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Influence of Soil-Transmitted Helminths in the Blood Biochemical Profile of Naturally Infected Rats

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ABSTRACT

Soil-transmitted helminths are geohelminths that induce infection in humans and animals. The irregular release of parasite eggs or larvae further complicates the diagnosis. This study aimed to establish the main alterations in the blood biochemical parameters such as creatinine, total bilirubin, and total protein levels of soil-transmitted helminth infected rats. Rats were exposed to soil samples contaminated with parasite eggs. Blood and stool samples were collected from the two groups of rats on the 15th and 30th days after exposure for biochemical analysis and stool analysis utilizing an automated biochemistry analyzer and formol-ether concentration technique, respectively. The results of this study revealed that on day 15, the creatinine (0.540 ± 0.055 mg/dl) and total bilirubin (0.183 ± 0.085 mg/dl) levels were significantly higher than the normal reference values, but the total protein level (6.080 ± 1.287 g/dl) has no significant difference. On day 30, a higher increase in the creatinine (0.600 ± 0.000 mg/dl) and total bilirubin (0.320 ± 0.091 mg/dl) levels were observed. The total protein level revealed a significant decrease with 4.980 ± 0.785 g/dl compared to the normal values and day 15 result. The biochemical parameters of the control group were observed to be within the normal physiological values. Without a clinical diagnosis, blood biochemical parameters can change in response to parasite invasions and the early stages of diseases. Therefore, it may aid in determining the current pathophysiology of soil-transmitted helminths among reservoir hosts.

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INTRODUCTION

One of the most detrimental phases in both animals and humans is the emergence of parasitic diseases, posing a severe danger to both health and the economy (Azizi *et al.*, 2007). Humans can become infected with soil-transmitted helminths (STH) through skin penetration by walking barefoot in a contaminated environment, accidental ingestion of embryonated eggs from contaminated soil and water, and by consumption of raw or undercooked meat from infected paratenic hosts including birds, pigs, chickens, ruminants, and rodents. After ingesting the infected paratenic hosts, larvae may pierce the intestinal wall and travel through the blood system to various organs including the lungs, muscles, liver, and the central nervous system (CNS) (Bowman, 2020).

The worldwide trend of raw meat and blood by-product intake increases consumers' susceptibility to zoonotic parasitic pathogens that

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can cause serious diseases (Ma *et al.*, 2018). The larvae of soil-transmitted helminths might be a cause of infection for humans as well as a threat to public health and food safety (Michelutti *et al.*, 2021). The degree of disease, the duration and pattern of larval migration, and the existence of symptoms significantly influence the clinical prognosis and intensity of soil-transmitted helminthiasis. However, due to a lack of trained professionals and relevant technology, the majority of these parasitic infections remain undetected. The intermittent release of parasite eggs or larvae complicates the diagnosis even more. As a result, quick and precise assays other than fecal examination for the diagnosis of STH are needed.

Blood is one of the most important markers for providing the status of metabolism in animals (Wu and Bowman, 2020). Biochemical blood parameters can alter in response to parasitic invasions and the early stages of infections with no clinical diagnosis. However, the complicated pathophysiology of soil-transmitted helminthiasis renders diagnosis difficult, hence the frequency is likely underestimated (Sterling, 2020).

This study aimed to establish the main alterations in the biochemical blood parameters such as creatinine, total bilirubin, and total protein levels of soil-transmitted helminth infected rats. The findings of this study will help in the initial assessment and current pathophysiology of soil-transmitted helminths among reservoir hosts.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from several locations in Malate, Manila, in January 2023. The study sites were chosen at random, considering stray cats that roam and deposit litter in the soil of these study areas. A total of 20 soil samples were collected at a depth of 10 cm, placed in labeled zip locks, and analyzed using the optimized sugar flotation technique. This procedure was used to identify soil-transmitted helminths that might spread the zoonotic infection to the rats. Positive soil samples were mixed to serve as a contaminated environment for the transmission of infection to rats. Then, 10 laboratory rats were obtained, and their feces were assessed to determine if they were clear of soil-transmitted helminthiasis. Five rats were subsequently placed in a contaminated environment with soil-transmitted helminths for 30 days. The control group was comprised of the remaining 5 rats which had no treatment. Fecal and blood samples were collected from the two groups of rats during the 15th and 30th day of exposure to infested soil for analysis. The samples were sent to the Zoology and Parasitology Laboratory of De La Salle University Science and Technology Research Center (STRC) for fecal and biochemical analysis.

