GASTRO INTESTINAL HELMINTH PARASITES IN STRAY AND PET DOGS OF KATHMANDU VALLEY, NEPAL

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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Date: 2079-05-08

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This is to recommend that the thesis entitled "Gastro-intestinal helminth parasites in Stray and pet dogs of Kathmandu Valley, Nepal" has been carried out by Govinda Prasad Bhattarai for the partial fulfilment of Master's Degree of Science in Zoology with special paper Parasitology. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor "Prof. Dr. Mahendra Maharjan" this thesis submitted by Govinda Prasad Bhattarai entitled "Prevalence of Gastro-intestinal helminth parasites in stray and pet dogs of Kathmandu Valley, Nepal" is approved for the examination and submitted to the Tribhuvan University in partial fulfilment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Govinda Prasad Bhattarai entitled "Gastro-intestinal helminth parasites in stray and pet dogs of Kathmandu Valley, Nepal" has been accepted as a partial fulfilment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
WHO	world health organization
GI	gastro-intestinal
PVT.LTD	private limited
Sp	species
PCR	polymerase chain reaction
CBS	central bureau of statistics
gm	gram
ml	milliliter
Sp.gr	specific gravity.
Nacl	sodium chloride
X^2	chai-square
DNA	deoxy-ribo nucleic acid

ABSTRACT

To ascertain the prevalence of gastrointestinal helminth parasites of dogs in the Kathmandu valley from March to August 2021, a cross-sectional study was carried out. For the investigation, a total of 120 fecal samples were collected: 60 from stray dogs and 60 from pet dogs. Out of 60 stray dog samples, 20 were obtained from the ground right after stray dogs defecated on it throughout the morning, and the remaining 40 samples were collected in collaboration with the rescue team of Sneha's Dog Care Center in Lalitpur. In contrast, 20 samples of pet dogs were taken from the animals brought to the central referral veterinary hospital in Tripureswor, Kathmandu, 20 from the city veterinary hospital pvt.Ltd. Satdobato, Lalitpur, and the remaining 20 samples were obtained through personal contact with dog owners or visits to their homes. The samples were subjected to direct, sedimentation, and floatation microscopy examinations. The central veterinary hospital in Tripureswor, Kathmandu, is where the laboratory work was completed. According to the study, the total prevalence of gastrointestinal helminth parasites is 27.5%, with stray dogs showing a higher prevalence (41.33%) than pet dogs (13.33%). *Ancylostoma sp.* (39.39%) showed the highest prevalence among the five helminth parasites that were identified in this study, followed by *Taenia sp.* (24.24%), *Ascaris sp.* (15.15%), *Toxocara sp.* (12.12%), and *Trichostrongylus sp.* (9.09%). However, only three helminth parasites were discovered in canine companions, with *Ancylostoma sp.* (6.66%) having a greater infection rate than *Ascaris sp.* (3.33%) and *Taenia sp.* (1.66%). In both stray and pet dogs, the age-based prevalence revealed higher frequency in dogs older than five years old than adult dogs and puppies. Male dogs (24.63%) had a lower overall sex prevalence than female dogs (31.37%). However, it was discovered that pet dogs, both male and female, were each afflicted. Dogs were more likely to have helminth parasites in Kathmandu, Lalitpur, and Bhaktapur districts than other areas. In this study, local breed stray dogs had higher helminth parasite infection rates than mixed breed dogs. In this investigation, it appears that helminth parasites are not present in the German shepherd and German retriever breeds.

Keywords: GI (helminth) Parasite, Cross-sectional study, dogs, Prevalence, Kathmandu valley

1. INTRODUCTION

The dog (*Canis familiaris),* the first domesticated creature, coexisted with humans (Briekner I, 2002). Out of the estimated 500 million canines globally, 400 million are thought to be stray dogs (Roldan *et al.,* 2010). This number is larger in developing nations, where stray dogs wander freely, increasing the danger of human intestinal parasite infection (Overgaauw&van, 2005). Dog parasite infection is a serious health concern for people everywhere (Naquirac, 2010). Increased zoonotic parasite prevalence results in significant human financial and bodily losses (Maha&Hossein, 2009). Dogs serve as a reservoir and transmitter of a variety of parasites that are crucial to public health (WHO, 1998). According to Bahrami *et al.* (2011), many gastro-intestinal parasites spread through their feces in the form of eggs, larvae, cysts, and oocysts, and in the majority of cases, this is the main cause of zoonotic parasitosis. Worldwide, helminth parasite prevalence in dogs ranges from 67.4% to 100%. (Schantz, 1991). Low levels of hygiene, insufficient veterinary care, and a lack of knowledge about zoonotic illnesses are other factors that contribute to the spread of disease to humans (Shimelese, 1994).

