SOIL TRANSMITTED HELMINTHES IN FREE-RANGE CHICKENS AND DOMESTIC HERBIVORES: POTENTIAL RESERVOIRS FOR HUMAN INFECTIONS IN A RWANDAN VILLAGE

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DECLARATION

This dissertation is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

I dedicate this work to all persons who have contributed in this study.

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GLOSSARY OF TERMS

The definitions given below apply to the terms as used in this report. They may have different meanings in other contexts.

Neglected Tropical Diseases (NTDs)

A group of diseases that historically have been overlooked, World Health Organization is working to overcome 17 neglected tropical diseases.

School Going Children

Children aged between 3 and 15 years.

Prevalence of STH Infection

The percentage of individuals in a population infected with at least one species of soil-transmitted helminthes.

Soil-transmitted Helminthes (STH)

Worms that cause intestinal infections in humans. Four species of nematodes are collectively referred to as soil-transmitted helminthes: *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Necator americanus* or *Ancylostoma duodenale* (hookworms).

ABBREVIATIONS

CIDC: Center for Infectious Diseases Control

STHs: Soil-transmitted Helminthes

NTDs: Neglected Tropical Diseases

ABSTRACT

Background: A disproportionate burden of soil transmitted helminthes (STH) in human populations occurs in marginalized, low-income, and resource-constrained regions of the world, with approximately two billion people infected worldwide. Infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, South America, China and East Asia. The control of STH infections in developing countries is of considerable public health importance. Infections with most STH are known to be closely linked with conditions of poverty, unsafe water, sanitation and hygiene. The possible contribution of domestic animals in the transmission of these parasites, if any, is largely unknown.

Objective: The objective of this study was to investigate the prevalence of STH infection in domestic free-range chickens and herbivores in a rural Rwandan village, as a potential source of transmission of infection from these animals to humans, and vice-versa

Significance of the study: This study will help in the understanding of the role that domestic free-range chickens and herbivores play in the transmission of human STH, in a rural Rwandan village

Methodology

Design: This was a community based cross-sectional study and used both qualitative and quantitative research designs.

Study area: This study was conducted in Nyarubuye village, a rural village in eastern province with about 750 households.

Data collection: Chickens droppings and stool of herbivores were screened for STH eggs and larvae and a standard questionnaire was used for demographic data collection.

Study population: The study population comprised of households keeping domestic free-range chickens and herbivores in the particular village. Only adult members of the households were enrolled for the study.

Sample size: The study used simple random sampling to select 75 homesteads; this was equivalent to 10% of the entire village population based on 2007 survey of homesteads.

Data analysis and presentation: Collected data were analyzed using SPSS and presented through frequencies and percentages.

Results and conclusions

Majority of the domestic animals were cows at 38% followed by goats at 35%. Free-range chickens formed 24% of the animals and the least group was sheep at 3%.The animals had eggs of hookworm, *Trichuris trichiura*, *Trichostrongylus* spp. and *Taenia* spp. in their faeces. The study found out that hookworm eggs were present in all herbivorous animals studied. Eggs of hookworm, *Trichuris trichuris, Trichostrongylus* spp. *and Taenia* spp. were also found in chickens' droppings.

Majority of the households, domestic animals grazed in open areas with only a small proportion of the population doing closed grazing. This practice may have been responsible for exposing the domestic animals to human STH.

Majority of the households had toilets (97.3%), about 72% were located outside their compounds and it is thus possible that they were not being used all the time, especially by young children who may not be able to walk the long distance out of the compounds. The presence of human STH in the stool of domestic animals may be attributed to the community not utilizing the toilets. Based on these findings the use of pit latrines by the villagers is recommended to minimize the spread of STH infections. The study also calls for community education on soil STH infections to equip the community with basic knowledge for better prevention. One of the limitations of the study is that it is not clear whether the eggs found in the faeces of domestic herbivores and free-range chickens are actually eggs of human STH and not of animals. This calls for more laboratory tests for confirmation. In addition, the sample size was small and a larger study is needed to confirm the findings.

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Soil-transmitted helminthes (STH) infections are among the most common causes of chronic infections in human beings worldwide (Brooker *et al.*, 2006). In developing countries there are more individuals infected with these worms than those not infected (Awasthi *et al.,* 2003). Infections thrive and persist in communities in need of better housing, clean water, appropriate sanitation, better access to health care, education and increased personal earnings (Crompton, 1999). This is typical of most rural communities and Rwanda has got no exception. Studies elsewhere have shown that children growing up in these communities are usually infected soon after weaning, and are re-infected constantly for the rest of their lives (Awasthi *et al.,* 2003).

Soil-transmitted helminthes infections are caused by different species of parasitic worms. They are transmitted by eggs or larvae present in human faeces, which contaminate the soil in areas where sanitation is poor (WHO, 2014). Most of the population in rural villages relies on crop farming and domestic animal keeping with the animals being allowed to roam freely, feed and defecate in or near human dwelling places (Jithendran and Bhat, 2001)**.**This life style can expose the population to various parasitic infections including soil transmitted helminthes and zoonotic infections such as trichostrongylosis, primarily found in herbivorous animals (Ghadirian and Arfaa, 1975, Watthanakulpanich *et al.,* 2013). In addition, human hookworm eggs have been shown to survive in the gut of chickens and retain their viability and thus chances of being spread in the environment in chickens' droppings (Ackert, 1922).