Formol-Ether Concentration Technique (FECT)

On the 15th and 30th day of contaminated soil exposure, rats were screened for soil-transmitted helminth infection. Rat fecal samples were collected and placed in a labeled stool container with 10% formalin. Soil-transmitted helminth infections were detected using the Formol-Ether Concentration Technique and identified with the aid of microscopy under the 40x objective (WHO, 2019). Identification of soil-transmitted helminths was done up to the genus level using bench aids, taxonomic criteria, and published scholarly articles (Guo *et al.*, 2012).

Biochemical Analysis of Blood Samples

Blood samples were taken on the 15th and 30th day of exposure to contaminated soils. Approximately 4 mL of venous blood samples were extracted via tail bleeding. After sampling, a portion of the collected blood samples was immediately transferred into tubes without anticoagulant for serum biochemistry analysis. The blood samples were centrifuged at 5000 rpm for 5 minutes for serum extraction. Then, the samples were analyzed using an automated biochemistry analyzer to measure creatinine, total bilirubin, and total protein levels.

Data Analysis

Data were analyzed by the diagnostic manual and atlas of J.W. Harvey (2012). A standard t-test was used to assess the validity of the results with one another and to the standard. $P<0.05$ values were considered significant. Data were expressed using tables and figures.

RESULTS AND DISCUSSION

Twenty (20) soil samples were collected from various locations in Malate, Manila in January 2023. The study sites were randomly selected because cats were allowed to move freely and frequently defecate on the soil of these important area sources. The soil samples from ten sites had an overall contamination rate of 100% (20/20). Four zoonotic helminths, namely: *Toxocara* spp. (roundworm), *Ascaris* spp. (roundworm), *Trichuris* spp. (whipworm), and hookworms were identified. *Toxocara* spp. was the most prevalent intestinal helminth species, with a prevalence rate of 100.00% (20/20), followed by *Trichuris* spp. with 65.00% (13/20), *Ascaris* spp. with 50.00% (10/ 20), and hookworm with 40.00% (8/20).

After the 15th day of natural infection in STH contaminated soil of the experimental group laboratory rats, all fecal samples were positive for at least one zoonotic intestinal helminth. *Ascaris* spp. was the most prevalent parasite with 100.00% (5/5) followed by *Trichuris* spp. with 60.00% (3/5) and hookworm with only 20.00% (1/5) (Figure 1).

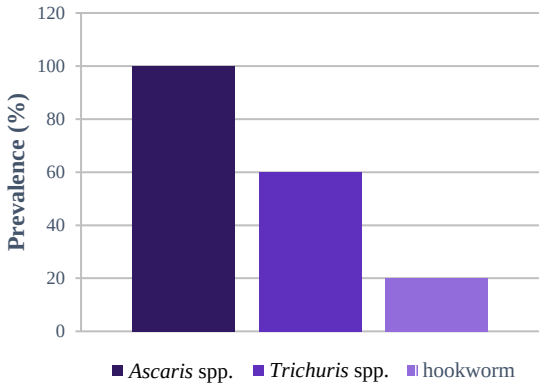

Figure 1. Prevalence of soil-transmitted helminths among rats exposed in contaminated soils detected using Formol-Ether Concentration Technique (FECT)

Blood biochemical analyses with parameters such as creatinine, total bilirubin, and total protein were conducted on the 15th and 30th day post-infection. During the 15th day post-infection, the average value of the creatinine (0.540 ± 0.055 mg/dl) and total bilirubin (0.183 ± 0.085 mg/dl) were significantly higher than the normal reference values, but the total protein level (6.080 ± 1.287 g/dl) has no significant difference with the normal reference value. The biochemical parameters of the control group were within the normal reference values (Table 1).

Following the 30th day post-infection, an increase in the average value of the creatinine (0.600 ± 0.000 mg/dl) and total bilirubin (0.320 ± 0.091 mg/dl) were observed. The values were significantly higher than the normal reference values and the 15th day post-infection. On the other hand, the total protein level revealed a significant decrease in average with 4.980 ± 0.785 g/dl. The biochemical parameters of the control group were still within the normal physiological values (Table 2).

The seroepidemiological study may reveal the local distribution of soil-transmitted helminths, particularly in urban areas where animal reservoir hosts are freely roaming. Most estimates of STH infections worldwide were linked to *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm infections (Pullan *et al.*, 2010). In the present study, all experimental rats were naturally infected with the said three most prevalent soil-transmitted helminths from contaminated soil as early as day 15 based on the fecal evaluation. The results indicate a rapid transmission of intestinal parasites from contaminated soil to animal hosts. Upon further FECT assessment, parasite load has increased from day 15 to day 30 post-infection but the same species of parasites were detected in the feces. An increase in parasitemia relates to an increase in the severity of the most frequent soil-transmitted helminthiasis complications (CDC, 2020). Despite having the highest prevalence in soil samples, *Toxocara* spp. was not detected in rat feces. This is mainly because rats may only serve as paratenic hosts for *Toxocara* spp. and worms cannot fully mature inside them making parasite eggs undetected in feces (CDC, 2019).