For quite a few parasites, dogs serve as the final host, but other animals may serve as intermediate hosts. *Giardia lamblia*, *Toxocara canis*, *Cryptosporidium* sp., *Ancylostoma duodenale*, *Echinococcus granulosus*, *Dipylidium caninum*, and *Toxoplasma gondii* are a few parasites that can spread from dogs to people (Nicolle and Manceaux, 1908) (Overgaauw *et al.,* 2009; Xhaxhiu *et al.,* 2011). In underdeveloped nations, diseases like hydatidosis, taeniasis, and echinococcosis are the most prevalent zoonotic diseases brought on by dog helminth parasites (Kaewthamasornk *et al.,* 2006). These intestinal parasite illnesses have a negative direct and indirect impact on human and animal health. Both stray and pet dogs play a part in the spread of helminth parasites. Human infection with helminth parasites is a developing health issue as a result of sharing an environment with animals, according to Dalimi *et al. (*2013)

The main cause of canine hookworm disease in the majority of tropical and subtropical regions of the world is *Ancylostoma caninum*. Male *A. caninum* measure 12mm in length, while females measure 15mm. Some species are smaller than others

(Aiello, 1998). This species can be found in the fox's and dog's small intestine. Fresh dog feces from infected dogs that float readily reveal the distinctive thin-shelled oval eggs. Young puppies may have acute anemia and infectious milk-borne mortality before eggs are discharged in their stool (Aiello, 1998). Severe infection is accompanied by diarrhea and black feces. Transmission can occur through the soil, skin invasion or puncture, ingesting infected larvae from the environment, from the milk of infected dogs, etc.

Dog cestodes (Tapeworms) include *Taenia* sp*, Echinococcus* sp*, D. caninum, and D. latum*, among others (Charkarborti, 2006). *Taenia* sp., a huge tapeworm with multiple proglottids, and *Echinococcus* sp, a tiny cestode, are two of them. They are located in dogs' tiny intestines. *Taenia* sp. can range in size from 70 cm to 500 cm, and *Echinococcus* sp. can range in size from 2.1 mm to 5.02 mm (Urquhart *et al.*, 1998). (Lapage, 1965). Rarely do these cestodes in the intestines of dogs and cats cause severe illness or clinical symptoms. The main clinical symptoms are fatigue, irritability, appetite loss, moderate diarrhea, abdominal pain, etc (Bhatia *et al.*, 2006). There may be signs of stunted development, malabsorption, a pot belly condition, diarrhoea, vomiting and anemia, rubbing of the anus on the ground, and biting at the anal region out of discomfort (Charkraborti, 2006). The proglottids or eggs found in the feces using the microscope approach provide the basis for the diagnosis.

In small animals like dogs and cats, the two most common roundworms are *Ascaris* sp. and *Toxocara* sp. They are the biggest nematodes and are mainly seen in pets. Veterinarians should pay attention to both the larval and adult stages. The larval stages can move in humans and can even infect newborn puppies fatally because of their migratory tendencies (Urquhart *et al.*, 1996). (Aiello,1998). Their eggs have a thick, smooth, sub globular shell and are dark brown. Transmission can happen through the placenta (Aiello, 1998), through the consumption of larvae found in the milk of heifers (Urquhart *et al.*, 1996), or through contact with contaminated excrement in the environment. Puppy deaths result from severe infections. As a result of a larva's migration to the lungs, pneumonia, nausea, and diarrhea with mucus may occur (Aiello, 1998). Clinical symptoms and the finding of eggs in their feces are used to make the diagnosis. Their eggs have a rounded, pitted shell (Aiello, 1998).

Dog parasites are extremely pathogenic not just to their particular host. They might also be the main contributors of zoonoses. Since humans and dogs coexist in close quarters, there are zoonotic parasites that can spread to humans and have negative effects. Zoonotic parasites can be spread through direct contact with animals, infected water, infected food, and indirect contact with animal secretions and excretions (Lappin, 2002). Small children, the elderly, and those with impaired immune systems are those most at risk of contracting a zoonotic infection (Juckett, 1997).

The capital and most populous city of Nepal, Kathmandu, has a large population of stray dogs (Kakati.k, 2010).Domestic dogs have received more attention for deworming than stray dogs. Due to this, stray dogs are more likely to contract parasites with serious medical implications. Studies on dogs are nearly limited to domesticated animals, and the absence of stray dogs from these studies biases the findings. Numerous studies have studied the prevalence of endoparasites in various regions' bovine, caprine, avian, etc. species. However, there aren't many studies on canine endoparasites, particularly those that focus on canine parasites. Therefore, the purpose of this study is to assess the prevalence of gastrointestinal parasites in dogs from various locations throughout the Kathmandu Valley.

1.2 Objectives of the study:

1.2.1 General objective:-

To determine the prevalence of gastro-intestinal helminth parasites of stray and pet dogs of Kathmandu valley.

Specific objectives:-

i. To compare the age, sex, location and breed wise prevalence of gastro intestinal parasites in stray and pet dogs

1.2 Rational of the study

Gastrointestinal parasites have great veterinary and public health importance that produce morbidity in both dogs and humans. The gastrointestinal parasites are known to be endemic in Nepal, especially in stray and semi domesticated dogs.. These parasites may be transmitted to human either directly through the ingestion of infective stages via close contact with a dog or indirectly through skin penetration or

ingestion of infective stages in the environment including those that may be food or water borne.

There appears a lack of widely accessible up to date information available on the prevalence and distribution of gastrointestinal parasites in Nepal. This study aimed to determine the prevalence and distribution of gastrointestinal parasites in stray and pet dogs from different localities in Kathmandu valley, Nepal.

2. LITERATURE REVIEW

2. 1 Background

According to Rhindali *et al.* (2006), dogs are linked to the spread of more than 60 zoonotic illnesses, including the helminth parasites that can have a significant impact on public health across the globe. Dogs are the undisputed hosts for a number of intestinal parasites that can transmit serious zoonotic diseases including hydatidosis, which is brought on by the *Echinococcus granulosus*. A potential risk of infection for humans is created by the growing trend of pet ownership and the large number of stray dogs that live with human homes in the city areas of developing countries. Due to the pollution of these locations by the feces of diseased dogs, playgrounds, parks, gardens, temples, and other public areas could potentially serve as sources of infection for both healthy dogs and humans. The diseased dog can transmit the disease to humans directly or indirectly through the intake of contaminated food and water. Furthermore, the danger of transfer of these diseases to humans is increased by the poor hygienic circumstances, insufficient veterinary care, and lack of knowledge about zoonotic diseases.