The presence of *Trichostrongylus* spp. especially in poor farming communities is as a result of people living in close proximity to domestic animals such as sheep, goats and cows (Watthanakulpanich *et al*., 2013).The burden of trichostrongylosisis considered to be rare unlike human hookworm infections. However, there is possibility of trichostrongylosis being higher than previously thought since the eggs of *Trichostrongylus* spp. Can be mistaken for hookworm eggs because of their close

resemblance (Megumi *et al.,* 2011; Robson *et al*., 2013).Over estimation of the prevalence of human hookworms can happen in any area where people associate closely with the infected domestic herbivores with *Trichostrongylus* spp. on one hand. On the other hand coprophagous chickens can contribute much in the high prevalence of hookworms in poor farming communities by contaminating the environment with hookworm eggs in their droppings from places away from human contact to places where children and adults get contact with contaminated soil or any other means such as contaminated kitchen utensils (Ackert *et al*., 1922; Olsen *et al*., 2001)

Evidence has been shown for the over estimation of the prevalence of human hookworm infections in a farming community by Megumi *et al*. (2011). According to the study common human hookworm infections may have been overestimated because of the difficulty of differentiating eggs of human hookworms and *Trichostrongylus* spp. while observing eggs under light microscope, the most commonly used tool to diagnose and identify intestinal parasites. A study by Robson *et al.* (2013) suggested the use of an ocular micrometer to measure the eggs since *Trichostrongylus* spp. eggs are larger than hookworm eggs.

The World Health Organisation (WHO) has come up with a control strategy for STH infections, which focuses on the morbidity control through periodic treatment of at-risk people living in endemic areas. According to the WHO, people at risk include preschool children, school-age children, and women of childbearing age (WHO, 2014). The organization uses health and hygiene education to reduce transmission and re-infection by encouraging healthy behaviours. In addition, WHO and other stakeholders in the health sector advocate for the provision of adequate sanitation, which is absent in resource-poor settings (Brooker *et al*.,2006; Horton, 2003; Stephenson *et al*., 1993; Bethony *et al*., 2006).

However, animals have a potential of passing human STH eggs in their faeces, thereby serving as transport hosts for these infections by contaminating the environment even in communities that are applying established control measures for the infections (Olsen *et al*., 2001; Ackert *et al*., 1922). If this is the case, control

strategies may be threatened by domestic animals such as free-range chickens and herbivores.

1.2 Statement of the Problem

Past epidemiological studies have shown that human STH infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa and means that Rwanda has got no exception. The prevalence of human hookworm infections and probably zoonotic *Trichostrongylus* spp. infections is high in rural Rwandan villages where this is attributed to several predisposing factors (CIDC Rwanda, 2008). Most of the population rely on mixed farming and live close to their chickens and domestic herbivores. Few homes use toilets all the time or source of clean water. All this make prevalence of STH infections at any given time-point to be high as a result of high transmission intensity and long duration of these infections. Although a number of studies have been done on human STH infections, none of the studies has focused on domestic animals as hosts in their transmission in rural Rwanda. Hence, this study examined the importance of free-range chickens and domestic herbivores in transmission of human STH in Rwandan villages.

1.3 Objectives of the Study

1.3.1 The General Objective

The objective of this study was to investigate the prevalence of human STHs in domestic free-range chickens and herbivores in a rural Rwandan village as a potential source of transmission of infection from these animals to humans and vice-versa.

1.3.2 Specific Objectives

1. To determine the presence of human soil transmitted helminthes in stool of domestic herbivores
2. To determine the presence of human soil transmitted helminthes in droppings of free-range chickens
3. To investigate the population excreta disposal that may lead to infection of domestic free-range chickens and herbivores with human soil transmitted helminthes

4. To investigate grazing of domestic animals that may lead to human soil transmitted helminthes infections from free-range chickens and herbivores

1.4 Research Questions

1. To what extent do domestic free-range chickens and herbivores harbor human soil transmitted helminthes?
2. What are the human/animal behaviors that may lead to transmission of STH infections from animal to human and vice versa?
3. What strategies can be adopted to minimize soil transmitted helminthes transmission through free-range chickens and domestic herbivores?

1.5 Significance of the Study

This study will help in the understanding of the role that domestic free-range chickens and herbivores play in the transmission of human STH, in a rural Rwandan village.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Soil Transmitted Helminthes Infections

The enormous global burden and poverty-promoting features of some parasitic, bacterial and viral diseases have put them on the international agenda as neglected Tropical Diseases (NTDs), since health has achieved prominence as a key driver of socioeconomic development (Horton, 2003; Bethony *et al*., 2006).Such diseases have a low profile and status in public health priorities, and are thriving mainly in poor areas of low-income tropical countries, due not only to unsafe water, poor sanitation and housing conditions, but also to the limited access of the populations to education and basic health care (World Bank, 1993; Ayanwale *et al.,* 1982).

Some of the NTDs are spread by soil infested with larvae and eggs of helminthes, whose transmission cycles are perpetuated under conditions of environmental contamination (Brooker *et al*., 2006). For this reason, they are also classified in the category of soil-transmitted helminthes (STH). STH (*Ascaris lumbricoides, Trichuris trichiura),* and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) are the most widespread species of the intestinal parasites infecting humans (Brooker *et al*., 2006; World Bank, 1993; Bethony *et al*., 2006).

The diseases affect more than 1.5 billion people, or 24% of the world's population (WHO, 2014). Such diseases not only impact iron status, growth, vitamin A status, and cognitive development, but may exacerbate other parasitic infections as well like in the case of STH and *Schistosoma mansoni* co-infection (Anne *et al*., 2008). Infected children may experience delays in psychological and physical development that impair their social functioning later in life (Stephenson *et al*., 1993).

2.2 Epidemiology of Soil Transmitted Helminthes Infections

Ascaris lumbricoides, Trichuris trichiura, and hookworm infections mainly occur in tropical and subtropical regions and are most common in developing countries, where personal hygiene is poor, there is insufficient access to clean water, and sanitation (disposal of human faeces and urine) is inadequate or absent (Bethony *et al*., 2006).

Humans acquire *Ascaris lumbricoides* or *Trichuris trichiura* infections when they ingest the parasite eggs after they have embryonated in the environment by eating raw, unwashed vegetables or by not washing their hands after handling contaminated soil (a common transmission route for children). In the case of hookworm, infective larval stages, which hatch from eggs in the soil, can penetrate human skin especially when one walks barefoot on contaminated soil. Mild infections with STH rarely have symptoms, but severe infections can cause abdominal pain and diarrhea, weakness, and malnutrition that can impair physical and mental development (Horton, 2003; Stephenson *et al*., 1993).

