg of worm species in rabbits

Audebert et al. (2002) stated that helminthiasis, or worm infestations, occurred in rabbits due to one or more worm species, and that different types of worms can infest each rabbit on the same farm. The absence of clinical symptoms of helminthiasis was likely owing to the smaller number of infectious worms than parasitic worms inhabiting the rabbit's digestive tract. *Trichostrongylus* is a nematode that usually attacks rabbits in the anterior part of the intestine. The prepatent period of *Trichostrongylus* is divided into two stages: larval and mature stages, with a total development time of 12 to 13 days. The only difference between the life cycles of *Trichostrongylus* in ruminants and *T. retortiformis* in rabbits is the length of the prepatent period. This comparable life cycle rise shows that the two species are related. *Cittotaenia* is a tapeworm that has two main implications: blocking nutritional intake and obstructing the intestines, resulting in nutrient loss. Different prevalence levels of *Cittotaenia* denticulata may be due to oribatid mites, which serve as a crucial intermediate host to complete its life cycle. High rates of tapeworm infestations also indicate high levels of oribatid mite infestation on agricultural crops (Frank et al., 2013). Egg of *Cittotaenia* in this research is presented in Figure 2a.

Trichuris is a whipworm prevalent in mammals around the globe, particularly in warm and humid climates. *Trichuris* commonly infests cows, sheep, goats, camels, South American camels, pigs, dogs, cats, rabbits, mice, and several species of wild animals. Some mammals may be infested by a single *Trichuris* species, while others (e.g., cats) are susceptible to infestation by many agents. *Trichuris* trichiura is the zoonosis *Trichuris* in humans. The life cycle of all *Trichuris* spp. is similar to a definitive host ingesting food or water contaminated with the embryonated egg, and the eggs hatch in the large intestine, either cecum and/or proximal colon (Else et al., 2020). *Trichuris* eggs from this research can be seen in Figure 2b.

There are 30 zoonotic and non-zoonotic species of *Toxocara* (Figure 2c) compatible with various hosts, including birds, rats, rabbits, pigs, and earthworms. *Toxocariasis* can be transmitted both vertically (transplacental and transmammary) and horizontally (eating *Toxocara* larvae). Humans can be infected orally by consuming *Toxocara* larvae or uncooked paratenic hosts (Ziegler & Macpherson, 2019). Meanwhile, Rodentia is a possible asymptomatic host for Hymenolepis (Figure 2d), a cestode worm infesting human (zoonosis). Due to the high likelihood of human infection, antiparasitic treatment is essential for controlling and preventing the spread of parasites among humans (De-Ovidio et al., 2015).

Passalurus (Figure 3) is the most common oxyurid worm found in naturally infected rabbits. Two species of *Passalurus* are *P. umbiguus* and *P. nonanulatus* (Sultan et al., 2015). *Passalurus* has two species: *P. umbiguus* and *P. nonanulatus*. While these nematodes can be detected with a conventional microscope, morphological study requires a scanning electron microscope (SEM) to differentiate them from other oxyurid species (Abdel-Gaber et al., 2019). *Passalurus* prevalence is dependent on the age and season of the definitive host. Younger rabbits are more frequently infected with *Passalurus* than older rabbits (Ili et al., 2018). This worm is present in the large intestine of rabbits and is often not highly contagious; however, it can cause deadly and severe infections in young rabbits (Hussein et al., 2021).