Dogs' gastrointestinal helminthes can have a devastating effect on both the host and people. They prevent dogs from being raised successfully and cause losses that show up as decreased resistance to infectious diseases, stunted growth, decreased work and feed efficiency, and general poor health (Soulsby, 1982). Symptoms displayed by parasitized animals vary according on the type and density of the parasites (Dunn, 1978).Certain things that resemble parasitic forms may be swallowed by animals. These are referred to as pseudo parasites. They include a variety of innocuous plant and animal waste, as well as pollen grains, plant hairs, grain mites, and mold spores. In feces, false parasites are discovered. As an illustration, coprophagy may cause a few feces of a scavenger or predator host to include parasite eggs or cysts from one species of host (Sloss, 1970).

Dogs may contract gastro-intestinal helminth parasites such as Ascarid worms (*Toxocara canis, T.leonia),* Hookworms (*Ancyclostoma canium, Uncinaria stenocephala),* Whipworms (*Trichuris vulpis),* and Tapeworms (*Diphyllobotherium latum, Diphylidium caninum, Echinococcus granulosus,* Chakraborty, 2006).

2.2 In Global Context

Distinct countries around the world have different helminth parasite infections in dogs. Studies on the parasitism of stray dogs housed in government shelters in various European nations are lacking. *A. caninum, T. canis, T. vulpis, Cryptosporidium* sp., *and Strongylidium* sp. were reported to be common in Portugal. (Otero *et al.* 2014; Silva 2010; Melo and Lebre 2011; Otero *et al.* When compared to the lower prevalence rates shown in other European nations, the overall prevalence of intestinal parasites recorded in one study in North Portugal was 57.2%, suggesting a significant presence of gastrointestinal parasites in stray and shelter dogs (Pullola et al. 2006; Becker *et al.* 2012; Dado *et al.* 2012; Zanzani *et al.* 2014). By Katagiri and Oliveira-Sequeira (2008), stray and housed dogs in Brazil both had similar values of 54.3%, while stray dogs in Nigeria had 52.6%. Malaysia scored 48% (Okoye *et al.* 2011, Mahdy *et al.* 2012), Canada scored 21%, and (Joffe et al. 2011). Iran (86%, Emamapour et al. 2015), Mexico (85%, Egua-Aguilar *et al.* 2005), South Africa (76%, Minner *et al.* 2002), Spain (71%, Martnez-Carrasco *et al.* 2007), and Poland (68%) all observed higher prevalence rates in stray dogs (Bajera *et al.* 2011).

The environment at shelters encourages the spread of parasites in dogs, and the main infections that cause sickness in shelter dogs are those that are spread by ingestion of parasitic developmental stages from a polluted environment (Ortuno, A., Castella, and J. 2011). Shelter and kenneled dogs transmit gastrointestinal parasites more commonly than owned dogs, according to studies comparing different dog populations (stray dogs, owned dogs, kenneled dogs, and shelter dogs). The increased prevalence in shelter dogs was attributed to increased exposure to parasites as a result of daily admissions of dogs from different origins, environmental contamination, and made worse by the possibility that the dogs' immune systems may have been compromised as a result of various stressors in the shelter environment (Palmer;2010). The highest prevalence of GI parasites was found in Mexico (98%; Alvarado-Esquivel *et al.,* 2015), followed by Serbia (75%; Sommer, *et al.*, 2017), and Iran (66%; Beiromvand, *et al.,* 2013), with lower prevalence in Ethiopia (-51%; Yacob, *et al.,* 2007), Malaysia (-48%; Mahdy, M.A.; 2012), and Portugal (39%). (Ferreira, F.S., 2011) Australia -37% (Palmer, C.S., 2010), Venezuela (36%; Ramirez-Barrios, R.A., 2004), and Canada (21%); (Joffe, D., 2011). In the UK, fewer but still sizable numbers of stray dogs are admitted into animal shelters.

Sub-Saharan Africa has a very high prevalence of canine helminthiasis. It is possible that insufficient dog deworming is to blame for the high frequency of gastrointestinal helminths (Adedoja A., 2014). The prevalence of *Ancylostoma* sp. was the highest. These results are comparable to research from Brazil (Katagiri, et al. 2008), Mexico (Canto, *et al*. 2011), Portugal (Ferreira, *et al.* 2011), and Argentina (Katagiri, *et.al* (Fontanarrosa, 2006). Toxocara sp. was identified as the most common helminth in studies conducted in the Czech Republic (Dubna *et al.*, 2007), Poland (Tylkowska *et al.*, 2010), Canada (Villeneuve *et al.*, 2015), Denmark (Al-sabi *et al.*, 2013), and southern Wisconsin (Coggins *et al.,* 1998). According to these results, the tropical/subtropical and temperate zones, respectively, had higher prevalences of the parasites *Ancylostoma* sp. and *Toxocara* sp. Cestode *Dipylidium caninum* was the most common kind. This is consistent with research from China (Selasine *et al.*, 2013), Mexico (Canto *et al.*, 2011), Brazil (Katagiri *et al.*, 2008), and Poland (Katagiri *et al.*, 2008). (Tylkowska, *et.al*, 2010).