Soil-transmitted helminths degrade the nutritional status of infected individuals in a variety of ways. The migration of soil-transmitted helminths' larvae passes through several organs such as the liver and kidneys which may induce organ damage or failure. In the biochemical blood tests, an increase in the creatinine and total bilirubin levels was observed on the 15th and 30th-day post-infection while protein levels were in the normal range for the 15th day but significantly decreased during the 30th day post-infection. Intestinal helminths prey on host tissues, particularly blood, causing iron and protein loss, particularly in severe infections (WHO, 2023). The decrease in total protein levels on the 30th day post-infection was specified by the liver's regulation of protein production and inadequate absorption of the small intestine's mucous membrane (Chala *et al.*, 2016).

The kidneys oversee a variety of functions, including homeostasis, regulation, excretion, and hormone synthesis (Matthew and George, 2011). Infectious diseases produced by pathogens such as viruses, bacteria, and parasites can impair kidney function. Creatinine levels in the blood were tested as a conventional biomarker of renal function (Lemos *et al.*,2013). After the 15th and 30th days of natural infection, differences in the levels of creatinine were observed between the experimental groups. There is a higher increase in the level of creatinine on day 30 than on day 15. A high creatinine result on a blood test may indicate poor kidney function or renal disease (Prastowo *et al.*, 2014).

Total bilirubin levels are suggestive of both red blood cell breakdown and adequate liver, gallbladder, and bile duct function. In general, elevated bilirubin levels are caused by hemolysis of parasitized and nonparasitized erythrocyte or hepatocyte impairment (Abro *et al.*, 2009). High bilirubin levels shown in this study may indicate that the red blood cells are degrading at an unusual rate or that the liver is not efficiently breaking down waste and removing the bilirubin from the blood. The findings of this study are congruent with the results of studies of Oyewole *et al.* (2010) and Onyesom and Onyemakonor (2011) which showed that most soil-transmitted helminth infected individuals have elevated serum activity, suggesting liver and kidney damage (Oyewole *et al.*, 2010; Onyesom and Onyemakonor 2011).

Table 1. Biochemical analysis of laboratory rats naturally infected with soil-transmitted helminths (Day 15)

Parameter	Unit	Normal Reference Value	Experimental Group (n=5)	Control Group (n=5)
Creatinine	mg/dl	0.200-0.400	0.540 ± 0.055	0.240 ± 0.055
Total Bilirubin	mg/dl	0.000-0.1000	0.183 ± 0.085	0.051 ± 0.022
Total Protein	g/dl	5.600-7.700	6.080 ± 1.287	5.680 ± 0.030

Table 2. Biochemical analysis of laboratory rats naturally infected with soil-transmitted helminths (Day 30)

Parameter	Unit	Normal Reference Value	Experimental Group (n=5)	Control Group (n=5)
Creatinine	mg/dl	0.200-0.400	0.600 ± 0.000	0.340 ± 0.055
Total Bilirubin	mg/dl	0.000-0.1000	0.320 ± 0.091	0.076 ± 0.018
Total Protein	g/dl	5.600-7.700	4.980 ± 0.785	5.900 ± 0.071

CONCLUSION

The results of this study revealed that soil-transmitted helminth infected rats had significantly altered biochemical markers when compared to healthy controls. It also demonstrates that quantitative metrics of biochemical blood parameters in individuals infected with soil-transmitted helminths can be used to estimate the intensity of infection, clinical manifestations, and disease pathophysiology, which are important for disease management. Attention to biochemical abnormalities as well as screening of infected individuals in endemic locations, are significant steps in reducing soil-transmitted helminthiasis morbidity and mortality and improving societal health. Long-term sustainability and control of parasitic infections also necessitate increased access to safe water and sanitation, as well as improved hygiene behavior via health education, cooperation with local government units, and strict implementation of programs for surveillance, diagnosis, and control of soil-transmitted helminth infections in the country.

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AUTHORS' CONTRIBUTIONS

JMC, HC, and MJF designed this study. HC and JMC were responsible for the data collection and statistical analysis. All authors worked on the interpretation of data and manuscript writing. MJF and JIJ critically reviewed and commented on the manuscript. All authors have revised and agreed on the final version of the manuscript before the submission for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ETHICAL APPROVAL

All animal experiments in this study were carried out in strict compliance with the ethical standards concerning the use of animal specimens approved by the Research Ethics Office (REO) of De La Salle University.

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