Trichostrongylus spp. is primarily a parasite of herbivorous animals. Several species of Trichostrongylus have been known to infect humans, including *T.orientalis, T.colubriformis*, and *T.axei* (Ghadirian and Arfaa, 1975)**.** Eggs are passed in the stool of the definitive host, usually herbivores, and hatch under favorable conditions. The released rhabditiform larvae grow in the soil or on vegetation, and after two molts they become filariform larvae that are infective. Infection of the human host occurs accidentally upon ingestion of these filariform larvae. The larvae reach the small intestine, where they reside and mature into adults. Most infections are asymptomatic. Heavy infections can cause gastrointestinal problems such as abdominal pain, diarrhea, anorexia, headache, fatigue, anemia and eosinophilia (www.cdc.gov/dpdx/trichostrongylosis-index).

Intervention against STH infection is based on regular anti-helminthic treatment, improved water supply, and sanitation and health education. Low-cost, high-coverage delivery of anti-helminthic treatment has been achieved in some settings, but improving sanitation is more complex. In rural Rwanda, for example, levels of access to improved sanitation are very low (5.4%), making evaluation of other components of intervention important. Many STH infections can be safely and effectively treated with anti-helminthic drugs, but there is rapid re-infection after successful treatment (Bethony *et al*., 2006).

2.3 Empirical Review

Approximately 740,000 people are infected with human hookworms globally and they are found to live in areas where sanitation and hygiene are poor and communities live close to domestic animals (Brooker*et al*., 2006).The highest prevalence occurs in sub-Saharan Africa, eastern Asia and in other areas characterized by poverty and poor sanitary conditions. A meta-analysis of the data from 36 publications on sanitation availability and/or use and the number of people in study populations infected with one or more of three types of STH indicates that, compared to people with no access to sanitation facilities, people with access to sanitation facilities were half as likely to be infected with STH (Brooker *et al*., 2006; Bethony *et al*., 2006). Specifically, the chances of infection with STH among people with access to latrines compared to people without access to latrines were higher for *Ascaris lumbricoides*, *Trichuristrichiura*, and hookworm, either separately or combined (Editor, 2004). The use of sanitation facilities also protected against these STH infections.

Combining data for both access and use of latrines, through a systematic review and a meta-analysis it had proven that sanitation is key in the prevention of STH infections (Utzinger, 2012).Conditions associated with poor sanitary disposal of human faeces could potentially lead to transmission of STH by animals acting as transport hosts if they ingest the eggs in human faeces and pass them in their stool (Thomas *et al*.,1924). Such an occurrence would create obstacles in control of STH as the animals would transfer the eggs from places with poor sanitation to places where human west is properly disposed as a control measure for these parasitic infections.

An experimental study of chickens and pigs as transport hosts for *Ascaris,Trichuris* and *Oesophagostomum* eggs by Olsen *et al*.(2001) showed that after passing non-embryonated eggs of *Ascaris suum, Trichuris suis* and *Oesophagostomum dentatum* are able to embryonate, develop normally and become infective to pigs (Olsen *et al*., 2001). According to the researchers it is highly probable that chickens and pigs as well as other domestic animals are able to act also as transport hosts for human parasites.

2.4 Critical Review of the Literature

Previous studies on the role of domestic animals in transmission of STH infections to humans have mostly been limited on pigs and very few in chickens. Most of these studies were experimental where domestic animals were fed STH eggs (*Ascaris lumbricoides, Trichuris trichiura* and hookworms) then recovered in their faeces after passage through their alimentary canals (Ackert, 1922; Olsen *et al.*, 2001). Some have used animal parasites eggs rather than using human parasites in their experiments and came up with an idea that the same scenario can happen when those domestic animals ingest human STH eggs (Olsen *et al*., 2001). Moreover, those studies used a very small number of animals to examine their role in transmission of human parasites. Hence, the present study was an observational, searching the evidence regarding the domestic free-range chickens and herbivores (cows, goats and sheep) in the transmission of human soil-transmitted helminthes.

CHAPTER THREE
METHODOLOGY

3.1 Study Design

This was a community based cross-sectional study. The study used both qualitative and quantitative research designs.

3.2 Study Area and Population

The study was carried out in a rural Rwandan village located in Nyarubuye sector of Kirehe District that is in Eastern Province of the country. Kirehe has 12 sectors, has a population of 229,468 inhabitants with a population density of187 inhabitants/$km^2$and is at an average altitude of 1500 meters. Nyarubuye alone has a total population of 10,832 inhabitants. Only 43% of the students who completed primary school are able to proceed to secondary level. As regards health, the population of Kirehe suffers much from malaria, no bloody diarrhea, lower and upper respiratory tracts infections, intestinal parasites etc. the distance travelled by the population to reach medical centers remains very long for a patient. Access to drinking water is also a major problem since the majority of the population finds water after having travelled a very long distance. In addition the District does not have hydroelectric energy in spite of its significant waterfalls and its economy is based primarily on agriculture and breeding (Kirehe, 2007). Therefore, the area was chosen because of the high prevalence of STH since most of the residents rely on agriculture and farming and live in close contact to their livestock and that lifestyle predispose them to various microbial infections including soil transmitted helminthes (CIDC Rwanda, 2008).

The study population comprised of households keeping domestic free-range chickens and herbivores in Nyarubuye village, Kirehe District. Only adult members of the households were enrolled for the study.

3.3 Sample Size

Nyarubuye village has a total of 750 households keeping domestic animals (National Institute of Statistics of Rwanda, 2012). The sample size is 10% of the population equivalent to 75 households keeping domestic animals.

3.4 Sampling Method

This study used simple random sampling to select 75 homesteads; this was equivalent to 10% of the entire population based on 2007 survey of homesteads (National Institute of Statistics of Rwanda, 2012).

3.4.1 Sampling Procedure

Simple random sampling method was applied to select the households to participate in the study. A local leader helped the investigator to randomly select and enroll 10 households keeping animals (chickens, goats, sheep and cows) every morning from 7Am to 12AM. During the data collection at household level, head of household was introduced to the objective of the study and asked if willing to participate in the study. After he/she had understood the objectives of the study, and its requirements and was willing to participate, and let his/her domestic animals be recruited as study subjects, he/she was given an informed consent form to sign or thumbprint for him/her. Thus, demographic data and domestic animals stool were collected after getting a signed written informed consent from respondents.