In Pakistan, there is very little knowledge about the frequency of canine gastrointestinal parasites and their environmental contamination. More parasites were found in stray dogs (34.4%) than in domestic dogs (-16.1%). (W.khan *et.al*, 2020). In other regions of the world, the prevalence rate was higher than that of this study, as reported by Puebla et al. (2015) for Cuba, Satyal *et al.* (2013) for Nepal, and Puebla et al. (2013) for Sri Lanka (Perera *et al.*, 2013). The most prevalent and crucial to canine pathogens hookworm is *Ancylostoma caninum.* The research done in Spain -6.2% shown the low incidence of this parasite (Causape *et al.,* 1996). Higher prevalence of this nematode was found in Australia (Jenkins and Andrew, 1993) at 100%; in the Galapagos at 57.7%; in Tanzania (Swai *et al.,* 2010); in Australia at 22.1%; and in Nigeria at 17.9% (Gingrich *et al.,* 2010). (Sowemimo and Asaolu, 2008). Hungary recorded the lowest prevalence rate at 8.1%. (Fok *et al.,* 2001). In Nigeria, Sowemimo, 2009 reported a maximum infection rate of 33.33% but Asaolu, 2008 found a prevalence of only 9% for the same parasite in the same nation. According to Papazahariadou et al. (2007), samples of owned shepherd and hunting dogs taken from Northern Greece have higher levels of Toxocara canis prevalence. The incidence of gastrointestinal helminth parasites was found to be 54.3% overall in stray dogs and domestic dogs from the Brazilian state of Sao Paulo (Katagiri *et al.*, 2007).

Today, helminth parasites are detected using a molecular technique called polymerase chain reaction (PCR). According to G. Suganya *et al.* (2018), India has a 23.72% prevalence of helminth parasites. The material that tested positive for Taenia sp by microscopy also tested positive for *Echinococus granulosus* using PCR. Rebecca *et.al.,* 2014 used PCR technique for the molecular detection of helminth parasites of dogs fecal samples collected from different parts of india and has found higher prevalence of *Ancylostoma ceylanicum* and *Ancylostoma caninum* either as single or mixed infection in sikim. W. Khan *et al.,* 2020 has recorded 26.8% prevalence of helminth parasitic infection from dogs stool samples collected from lower Dir. district Pakistan. *Dipylidium caninium* (11.8%) was more prevalent helminth parasitic infection at that study carried out by microscopy method.

2.3 In the National Context

In the context of Nepal, there are very few studies on canine gastrointestinal parasites. The majority of research done to this point have used microscopy techniques. Although molecular techniques are more standard and advanced than microscopy method, the results for the detection of canine helminth parasites from their feces are not extensively established. In Rupandehi district, Yadav KK & Shrestha B, 2017 reported 58.75% positive samples for the presence of at least one helminth parasite. In that study, stray dogs had a considerably greater prevalence of helminth parasites (78.5%) than pet dogs (39%) did. In a similar vein, companion dogs younger than one year of age had higher infection rates than dogs older than one year. Male pet dogs had a 46.97% higher prevalence of helminth parasites than female dogs, which was 23.53%. Pet dogs of mixed breeds had higher infection rates than purebred canines. The *Ancylostoma sp* infection had the greatest rate of occurrence (46.8%) among the species studied in the same study. However, Satyal et al. (2013) found that dogs in the Kathmandu district had a 46.7% prevalence of gastrointestinal helminth parasite infection. The prevalence of *Ancylostoma* sp. was highest (52%) in that study as well. More female stray dogs than pet dogs had the infection. The infestation rate was higher in puppies under the age of two. In 2010, Giri DR reported that samples of dog feces presented to Tripureswor Central Referral Hospital in Kathmandu had a prevalence of gastrointestinal helminth parasites of 41.3%. The prevalence of *Ancylostoma* sp. was higher in that study as well, and pups were more likely to contract helminth parasites than older dogs. It was discovered that female

dogs were more diseased than male dogs. According to Shrestha NR (2011), more cross breeds (28.57%%) than pure breeds (23.94%) and mongrel breeds (18.5%) were found to be positive. In the investigation, it was discovered that female dogs and puppies were more infected than male dogs and older canines.

As a result, the parasite problem in dogs, both stray and pet, varies by country. In general, Asian and African nations have more stray dogs with gastrointestinal parasites than European and American nations. Similar to this, in developed nations, a molecular approach has been adopted for routine tests to identify intestinal parasites in dog feces samples. In comparison to microscopy, the molecular approach is more trustworthy and has a higher level of specificity for the detection of parasite species. In general, it was discovered that female dogs and dogs of mixed breeds were more sick than male and purebred canines. *Ancylostoma* sp. was also discovered to be more common than other parasites in both stray and domesticated dogs all over the world.

3. MATERIALS AND METHOD

3.1 Study area and sample collection site

From March to August 2021, a cross-sectional survey was conducted for canine helminth parasites in the Kathmandu Valley of Nepal's Bagmati Province. The valley is situated at an altitude of 1400m above sea level in the middle of the country's hilly region, between 85.3240oE and 27.7172oN. (CBS, 2007). The study region included Kathmandu, Lalitpur, and Bhaktapur, three districts in the valley. The fecal samples were brought to the Central Referral Veterinary Laboratory Hospital in Tripureshwor, Kathmandu, from various locations throughout the valley. Following the rescue team of Sneha's Dog Care Center Lalitpur, feces of stray dogs were collected from various locations, including Kalanki, Shantinagar, Baneshower, Satdobato, Emadol, Balkhu, Baisepati, Sallaghari Bhaktapur, Thimi and Lokanthali of the Kathmandu valley, as well as from the ground immediately after stray dogs urinated during the early morning from BodeThimi, Lokanthali, Balkot, Chagunarayan and Tikathali.Pet dog's samples were collected from Central referral Veterinary Hospital Tripureshwor, Kathmandu, City Veterinary Hospital Pvt.Ltd, Satdobato and by Individual household visits of the public people from different localities such as Thimi chapacho, Kausaltar, Koteswor and Emadol.

