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# Investigation of Anthelmintic Property of Cow Urine Distillate on *Ascaris* and *Trichuris* eggs Isolated from Soil

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## Abstract

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**Background:** Helminth infection occurs due to contact with contaminated soils and poor hygiene practices. Even though there is immense progression in human medicine, infections that occur due to bacteria, fungi, viruses, and parasites persist as a crucial threat to public health. Over the years, many studies indicated cow urine has antimicrobial activity. Hence, this study looks into anthelmintic property of cow urine in *Ascaris* spp., and *Trichuris* spp., eggs.

**Methods:** The soil sample was collected at the riverside, near the toilet area and at the pond area at an aborigine village in Malaysia. Next, flotation technique was used to isolate the helminth eggs and it was identified using a light microscope. The cow urine was prepared as raw cow urine (RCU), evaporated raw cow urine diluted with distilled water (ERCUD), evaporated raw cow urine diluted with saline (ERCUS) and evaporated filtered cow urine diluted with saline (EFCUS).

**Results:** From the soil samples, mainly two types of helminths were found which were *Ascaris lumbricoides* and *Trichuris trichiura*. Evaporated cow urine diluted with saline showed the most prominent result in the destruction of helminth eggs.

**Conclusion:** Evaporated cow urine possesses biochemical agents that can be used in the future as anthelmintic agents whereby further studies should be conducted to further explore its potential to use cow urine as a potential anthelmintic agent.



## Introduction

Despite immense progression in human medicine, infections that occur due to bacteria, fungi, viruses, and parasites persist as a crucial threat to public health. This issue is exacerbated in developing countries due to the unavailability of medicine and the emergence of widespread drug resistance [1]. Contamination of the environment containing parasite eggs or oocyst poses a critical risk to public health as a worm can lay up to 200,000 eggs per day contributing to environmental contamination [2]. Helminth infection can occur easily due to contaminated objects and poor hygiene [3]. Many treatments are possible to treat STH such as mebendazole, albendazole, and piperazine citrate drug, but there is no remedy to abolish helminth eggs that is cost-effective. Over the past few years, there has been an abundance of research associated with cow urine treatment and research centers and it has been proven that cow urine (Gomutra) is effective in curing many diseases such as blood pressure, heart attack, thyroid, asthma, psoriasis, eczema, migraine, ulcer, constipation, and even cancer. Cow urine has demonstrated effectiveness as both a pest controller and larvicide across various fields. Previous research shows that cow urine can cause paralysis and death in worms depending on the dose which indirectly shows the anthelmintic activity of the cow urine [4]. Hence, in this study, cow urine was explored as a potential anthelmintic agent that may affect the structure of the eggs when exposed to cow urine within a period of time. Hence, this study was designed to look into the potential of using cow urine as an environmentally friendly anthelmintic agent.

## Methods

### Study Design and Sampling

Around 300g of the soil was collected at an aborigine village in Ulu Semenyih, Selangor, Malaysia. The soil sample was collected at the riverside, near the toilet area, and at the pond area. Contaminated soil with helminth eggs is expected within these areas knowing practice of the aborigine community practicing open defecation. Cow urine (CU) samples were obtained from a cow farm located at Lanchang, Pahang, Malaysia in the early morning from the cow caretakers. Around 250mL of CU underwent an evaporation process by using a hot plate at 60°C to concentrate the compound in the cow urine.

### Soil Sample Collection

A small shovel and a plastic bag were used to collect the soil samples. Roughly around 200-300 grams of soil within a depth of 4-6 inches (optimized) was collected. The soil samples were collected from the toilet area as the soil from the toilet area is loamy which is the

optimum environment for the helminth eggs to grow. The preservation of soil samples took place in a refrigerator to keep the soil in its state prior experiment.

### Helminth Egg Isolation from Soil Samples

Soil samples undergo floatation techniques to isolate helminth eggs from the soil. After the detection of eggs using McMaster Chamber, the eggs were cultured by using 0.1% sulfuric acid in a glass petri dish. The eggs were cultured at a temperature of 30-35°C for three weeks. A few drops of 0.95% sodium chloride were added to prevent the dryness of the sample within the duration.