3.5 Data Collection Methods

3.5.1 Laboratory Tests

Domestic free-range chickens and herbivores (cows, goats and sheep) were identified. From each homestead, three samples of chickens' faecal material, three samples of sheep and goats and two samples of cows' faecal material were collected randomly from the dens. The collected stool samples were brought to Nyarubuye health center laboratory and samples were screened for STH eggs using standardized, quality controlled parasitological tests. All faecal material samples were examined using Kato-Katz technique for the concentration and identification of STH.

3.5.2 Procedure

A small amount of faecal material was placed on the newspaper or the glazed tile then screen nylon was pressed on top so that some of the faeces filter through. A flat-sided spatula scraped across the upper surface of the screen nylon to collect the sieved faeces. A template placed on the slide and the sieved faeces were added with the spatula so that the hole in the template is completely filled. The spatula was passed over the filled template to remove excess faeces from the edge of the hole. The template was removed carefully so that a cylinder of faeces left on the slide. The faecal material was covered with a pre-soaked cellophane strip. Then the slide inverted and the faecal sample was pressed firmly against the hydrophilic cellophane strip to spread evenly. The slide was placed on the bench with cellophane upwards to enable the evaporation of water while glycerol cleared the faeces. For all helminthes, except hookworm eggs, the slide was kept for one or more hours at room temperature to clear the faecal material, prior to microscopic examination. About 41.7 mg K-K thick smears were read under a microscope within 20–40 minutes to avoid over-clearance of hookworm eggs in glycerol. At least 5 samples were collected and tested daily in a period of 2 months. Hookworms,*Trichostrongulus* spp., *T. trichiura* and *Taenia species* eggs were counted and recorded separately. For quality control, a senior laboratory technician re-examined a random sample of 10% of the K-K thick smears daily (Yvette *et al*., 2005).

3.5.3 Questionnaire

Participants were asked questions in Ikinyarwanda with a pre-tested questionnaire by the researcher. A qualified translator was available for translation purposes. In addition, the translator was a person who can be trusted in order to achieve maximum reliability of the study. During faecal material collection, the questionnaire was administered to the household to obtain demographic information, and ownership of animals (cows, goats, chickens and sheep). Risk factors for soil transmitted helminthes infections were determined via response to the presence and location of toilet at home (no toilet–using “the bush” or latrine). The pretested data was not used in the main study sample.

3.6 Data Management, Analysis and Interpretation

3.6.1 Management

Data from the interviews was recorded in questionnaires and Laboratory findings were recorded in data capture sheet for laboratory results. Data was checked for completeness and accuracy after collection and analysis, on a daily basis, and entered into a personal computer.

3.6.2 Analysis and Interpretation

Collected data was analyzed using SPSS and presented through frequencies and percentages. The information is presented by use of bar charts, graphs and pie charts and in prose-form. This involved tallying up responses, computing percentages of variations in response as well as describing and interpreting the data in line with the study objectives through use of SPSS. Data interpretation was done in each data representation. Content analysis was used to analyze qualitative data or the data collected from the open-ended questions.

3.7 Ethical Considerations

Ethical clearance of the study was obtained from Kenyatta National Hospital-University of Nairobi Ethics and Research Committee, Rwanda National Health Research Committee and College of Medicine and Health Sciences Institutional Review Board. Permission from the community was sought before initiating the study by communicating the responsible health management offices. Individual informed consent was sought from participating members of the households in the local language, ikinyarwanda, for all literate heads of homesteads to enroll them and their free-range chickens and herbivores into the study voluntarily. Since minors are not included in the study, no consent was sought from their parents for participation. Data collected during the survey from each study participant and results of laboratory tests were kept confidential and were not disclosed to anyone. Study free-range chickens and herbivores were given a unique study identification number.

CHAPTER FOUR
RESULTS

4.1 Introduction

This chapter presents the findings. It comprises of the response rate, respondent's demographic information and the laboratory results.

4.2 Presentation of Results

4.2.1 Questionnaire Response Rate

The study targeted 75 households out of which all of them were visited. This constituted 100% of the sampled population.

4.2.2 Distribution of the Domestic Animals

More than 1 animal species were found in a household and none of the households had closed chickens. Households that had free-range chickens were 29(24.3 %). Those that kept cows were 45(37.8%) while those that kept goats were 42(35.2%). The households that kept sheep were 3(2.7%).See Table 4.1.

Table 4.1Distribution of the Domestic Animals

Domestic animal	**Frequency**	**Percentage**
Free range chicken	29	24.3
Cow	45	37.8
Goat	42	35.2
Sheep	3	2.7
Total	**119**	**100**

4.3. Population Excreta Disposal that may lead to Infections of Domestic Free-range Chickens and Herbivores with Human Soil Transmitted Helminthes.

Table 4.2 shows the number of households that had toilets. The majority 54 (72%) of the households participating in the study had toilets outside their compounds. Another 19 (25.4%) of the households had toilets in their compounds. Only 2 (2.6%) of the households lacked toilets.

Table 4.2 Presence of Toilet

Presence of toilet	Frequency	Percentage
No	2	2.6
Yes:-in the compound	19	25.4
Yes:-out of the compound	54	72
Total	**75**	**100**

4.4 Grazing of Domestic Animals that may lead to Soil Transmitted Helminthes Infections from Free-range Chickens and Herbivores

Table 4.3 shows the grazing behavior of herbivorous animals among the households. The majority 66 (88%) of the households grazed their herbivores in open areas. Only 9 (12%) of the households grazing these in closed areas. All the animals that were grazed in closed area were sheep and goats. All free-range chickens grazed in open area.