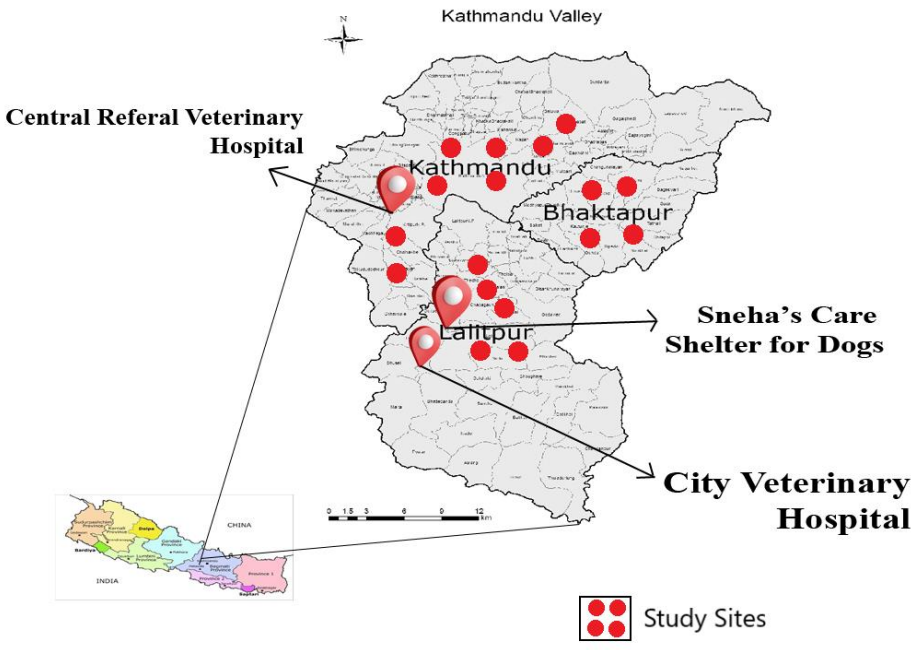

Figure 1: Study Area

3.2 Sample size and sample collection technique

There were 120 fecal samples taken in all (60 from stray dogs and 60 from pet dogs). Out of 60 fecal samples from stray dogs, 40 samples were collected by the Sneha's Dog Care Center Lalitpur rescue team from various locations in the Kathmandu valley, including Kalanki(6), Shantinagar(5), Baneshower(4), Satdobato(3), Emadol(3), Balkhu(3), Baisepati(3), Sallaghari Bhaktapur(3), Thimi(5), and Lokanthali (4). The use of muzzles helped control the dogs. With the gloves on and the index finger turned inside out from their rectum/anus, samples were taken. The remaining 20 fecal samples were purposefully taken from the ground in the early morning hours in the following locations: BodeThimi(5), Lokanthali(3), Balkot(4), Chagunarayan(5), and Tikathali (5). Sterile plastic vials (samples marked as male/female distinguished by their external organs). In case of pet dogs, 20 samples were taken from the dogs admitted by owners themselves in the central Referral veterinary laboratory Hospital Tripureshwor Kathmandu Nepal, Other 20 samples were collected from city Veterinary Hospital pvt.Ltd, Satdobato, Lalitpur and remaining 20 samples were taken from the other different localities such as Thimi chapacho(5),Kausaltar(7),Koteswor(4) and Emadol(4). of the valley through direct personal contact or visiting.

3.3 Sample preservation and transportation

Samples were maintained by thoroughly dipping sterile plastic vials in 10% formalin solution. Age, sex, gender, bread, location, date, and time of collection were all noted on the labels of each vial. The samples were physically examined by hand to determine their color, condition, whether they were bloody or not, and whether they were mucus- or non-mucus-producing. Following that, the conserved samples were brought to the central referral veterinary hospital in Kathmandu, Tripureshwor, where they were kept in a refrigerated box for testing.

3.4 Laboratory examinations

Laboratory examinations of fecal samples from both stray and pet dogs were conducted separately as follow:

3.4.1 Direct wet mount smear technique

This method was employed when little material was available or when the fecal investigation needed to be finished quickly. With the help of a wooden applicator stick, fresh feces samples (2–3 grams) were applied to a slide. For semi-solid and diarrhoeic samples, the material was emulsified with a drop of physiological saline (0.85%), and for formed stool samples, iodine was utilized. They were then covered with a cover slip and inspected under a microscope at first a 10x and then a 40x magnification (Kirk&Bistner, 1969).

3.4.2 Sedimentation method

The fecal material was ground to around 2-3 grams, added to a beaker with 100 ml of water. The liquid was transferred to a beaker after passing through a tea sieve. The material that was still in the sieve was thrown out. About 70% of the supernatant was removed after 10 minutes and replaced with fresh water until the supernatant was cleared. The bottom sediment was then studied under a microscope (Soulsby, 1978).