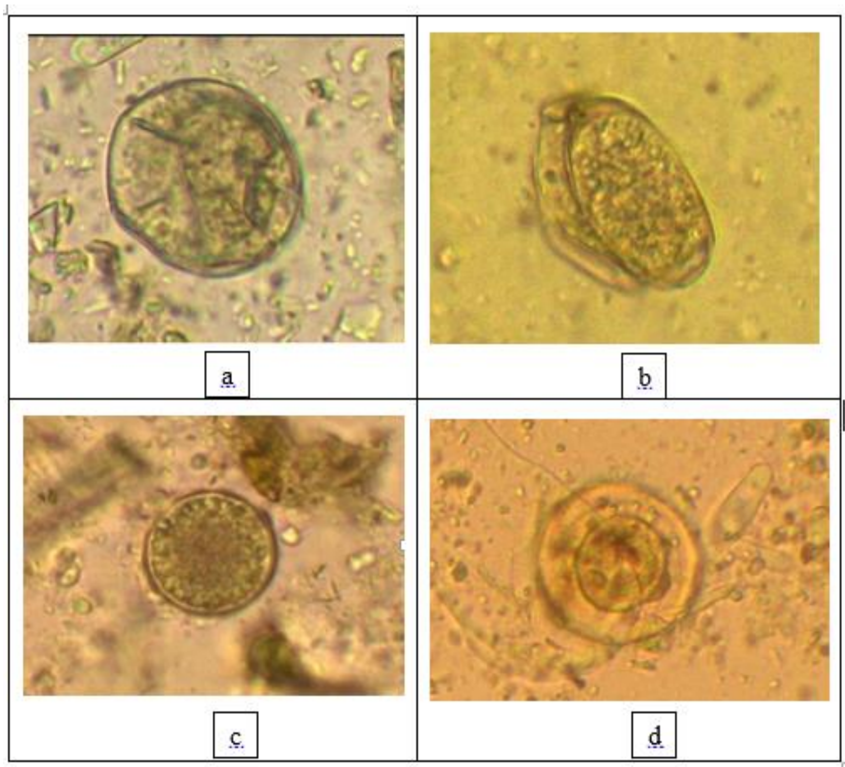

Figure 2. Worm eggs of in rabbit gastrointestinal tract: a. *Cittotaenia* sp, b. *Trichuris* sp c. Taxocara sp, d. Hymenolepis sp

Figure 3. Worm of *Passalurus sp* in rabbits

In our study, we found that the occurrence of gastrointestinal parasites caused by helminthiasis in rabbits was relatively low at 9.87%, especially when compared to the strikingly high occurrence of coccidiosis at 90.11%, as reported previously (Indrasanti et al., 2019). While we know that there is an interaction between microbiota and intestinal parasites, the exact nature of this relationship remains unclear. In fact, coccidiosis infection has been linked to a decrease in the diversity of intestinal microbiota (including bacteria, viruses, fungi, protozoa, and parasites) and an increase in harmful bacteria (Lu et al., 2021). Our study uncovered a compelling area for further investigation: the potential relationship between rising coccidiosis infestations and declining helminthiasis levels in rabbit intestines. This suggests that there may be competition among microorganisms for resources or possibly an unknown inhibitory mechanism at play. This phenomenon doesn't just occur among parasite species; it also happens among various microorganisms within the digestive tract. Hauck (2017) stated that there was an interaction between Eimeria spp and Clostridium perfringens in chickens, Ascaridia galli with various bacteria in chicken intestines and Blastocystis with intestinal microbiota in humans. Gaining a comprehensive understanding of the complex interactions involving the host immune system, gut microbiota, enteroendocrine system, and parasites is crucial for developing strategies to control gastrointestinal diseases in the post-antibiotic era and for vaccine development (Lee et al., 2022; Wickramasuriya et al., 2022).

Table 2. Results of an examination for gastrointestinal worms in rabbits of various ages and sexes

Sex	Period	Age	Total samples (rabbits)	Results		Prevalence (%)
				Infested (samples)	Not infested (samples)	
Male	Wean 1	born - 1 months	74	7	67	9,46
	Wean 2	> 1 – 6 months	72	5	67	6,94
	Young	> 6 – 18 months	78	11	67	14,10
	Adult	> 19 months	5	0	5	0
Total male rabbits			**229**	**23**	**206**	**10,04**
Female	Wean 1	born - 1 months	83	9	74	10,84
	Wean 2	> 1 – 6 months	60	5	55	8,33
	Young	> 6 – 18 months	92	12	80	13,04
	Adult	> 19 months	12	1	11	8,33
Total female rabbits			**247**	**27**	**220**	**10,93**
Subtotal			**476**	**50**	**426**	**10,50**

Correlation between age and sex with infestation of gastrointestinal worm in rabbits

Rabbits of any sex or age are susceptible to gastrointestinal helminthiasis infestation. The results of gastrointestinal worm examination are presented in Table 2. The findings from our fecal examinations for gastrointestinal worm eggs (Table 2) revealed that younger rabbits exhibited the highest rates of infestation. This could be attributed to their diet, which primarily consists of green forage and grass—both of which can carry endoparasites, including worms. In contrast, nursing bunnies are likely to receive antibodies from their mother's milk. According to Ola-Fadunsin et al. (2018), younger rabbits are more susceptible to infections than adults, likely due to their developing immune systems, while adult rabbits often have stronger immunity from past infections. However, (Ola-Fadunsin et al., 2018) reported that age, sex, and seasons showed a very significant effect on infections. This finding may be attributed to similar care and management practices across different ages and sexes. Ultimately, environmental factors play a crucial role, as increased contact with sources of infection is likely to determine the incidence of worms (Zulfikar, et al., 2024).

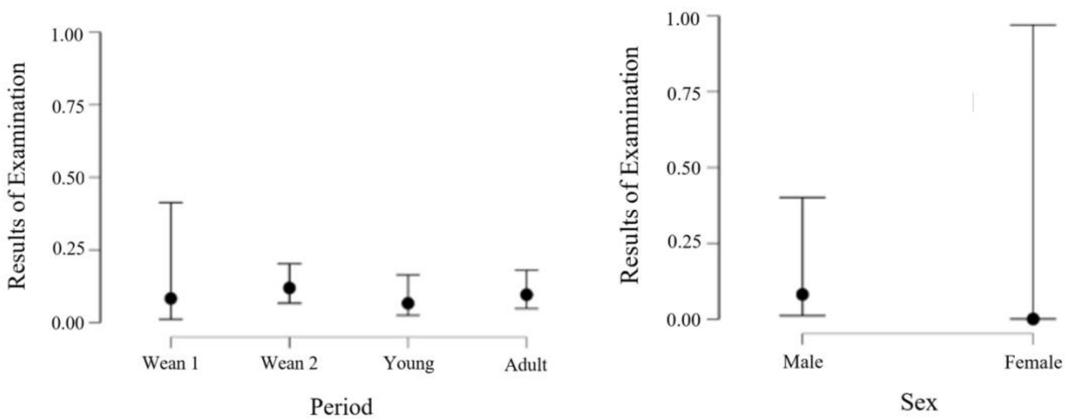

Figure 4. Correlation between age and sex with infestation of gastrointestinal worm in rabbits