Table 4.3 animal Grazing in households

Grazing in the open area	Frequency	Percentage
Yes	66	88
No	9	12
Total	**75**	**100**

4.5 Laboratory Results

4.5.1 Human Soil transmitted Helminthes in Chickens 'Droppings

Chickens' droppings obtained from 3 households out of the 29 (10%) households that kept free-range chickens were found with hookworm eggs. The same number of chickens' droppings examined was found to have *Trichuris trichiura* eggs .In addition to these STH *Taenia* eggs were found in chickens droppings obtained from 1household (3.4%). *Trichostrongylus* spp. eggs were also found in chickens droppings obtained from another 1 household (3.4%).See Table 4.4

Table 4.4 HumanSoil transmitted Helminthes in Chickens' Droppings

Helminthes eggs	Frequency	Percentage
Hookworms	3	10.3
Trichuris trichiura	3	10.3
Taenia spp	1	3.4
Trichostrongylus spp	1	3.4
Total	**29**	

4.5.2 Human Soil Transmitted Helminthes in Stool of Domestic Herbivores.

Cows stool samples obtained from 5 households out of the 45 (11.1%) households that kept cows were found with hookworm eggs. *Trichostrongulus spp.* eggs were also found in cows stool samples obtained from 3 households (6.6%). None of the cows stool examined had *Trichuris trichiura* eggs. See Table 4.5.

Table 4.5 Human Soil Transmitted Helminthes in the Stool of cows

Helminthes eggs	Frequency	Percentage
Hookworms	5	11.1
***Trichostrongylus* spp**	3	6.6
Total	45	

Goats stool samples obtained from 11 households out of the 42 (26.1%) households that kept goats were found with hookworm eggs. *Trichuris trichiura* eggs were found in goats stool samples obtained from 3households (7.1%). *Trichostrongulus spp.* eggs were also found in goats stool samples obtained from 15 households (35.7%).See Table 4.6.

Table 4.6 Human Soil Transmitted Helminthes in the Stool of Goats

Helminthes eggs	Frequency	Percentage
Hookworms	11	26.1
Trichuris trichiura	3	7.1
Trichostrongylus spp	15	35.7
Total	42	

Sheep Stool samples obtained from one household out the 3(33.3%) households that kept sheep had hookworm eggs. None of the sheep had the other type of soil transmitted helminthes eggs.

Hookworm eggs were differentiated from those of *Trichostrongylus spp*. by observing and comparing their sizes and shapes under light microscopy. Hookworm eggs were smaller in size and bluntly rounded at the ends compared to *Trichostrongylus* eggs, which were bigger and tapered at one end. *Trichuris trichiura* eggs were also identified from other *Trichuris* spp eggs normally found in animals by looking at their polar plugs. *Trichuris trichiura* were found with clearly prominent polar plugs.

CHAPTER FIVE
DISCUSSION

5.1 Human Soil Transmitted Helminthes in Stool of Domestic Herbivores

The study sought to find out whether there were human STH in the stools of domestic herbivores. Hookworm eggs were found in stool samples obtained from cows, goats and sheep. These were distinguished from *Trichostrongylus* spp eggs which were only in cows and goats by the morphological characteristics under microscope as described above .To the best of my knowledge this is the first time that hookworm eggs have been identified from stool samples obtained from these animals. However an experimental study by Ackert & Payne (1922) in which pigs were fed with eggs of human hookworms found a high proportion of the hookworm eggs swallowed by the pigs were recovered from their faeces. This suggests that herbivorous animals may act as transport hosts for hookworms. Furthermore in a study by Olcen *et al*.(2001) demonstrated that pigs can act as transport hosts for *Ascaris suum, Trichuris suis* and *Oesophagostomum dentatum*. The study cited that there is high probability that domestic animals are also able to transport human parasites.

Trichuris trichiura eggs were only identified from goats. Similarly no previous study has described these findings in herbivores although herbivorous animals have intestinal parasites that produced eggs that resemble *Trichuris trichiura* eggs such as *Trichuris suis* none of these parasites have eggs with clearly demonstrated prominent polar plugs like the ones seen in this study. It is possible that *Trichuris trichiura* eggs ingested by goats passed safely through the digestive tracts. A study by Olcen *et al*.(2001) showed that when pig ingests *Trichuris suis* eggs some of the eggs may be passed intact in their stool. *Trichostrongylus spp* eggs were also obtained from cows and goats stool specimens as expected since these are parasites for herbivorous animals and human get infections from these parasites accidentally (Megumi *et al*., 2011).

The community in which this study was conducted has previously shown to have a high prevalence of STH infections among school children. The study found out that 191/240 (79.6 %) of school children in Kirehe District had STH eggs in their stool

samples. Hookworms, *Trichuris trichiura* and *Ascaris lumbricoides* eggs were found in 77.9 %, 8.8% and 0.8% of school children studied respectively. No other species were found (CIDC Rwanda, 2008). The present study showed the presence of hookworm eggs in stool specimens obtained from cows, goats and sheep. *Trichuris trichiura* eggs were only found in goats, no *Ascaris lumbricoides* were found. However, for logistic reasons, the present study did not examine children for these households for STH in order to correlate household infections and presence of infection in animals. In addition *Trichostrongylus* spp. eggs were found in both cows and goats stool specimens. Therefore, it is possible that these domestic animals act as transport hosts for human STH in this farming community mainly hookworm infection since it is highly prevalent in that community and the present study determined its presence in cows, goats and sheep. However, a study by Megumi *et al.*(2011) has shown clearly that *Trichostrongylus* spp. eggs can be misdiagnosed as hookworm eggs. Where by 46 villagers who were found to harbor hookworms by microscopic examination, only 3/46 had common human hookworms after performing molecular examination and 43/46 was found with *Trichostrongylus* spp. Researchers went a head examined and identified *Trichostrongylus colubriformis* in a goat from the same village. The results suggested that the letter was causing human hookworm infections in that village (Megumi *et al*., 2011).