3.4.3 Floatation method

Any floating technique is based on the principle that worm eggs will float to the surface when suspended in a liquid with a specific gravity greater than that of eggs. Eggs from nematodes and cestodes float in fluids with specific gravities of 1.10 to 1.20. The significantly heavier trematode eggs need a specific gravity of 1.30-1.35. For nematode and cestode ova, sodium chloride (specific gravity=1.20) and magnesium sulphate (specific gravity=1.28) are employed as floating solutions. Trematode eggs are floated in a saturated solution of zinc chloride (specific gravity=1.18) or zinc sulphate (specific gravity=1.18) (Bhatia *et al.*,2006).About 2-5 gm of faecal sample was taken and mixed with 10 ml floatation solution. The mixture was poured through a tea strainer into a beaker. The strained solution was poured into a 15ml centrifuge tube and it was filled with floatation solution. Cover slip was placed on the tube and centrifuged at 2500rpm for 5 minutes. Then after, tube was removed from centrifuge and let it stand for 10 minutes. The supernatant was discarded and remaining sediments was mixed with small amount of saline. Again, One drop of sediment was transferred on the glass slide along with a drop of saline and covered by cover slip. Then after, slide was examined under light microscope at 10x magnification and then at 40x magnification (Bhatia *et al.*, 2006).

3.5 Identification of parasitic eggs

With the aid of a microscope and 10x and 40x objective lenses, parasite eggs observed in the fecal samples were identified based on their form, shell composition, colors, and other exterior features. According to Foreyt, 2001; Bhatia *et al.,* 2006; Thienpont *et al.,* 1986; and with reference to the text book of Veterinary Clinical Parasitology, the identification of eggs was carried out.

3.6 Data entry and analysis

The data was entered in MS-excel sheet for statistical analysis and coded for SPSS. The coding of the data was done for breed, location, sex and age of the dogs. Effects of age, sex, location, breed and prevalence of parasites was calculated by chi-square test.Value of $p<0.05$was considered significant at 95% level of confidence.

3.7 Ethical approval

Any research related to human, animals, plants or any forms of biomes should have pre-informed consent from the concerned authority. So, the ethical approval for the study was taken from veterinary council. This approval letter was submitted to Snhea′s dogs care center for the ethical approval of the provision for the collection of samples along with their rescued team.

4. RESULTS

4.1 Overall Prevalence

Out of 120 dogs fecal samples examined, 33 (27.5%) were positive for the presence of at least one helminth parasite in Kathmandu valley. The prevalence of helminth parasites was 41.66% (n=25) for stray dogs and 13.33 %(n=8) for pet dogs (Fig1) respectively.

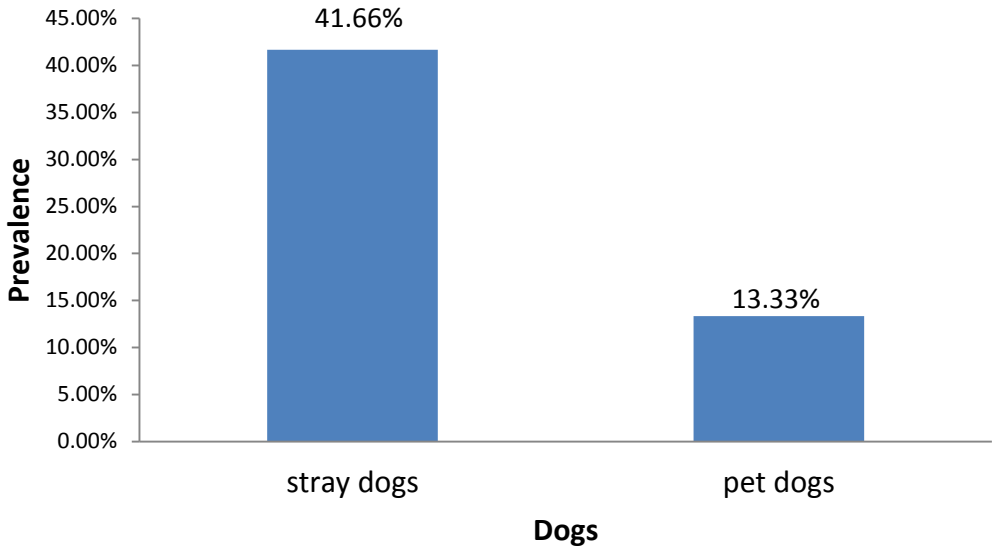

Figure2: Overall prevalence of helminthes parasites in stray and pet dogs of Kathmandu valley

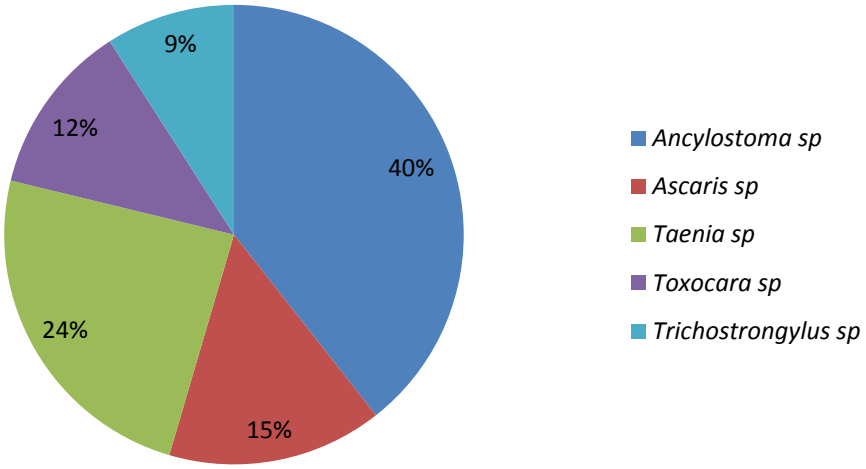

Figure 3: Overall species wise prevalence of helminthes parasites in dogs of Kathmandu valley

In both pet and stray dogs of Kathmandu valley altogether five different helminth parasites were recorded. Among them, *Ancyclostoma* sp showed the highest (40%) prevalence followed by *Taenia* sp (24%). *Trichostrongylus* sp. infection was found to be comparatively less prevalence (Fig 3).