### Cow Urine Preparation




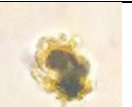
Cow urine samples were replicated and separated into 4 types of preparation. The preparations are called raw cow urine (RCU), evaporated raw cow urine diluted with distilled water (ERCUD), evaporated raw cow urine diluted with saline (ERCUS) and evaporated filtered cow urine diluted with saline (EFCUS). Cow urine repeatedly undergoes different processes in quadruple. The evaporation process was done by using a hot plate with a temperature of 60°C. As for the filtration process, Whatman filter paper was used to filter cow urine. Cow urine samples were filtered and evaporated by using a hot plate with a medium temperature (60°C). It took approximately 4 to 5 hours for the cow urine samples to evaporate completely. Around 5ml of distilled water and 5ml of saline were added respectively for each sample to prepare ERCUD, ERCUS, EFCUD, and EFCUS. Different preparations of cow urine were done to investigate how different preparations of cow urine can affect the anthelmintic properties of cow urine.

## Results



It took around 3 weeks to isolate and culture both helminth eggs. From the soil samples, two types of helminths are commonly found which are *Ascaris lumbricoides* and *Trichuris trichiura*. Table 1 below shows all the summarized results post 24 hours of various treatments using the most commonly found helminths in this study.

Based on Table 1, for raw cow urine (RCU), both *Ascaris* and *Trichuris* eggs can be found after being exposed to the sample for 24 hours. There is no indication that the RCU affects both types of helminth eggs as the eggs are still attached to their structure and can be found under the microscope using the McMaster Chamber after 24 hours. In addition, for evaporated raw cow urine diluted with distilled water (ERCUD) and evaporated filtered cow urine diluted with saline (EFCUS), both results for *Ascaris lumbricoides* and *Trichuris trichiura* indicate there are no eggs that can be detected after 24 hours of being exposed to the sample. However, for evaporated raw cow urine diluted with

saline (ERCUS), helminth eggs can be monitored before and after the exposure to the sample.

Types of Preparation	Methodology	Observation after 24 hours	
		<i>Ascaris lumbricoides</i>	<i>Trichuris trichiura</i>
Raw Cow Urine (RCU)	A few drops of RCU were placed on the helminth eggs and cultured	 AL eggs were detected	 TT eggs were detected
Evaporated raw cow urine dilute with distilled water (ERCUD)	A few drops of ERCUD were placed on the helminth eggs and cultured	No eggs detected	No eggs detected
Evaporated raw cow urine dilute with saline (ERCUS)	A few drops of ERCUS were placed on the helminth eggs and cultured	 AL eggs were detected.	 Distorted eggs were detected.
Evaporated filtered cow urine dilute with saline (EFCUS).	A few drops of EFCUS were placed on the helminth eggs and cultured	No eggs detected	No eggs detected

**Table 1:** Observation of helminths egg after exposure to different types of cow urine after 24 hours.

Healthy <i>Ascaris lumbricoides</i>	Distorted <i>Ascaris lumbricoides</i>
	

**Table 2:** The effect of evaporated cow urine dilutes with saline (ERCUS) before and after exposure to helminths egg.

Based on the result shown in Table 2, the structure of healthy *Ascaris lumbricoides* is equivalent to a compressed oval shape layered by a thick shell that consists of three layers of membrane. Regarding distorted *Ascaris lumbricoides* structure, the outer layer of the membrane is visible where the membrane is impaired. Thereupon, it can be inferred that the exposure of ERCUS is proficient in disrupting the structure of helminth eggs by giving ample time for the anthelmintic property to elapse.