Correlation between hygiene and gastrointestinal worm infestations in rabbits

In this study (Figures 5 and 6), the parameters of hygiene that affect helminthiasis occurrence (P<0.01) in rabbits include the frequency of cage cleaning and method of cage cleaning, but not the separation of feces from urine in the cage (P>0.05). It is because hygiene is a very important factor to inhibit the spread of gastrointestinal worm infestation. We found that more frequent cage cleaning (twice a day) with disinfectant and feces-urine separation are crucial in preventing the spread of gastrointestinal worm infestation.

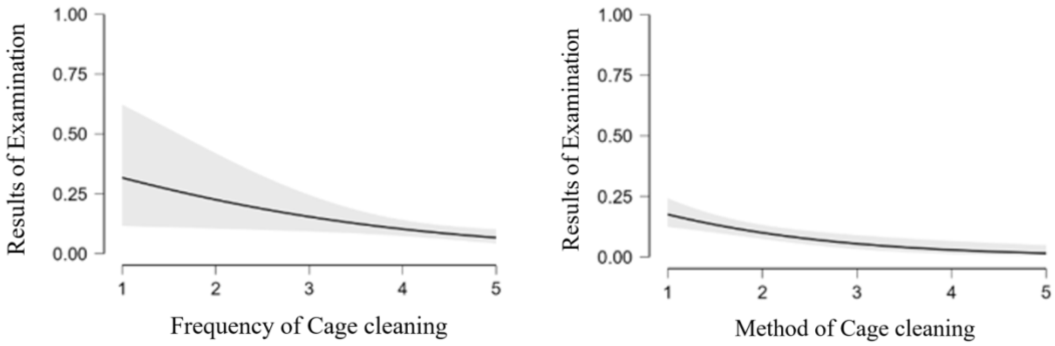

Figure 5. Correlation between frequency and methods of cage cleaning and gastrointestinal worm infestations in rabbits

Age and sex did not influence the occurrence of gastrointestinal helminthiasis; however, hygiene practices remain the primary contributing factor to these cases. Worm infestations in rabbits may result from an unclean environment. The study collected all feces samples from rabbits bred by farmers in traditional community farms. The overall application of biosecurity in a rabbit breeding business has not been carried out perfectly, and hence, there is non-optimum disease control.

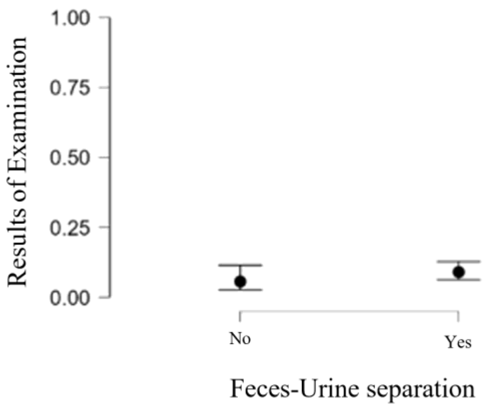

Figure 6. Correlation between feces-urine separation and gastrointestinal worm infestations in rabbits

In addition, according to Szkucik et al. (2014), hygiene and supervision are the most important factors to prevent invasion of parasitic gastrointestinal so that the quality and quantity of carcasses will be guaranteed. While anthelmintics remain the main choice to control helminthiasis infections, worm infections in rabbits can be treated using drugs from the benzimidazole group: fenbendazole, mebendazole, thiabendazole, niridazole, albendazole, diethylcarbamazine, praziquantel, and ivermectin (Manke et al., 2015), (Ilić et al. 2018). However, the current use of synthetic anthelmintics is questionable due to the emerging resistance to these molecules (Collas et al., 2018). Traditional medicines and plant extracts is viable to reduce the side effects of these drugs (Manke et al., 2015; Indrasanti et al., 2017; 2019). Comprehensive biosecurity measures, particularly regarding hygiene in livestock businesses, are expected to reduce the rate of worm infection in rabbits and simultaneously decrease the use of anthelmintics.

Conclusions

Prevalence of gastrointestinal helminthiasis is relatively low. Age and sex had no effect on the prevalence of gastrointestinal helminthiasis in rabbits. Frequency and technique of cage cleaning had a highly significant effect on the occurrence of gastrointestinal helminthiasis; however, there was no significant effect with the separation of feces and urine.

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