Table 1: Species wise prevalence of helminth parasites in pet and stray dogs of Kathmandu valley.

Helminth parasites	**Pet dogs (N=60)**			**Stray dogs (N=60)**			**Prevalence (N=120)**
Ancylostoma sp	6.66%	X^2=3.22	P=0.782	15%	X^2=0.933	P=0.817	39.39%
***Ascaris* sp**	3.33%			5%			15.15%
***Taenia* sp**	1.66%			10%			24.24%
***Toxocara* sp**	0%			6.66%			12.12%
Trichostrongylus sp	0%			5%			9.09%

Pet dogs were found to be less infected than stray dogs with helminth parasites .Only three species of helminth parasites were detected on them. Out of them, *Ancylostoma* sp infection was found to be higher. Whereas stray dogs were found highly infected with five different species of helminth parasites. Comparatively, *Ancylostoma* sp infection was found high followed by *Taenia* sp infection. Prevalence of *Ascaris* sp, *Toxocara* sp and *Trichostrongylus* sp was found comparatively less (Table 1).

Table 2: Age wise prevalence of helminth parasites in dogs of Kathmandu valley.

Age group of dogs	**No. of samples**	**Prevalence**
Puppy(0-2 years)	8	6.66%
Adult(2-5 years)	8	6.66%
Senior(above 5 years)	17	14.16%

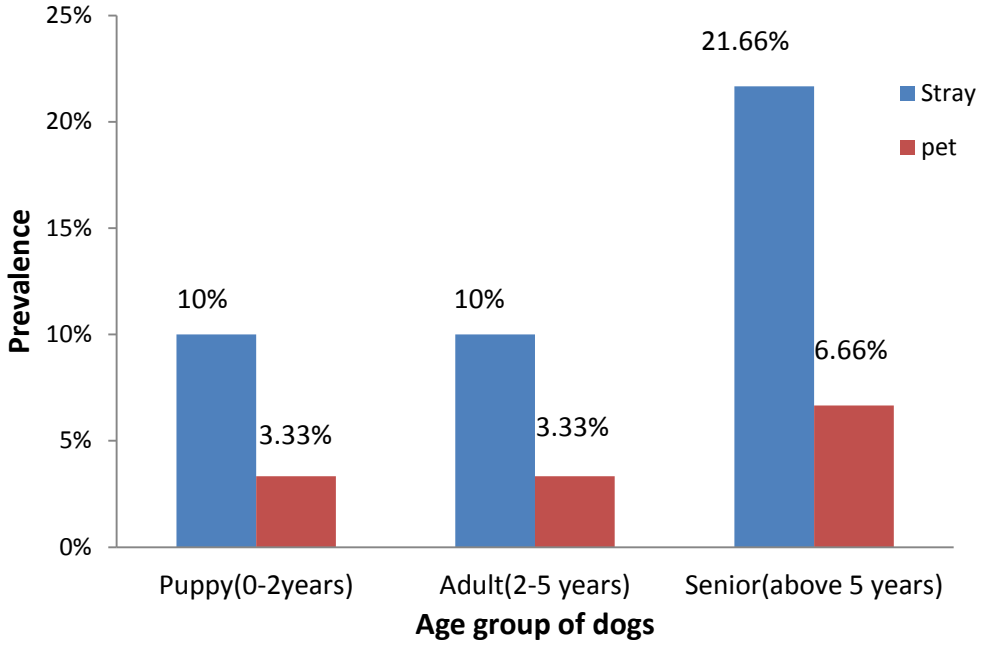

Figure 4: Age wise prevalence of helminth parasites in stray and pet dogs of Kathmandu valley.

The overall prevalence of different age group showed that dogs of senior stage (above 5years) had high prevalence of helminth parasites followed by puppy stage (0-2 years) and adult stage of 2-5 years (table 2).There is high prevalence of helminth parasites in stray dogs in all the stages. The highest prevalence is in stray dog of age above 5years. (Figure 4)

Table 3: Overall sex wise prevalence of helminth parasites in dogs of Kathmandu valley.

Sex of dogs	**No. of samples**	**Prevalence**
Male	69	24.63%
Female	51	31.37%
Total	120	27.37%

The overall prevalence of helminth parasites is comparatively high in female dogs (31.37%) than that of male dogs of Kathmandu valley. High infection was recorded in stray dogs than pet dogs. In stray dogs high prevalence is in male than female but in case of pet dogs, high prevalence was in female than male(Table 3 and Fig 4).