5.2 Human Soil Transmitted Helminthes in Chickens Droppings

The present study also sought to find out the presence of human STH in chickens 'droppings. Eggs of hookworm, *Trichuris trichiura* were found in chickens' droppings obtained from households. In addition to STH *Trichostrongylus* species and *Taenia* eggs were also found. Hookworm eggs have been previously demonstrated in chickens' droppings in an experimental study by Ackert (1922) in Trinidad where by some hookworm eggs were able to remain viable while passing through the alimentary canal of chickens and were able to hatch. *Trichuris trichiura* eggs probably from human origin have been previously demonstrated in chickens' droppings Otto *et al*., (1931). Furthermore Olsen *et al*. (2001*)* demonstrated that the eggs of *Trichuris suis* when ingested by chickens can be passed in their droppings intact. *Trichuris suis* are parasites similar to *Trichuris trichiura* thus chickens may be transport hosts for *Trichuris trichiura*. The *Taenia* eggs have not been identified

previously from chickens' droppings. Although Otto *et al*. (1931) demonstrated *Ascaris lumbricoides* eggs in chickens' droppings the present study did not identify them. Eggs of *Trichostrongylus* spp. In chickens' droppings have also been previously identified in the study by Orunc and Bicek (2009).

5.3 Population Excreta Disposal that may lead to Infections of Domestic Free-range Chickens and Herbivores with Human Soil Transmitted Helminthes

The study sought to find out how the community excreta disposition may lead to infections of domestic free-range chickens and herbivores with STH. Although the majority of the households had toilets, these were located outside their compounds and it is thus possible that they were not being used all the time, especially by young children who may not be able to walk the long distance out of the compounds. It can be speculated that the domestic herbivores and free-range chickens acquired the STH from ingestion of the eggs in contaminated environment. Therefore, the presence of STH in the stool of domestic animals may be attributed to the community not utilizing the toilets. Since free-range chickens and herbivores move freely in the communities searching for food it is unavoidable that they can accidentally ingest STH eggs, which have been deposited in human faeces.

The results of the study match also those of a study by Ackert (1922)showing that in feeding about the yard and edges of the fields free-range chickens are likely to swallow material containing infective hookworm larvae and the later deposit it about the door yard. This may be attributed to the community not utilizing the toilets or the culture of using human excrements as fertilizer.

These findings differ with the findings of Ulukanligil (2001) which, did not link domestic animals with the transmission. According to the study, in areas where infected individuals excrete STH eggs in their faeces, in regions where people regularly defecate in the open, the soil becomes contaminated with eggs. People pick up *Ascaris lumbricoides* or *Trichuris trichiura* infections when they ingest these eggs after they have matured in the environment by eating raw, unwashed vegetables or by not washing their hands after handling contaminated soil (a common transmission route for children).

5.4 Grazing of Domestic Animals that May Lead to Soil Transmitted Helminthes Infections from Chickens and Herbivores

The study sought to find out the grazing of domestic animals that may lead to STH infections from chickens and herbivores. The study found out that majority of the households graze in open areas with the least number of the population doing closed grazing. Eggs passed intact in chickens' droppings and stool of herbivores may be a potential source of infections to human beings if accidentally ingested like in the case of *Trichuris trichiura* or if the eggs hatch to release larva which can penetrate the skin like in the case of hookworms therefore the fact that chickens' droppings and stool from cows, goats and sheep were found with eggs of human STH suggest that these animals contributed to transmission of these infections to humans. However the study by Ackert (1922) showed that the great majority of hookworm eggs ingested by chickens fail to produce infective larvae suggesting that in fact chickens ingesting hookworm eggs may actually contribute to the reduction in transmission of hookworm infections to human beings.

Findings are also supported by the study done in Laos village by Megumi *et al.* (2011).The village had semi-domesticated goats, cows, fowl, and dogs which wondered freely everywhere. According to the researchers the risk of contaminating vegetables in the fields and sources of water with animal faeces was high. In the same village many people were diagnosed with common human hookworm eggs although almost all were later identified as *Trichostrongylus spp*. after performing molecular diagnosis.

CHAPTER SIX
CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The study has found out that the domestic animals had eggs of human hookworms and *Trichuris trichiura* in their faeces. In addition to these STH, eggs of *Trichostrongylus species* and *Taenia species* were also found. From the literature, this can be attributed to the fact that when infected individuals excrete helminthes eggs in their faeces, in regions where children and irresponsible persons regularly defecate in the open, the soil and pasture become contaminated with eggs. The findings suggest that domestic herbivores and free-range chickens may be serving as a reservoir for these infections. This has an implication in the control of these STH infections since even if mass chemotherapy is to be implemented as a method of controlling these infections, the population could still become at risk of acquiring infection from soil contaminated with the human STH eggs, but coming from the domestic animals, by eating raw, unwashed vegetables or by not washing their hands after handling contaminated soil (a common transmission route for children) or by skin penetration of filariform larvae developing in the soil from the hookworm eggs passed from the animals. The free-range chickens and open grazing for the cows, goats and sheep plus the community recreating in the bush may have enhanced the spread of soil transmitted helminthes infections.

6.2 Recommendations

This study recommends the use of pit latrines for the villagers to minimize the spread of STH infections. This is so because the households cannot manage to practice closed grazing for their animals since the land is communal. In addition, the households cannot afford closed range system for their chickens as they don’t have enough funds to purchase chickens feeds. The study calls for community education on STH infections to equip the community with basic knowledge for better prevention.

6.3 Study Limitations

One of the limitations of the study is that it is not absolutely clear whether the eggs found in the domestic herbivores and free-range chickens' droppings are actually eggs of human helminthes and not of domestic animals. This will need more laboratory tests for confirmation. In addition, the sample size was small and will need to be confirmed in a larger study.

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APPENDICES

APPENDIX I: CONSENT FORM

Respondent study Number: ---------- **Date: ----------**

Study Title:

Soil Transmitted Helminthes in free-range Chickens and domestic Herbivores: Potential Reservoirs for Human Infections in Rwandan Village.

Investigator: MS. Gahongayire Fatuma, Medical Microbiology, University of Nairobi.

Cell phone: 0788453355

Supervisors: 1. Prof. Walter Jaoko, Medical Microbiology, University of Nairobi

2. Prof. Kato Njunwa, College of Medicine and Health Sciences, University of Rwanda

Investigator's statement:

I am asking you and your domestic animal to kindly participate in this study. The purpose of this consent form is to provide you with the information you will need to help you decide whether or not to participate in the study.