## Discussion

The result above shows that all tests using evaporated raw cow urine dilute with saline compound shows more pronounced results than using raw cow urine. Preliminary studies indicated that cow urine in its distilled form has greater larvicidal potency than raw cow urine [5]. This may be due to the phenol substance in cow urine compositions. Phenols have been substantiated as anthelmintic agents in previous

research due to their complex form and their hydrogen binding [6]. Furthermore, distillation is an optimal water removal process as 97% of water and 50% of salts can be removed without losing a considerable amount of nutrients which can be advantageous for the final product use as fertilizer [7].

In cow urine, there are more than 10 types of enzymes which are alkaline phosphatase (110.110 KA unit), lactate-dehydrogenase (21.780 unit/L), amylase (90.236 unit), acid phosphatase (456.620 XA unit), vitamin B1 (444.125 mg/L), vitamin B2 (0.6339mg/L), vitamin C (216.408mg/L), uric acid (135.028 mg/L), protein (0.1037 g/L), lactate (3.7830 mmol/L), creatinine (0.9970 g/L), aromatic hydroxyl acid (2.7030mg/100ml), calcium (5.735 mmol/L), phosphorus (0.4805 mmol/L), phenol (4.7580mg/100ml), free volatile phenol (0.7130 mg/100ml) and combined volatile phenols (1.3420mg/100ml) [8]. Cow urine properties such as volatile and non-volatile components which are urea, aurum hydroxide, phenols, carboxylic acid, creatinine, calcium, and manganese are well known for their ability as antimicrobial, antifungal, and germicidal [9]. Moreover, both biogenic volatile inorganic and organic compounds such as methanol, propanol, acetone, CO<sub>2</sub>, NH<sub>3</sub>, CH<sub>4</sub>, and some metabolic secondary nitrogenous products are formed during the distillation process of cow urine [10].

Previous research shows that cow urine can cause paralysis and death in worm's dependent on a dose which indirectly shows the anthelmintic activity of cow urine were affected by its concentration [10]. In this study, cow urine demonstrated anthelmintic activity by affecting the structure of the eggs within a period. Cow urine has demonstrated effectiveness as both a pest controller and larvicide across various fields [11]. Cow urine is a substantial source of macronutrients and micronutrients and thus purifies the environment and increases soil fertility with disinfectant and prophylactic properties [12]. With so many benefits of cow urine, the ability to enrich the soil, fertility of the soil, and productivity of the soil is important. It modifies the texture of the soil and has a high content of oxygen and nitrogen that is used to produce superior-quality compost to improve agricultural yields and save humans from the residual effects of harmful pesticides and fertilizers [13].

The beneficial effect of cow urine on health is caused by its chemical components, therefore ingestion of cow urine distillate or any other form is beneficial not only for human physiology but also as an antiseptic, fertilizer, and other therapeutic values [14]. Moreover, cow urine has been granted U.S patents (No. 6,896,907 and 6,4,10,059) for its therapeutic properties, especially for its ability as an antibiotic agent to control bacterial infections and to fight cancer [15]. It has struck a stand

out as its therapeutic value has been proven across every medical field including as an anthelmintic agent. Cow urine is well known for its many therapeutic value, and great pharmacological significance, and its medicinal usefulness has been extensively described in Ayurveda in detail [16]. Thus, cow urine is highly valuable for the use of its anthelmintic property in all other spheres.

It is hypothesized that evaporated raw cow urine possessed biochemical agents that can be used in the future as anthelmintic agents. It has been proven that cow urine can affect helminth egg structure in its evaporated form. Further studies can explore more possibilities to investigate cow urine as a potential medicine. Different methods such as photo-activation of cow urine, high-performance liquid chromatography (HPLC), distillation, and evaporation can be done in the future to compare the result and provide a better recommendation.

## Author Contributions

Dr. Mehru Nisha and Ruziana conceived of the presented idea. Ruziana developed the theory and performed the computations. Dr. Mehru Nisha and Dr. Pang Jyh Chyang verified the analytical methods and encouraged Ruziana to investigate anthelmintic property of cow urine and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript. Ruziana carried out the experiment and wrote the manuscript with support from Dr. Mehru Nisha and Dr. Pang Jyh Chyang.

## Conflict of Interest

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

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