Introduction:

The control of STH infections in developing countries is of considerable public health importance. Infections with STH share for a long time closely linked with conditions of poverty, unsafe water, sanitation and hygiene and probably domestic animals play a role in the transmission of STH infections and this study seeks to establish the exact role of domestic animals. You will be asked a number of questions that will take an average about 20 minutes of your time. We will collect animal droppings in your compound and use them for the study. No laboratory tests relating to this study will be carried out on you and the laboratory tests from the animal droppings will be treated with utmost confidentiality. Your daily duties will not be interrupted by your agreement to participate in the study.

Benefits:

The results of the study will help all the stake holders in the health sector have a glimpse on the extent to which domestic free-range chickens and herbivores transmit STH and to make recommendations for control.

Risks:

No direct or indirect risks are anticipated in this study. Only a bit of your precious time will be taken by the interviewer.

Voluntariness:

Participation in the study will be fully voluntary. You are free to refuse to participate or withdraw from the study at any time. There will be no financial reward to you for participating in the study.

Confidentiality:

The information obtained about you and your domestic animal will be treated with utmost confidence and information to identify you or domestic animal will not be released to any person or forum without your permission.

Questions:

If you ever have any questions regarding the study you can contact the investigator, Ms. Gahongayire Fatuma, Tel: +250788453355.

- Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee, Tel: 020 726300-9
- University of Rwanda/College of Medicine and Health Sciences Institutional Review Board,
  Fax: +250571787,phone :(250)788490522

Participant's statement:

I _____having received adequate information regarding the study research, risks, benefits hereby AGREE to participate in the study with domestic animal. I understand that our participation is fully voluntary and that I am free to withdraw at any time. I have been given adequate opportunity to ask questions and seek clarification on the study and these have been addressed satisfactorily.

Respondent's Signature: _____ Date _____

I _____ declare that I have adequately explained to the above participant, the study procedure, risks, and benefits and given him /her time to ask questions and seek clarification regarding the study. I have answered all the questions raised to the best of my ability.

Interviewer's Signature _____ Date _____

CONSENT FORM (IKINYARWANDA VERSION)

Numero y'usubiza: ----------Tariki: ----------

Umutwe w'ubushakashatsi:

Soil Transmitted Helminthes in free-range Chickens and domestic Herbivores: Potential Reservoirs for Human Infections in Rwandan Village

Umushakashatsi: Gahongayire Fatuma, Medical Microbiology, University of Nairobi.

Numero y'itumanaho: +250788453355

Abayobozi1. Prof. Walter Jaoko, Medical Microbiology, University of Nairobi

2.Prof. Kato Njunwa, College of Medicine and Health Sciences, University of Rwanda

Ijambo ry'ukora ubushakashatsi: Muraho! Nitwa Gahongayire Fatuma, ndi umunyeshuli muri kaminuza ya nayirobi nkaba niga ibirebana na mikorobe mu buvuzi bwa kizungu.

Nasabaga wowe n'amatungo woroye kwitabira ubu bushakashatsi. Iyinyandiko igamije kugirango usobanukirwe neza ibijyanye ni ubu bushakashatsi hanyuma utwemerere cg se wange wowe ni amatungo gukorerwaho ubushakashatsi.

Gusobanura ubushakashatsi:

Guhangana n'ikibazo cy' imyandurire y'inzoka mu bihugu biri munzira y'amajyambere ni uRwanda rurimo bishingiye kukwita kubuzima bw'abaturage muri rusange. Ubwandu bw'inzoka rero bwagiye buhuzwa n'ubukene, amazi yanduye, isuku idahagije ndetse no ku bushakashatsi bwakozwe hirya no hino bugaragaza ko amatungo yororerwa mu rugo nk'inkoko, ihene, intamani andi yakwirakwiza izo nzoka mu baturanyi batari banduye bakandura. Muri make ubu bushakashatsi bugamije kureba koko niba aya matungo yaba afite urahare mu ikwirakwiza ry'izinzoka. uribubazwe ibibazo bike biza gufata iminota makumyabiri yonyine ikindi ni uko turibuze gufata umusarani w'ayo matungo (inkoko, ihene intama n'inka) bikazadufasha mu bushakashatsi turi gukora. Mwe nta musarani cg se ibindi bizamini bikorwa kwa muganga musabwa gutanga ndetse ibizamini by'amatungo yanyu biraza gukorwa neza kandi mubu ryo bw'ibanga. Ikindi ni uko tutari burogoye imirimo yanyu ya buri munsi kugirango mu itabire ubushakashatsi.

Inyungu:

Ibizava muri ubu bushakashatsi bizafasha abakozi b'urwego rw'ubuzima kongera ubumenyi ku bijyanye ni uburyo inkoko, ihene, intama n'inka zikwirakwiza inzoka mu bantu cyane cyane aboroye ndetse n'uburyo hafatwa ingamba zo kurwanya iryo kwirakwizwa.

Ingaruka:

Ntangaruka zavuba cg z'igihe cyizaza kubantu bari bwitabire ubu bushakashatsi cg amatungo yabo. Keretse gusa igihe cyanyu muri buduhe.

Ubushake:

Kwitabira ubu bushakashatsi ni ubushake gusa. Ni uburenganzira bwawe kwitabira cg kubyanga igihe ushakiye.Kandi nt'amafaranga ateganijwe kugirango witabire.

Ibanga:

Nkwijeje kugira ibanga ku makuru uri bumpe yaba akwerekeye ndetse ni amatungo yawe kandi ayo amakuru yatuma umuntu agusobanikirwa ntabwo azerekwa undi muntu uwo ariwe wese keretse abonye uruhushya rwawe.

Ibibazo:

Ugize ibibazo ushaka kubaza kuri ubu bushakashatsi, wabaza nyir'ubushakashatsi Ms. Gahongayire Fatuma, Tel: +250788453355.

-Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee, Itumanaho: 020 726300-9

-University of Rwanda/College of Medicine and Health Sciences Institutional Review Board, Fax: +250571787, phone :(250)788490522

Amagamboy'uwitabira:

Jye_____maze kumva neza ibijyanye ni ubu bushakashatsi, ingaruka, inyungu nemeye hamwe n' amatungo yanjye kwitabira ubu bushakashatsi. Nasobanuriwe neza ko kubwitabira cg kutabwitabira ari uburenganzira bwanjye ndetse ni igihe cyose nashakira navamo. Nahawe ni uburyo busesuye bwo kubana baza ibibazo byo kugirango mbashe gusobanukirwa neza n' ubu bushakashatsi.

Umukono w'usubiza: _____ Tariki _____

Jye_____ndemeza ko nasobaniriye bihagije uvugwa haruguru witabiriye ubu bushakashatsi, ibyerekeye ubu bushakashatsi, ingaruka, ni inyungu ndetse muhani igihe ngo abaze ibibazo bishoboka byose kugirango asobanukirwe cyane n'ubu bushakashatsi. Nasubije neza uko nshoboye kose kugirango asobanukirwe.

Umukono w'ubaza _____ Tariki _____

APPENDIX II:

QUESTIONNAIRE

SOIL TRANSMITTED HELMINTHES IN FREE-RANGE CHICKENS AND DOMESTIC HERBIVORES: POTENTIAL RESERVOIRS FOR HUMAN INFECTIONS IN RWANDAN VILLAGE

A CASE STUDY OF RWANDAN VILLAGE

This research is meant for academic purpose. You're kindly requested to provide answers to these questions honestly and precisely as possible. Responses will be treated with utmost confidentiality.

Gender of the respondent

[ ] Male

[ ] Female

Age in years of respondent

[ ] 18-25

[ ] 26-35

[ ] 36-45

[ ] 46-50

[ ] Above 50

Education level

[ ] None

[ ] completed Primary

[ ] Secondary Certificate

[ ] Post-Secondary Qualification

4. Number of people living in house ☐ =1 ☐ =2 ☐ =3 ☐ =4 ☐ = 5

5. Do you have a toilet?

[ ] No

[ ] Yes; which type? 1. Toilet in compound

2. Outside shared facility or latrine

6. Which domestic animals do you keep?

☐ 1. Free range chickens ☐ 2.enclosed chickens ☐ 3.herbivores (cow, goat, sheep, and pig)

7. Do your domestic animals graze in the open areas?

[ ] Yes

[ ] No

8. To what extent do you understand helminthic infections? Please rank between 1-5 (5 being the highest).

Not at all	Small Extent	Not Sure	Some Extent	Large extent
□□	□□□	□□	□□	□□

9. Have you or any other member of the family been diagnosed with any helminthes?

[ ] Yes

[ ] No

10. Which strategies do you think that can be adopted to minimize helminthes transmission through free-range chickens and domestic herbivores?

……

……

……

Thank you for your participation in this study.

QUESTIONNAIRE (I KINYARWANDA VERSION)

SOIL TRANSMITTED HELMINTHES IN FREE-RANGE CHICKENS AND DOMESTIC HERBIVORES: POTENTIAL RESERVOIRS FOR HUMAN INFECTIONS IN RWANDAN VILLAGE

A CASE STUDY OF RWANDAN VILLAGE

Ubu bushakashatsi bugamije kumenya niba amatungo yororerwa mu rugo kandi yemererwa kugendagenda ashaka ibyo arya kumanywa hanze y'aho arara yaba akwirakwiza amagi y'inzoka zo munda aho abantu bashobora kwandura indwara ziterwa no kumira amagi cg gukandagira inzoka zibasha kwinjira mu ruhu.Urasabwa gutanga ibisubizo by'ukuri kandi udatandukiriye ku bibazo biri bubazwe. Ndakwizeza ko ibisubizo uri butange bizakoreshwa neza bigatuma hafatwa ingamba mu kurwanya indwara ziterwa n'inzoka zo munda.

1. Igitsina cy'usubiza.
   [ ] Gabo
   [ ] Gore
2. Imyaka y'usubiza
   [ ] 18-25
   [ ] 26-35
   [ ] 36-45
   [ ] 46-50
   [ ] hejuru ya 50
3. Amashuri yize
   [ ] ntiyize cg yacikirije abanza
   [ ] yarangije abanza, yacikirije ayisumbuye cg yize imyuga
   [ ] yarangije ayisumbuye
   [ ] yakomeje kwiga arangije ayisumbuye

4. Umubare w'ababa mu rugo =1 ☐ =2 ☐=3 ☐ =4 ☐= 5 ☐

5. Mufite umusarani?

[ ] ntawo

[ ] yego; uteye ute? (i). Umusarani uri imbere mu rugo

(ii) Uri inyuma y'urugo ndetse tuwuhuriyeho ni abandi

6. Ni ayahe matungo mworoye?

☐1.Inkoko zitembera kumanywa ☐ 2.inkoko zifungiranwa ☐3.amatungo arisha ibyatsi (inka, ihene, n'intama)

7. Amatungo yanyu arisha hanze y'urugo?

[ ] yego

[ ] oya

8. Ni kukihe kigero wumva uburyo bwo kwandura indwara ziterwa ni inzoka zo munda?

Nta na gito	Gito cyane	sinkizi	Kinini	Kinini cyane
☐☐	☐☐☐	☐☐	☐☐	☐☐

9. Wowe cg uwo mu bana haba hari uwigeze asuzumwa inzoka zo munda?

[ ] yego

[ ] oya

10. Ni izihe ngamba utekereza ko zafatwa zikaba zagabanya ubwandu bw'inzoka zo munda zaba ziterwa n'amatungo yororerwa mu rugo ndetse akemererwa no kugendagenda ashaka ibyo kurya hanze y'urugo

……

……

Murakoze ku ruhare rwanyu muri ubu bushakashatsi

TIME FRAME

Number	Activity	Estimated time
1	Proposal Development	January to March 2014
2	Submission of proposal for ethical approval	April 2014
3	Data Collection	July 2014
4	Data Analysis	August 2014
5	Thesis development	September 2014
6	Presentation	October 2014