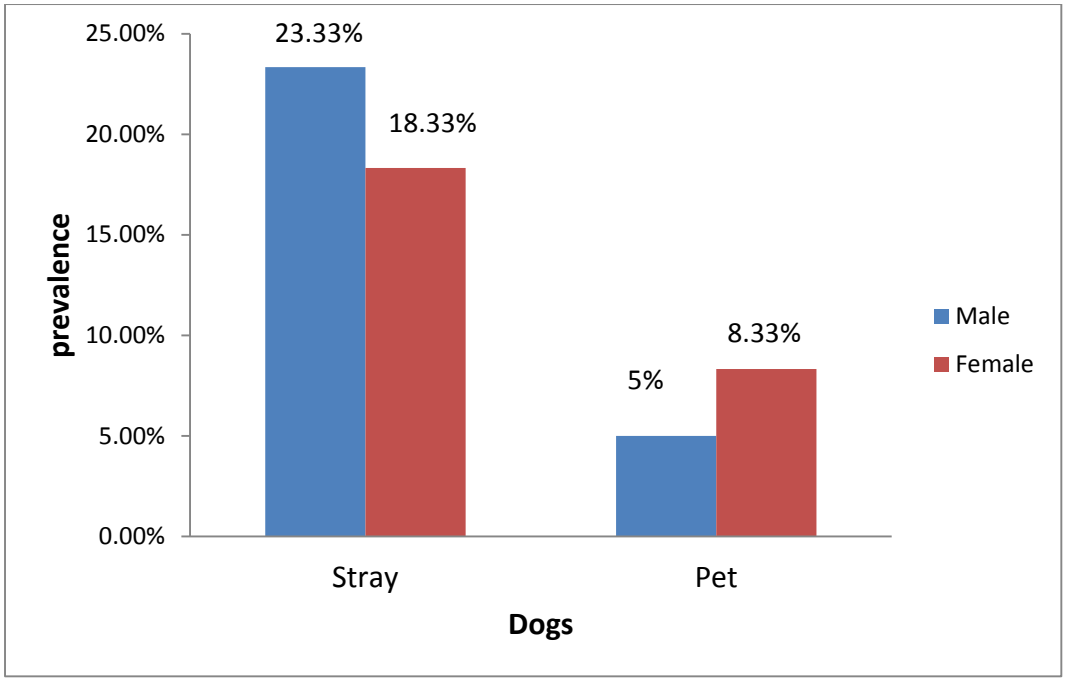

Figure 5: Sex wise prevalence of helminth parasites in dogs of Kathmandu valley.

The overall helminth parasitic infection in dogs was found almost similar in Kathmandu and Lalitpur districts but comparatively less in Bhaktapur district (Figure 6).However, this prevalence is higher in the stray dogs than pet dogs(Table 4).

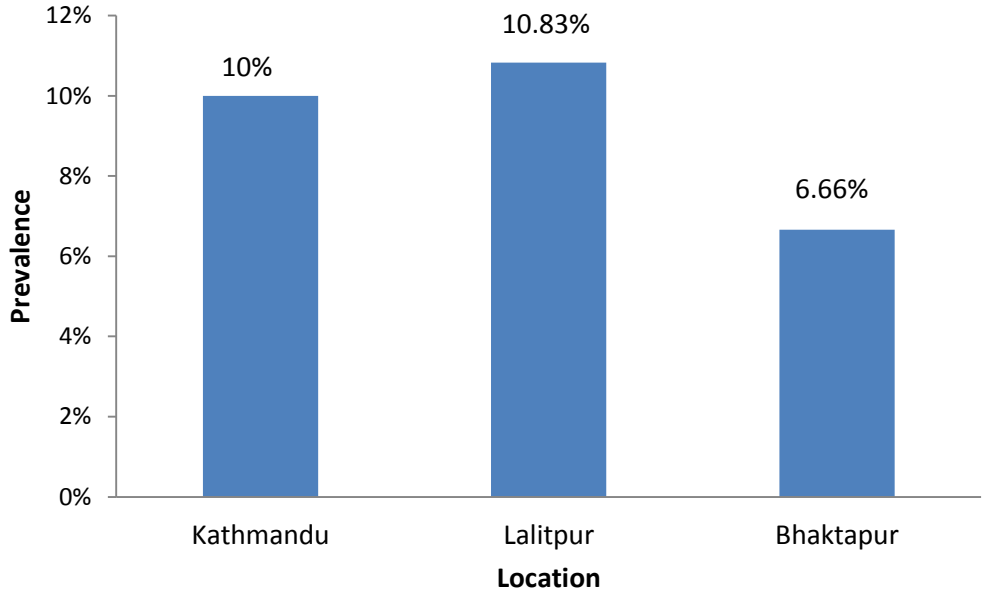

Figure 6: Overall location wise prevalence of helminth parasites in dogs of Kathmandu valley.

Table 4: Location wise prevalence of helminth parasites in stray and pet dogs of Kathmandu valley.

Location	**Stray dogs (N=60)**	**Pet dogs (N=60)**
Kathmandu	15%	5%
Lalitpur	16.66%	5%
Bhaktapur	10%	3.33%

The breed wise prevalence showed that local breed stray dogs were mostly infected by helminth parasites followed by mixed breed. The helminth parasitic infection was not recored in other breed of stray dogs during this study time. But in pet dogs similar type of infection was observed in local, mixed and other breeds. The parasitic infection was not recorded in the common pet dogs breed like German.sepherd and German.retriver in the study area.

Table 5: Breed wise prevalence of helminth parasites in dogs of Kathmandu valley.

Breed of dogs	**Stray dogs (N=60)**	**Pet dogs (N=60)**	**Total (N=120)**
Local	25%	3%	14.16%
Mixed	16.66%	3%	10%
L.retriver	0%	2%	1.66%
Others	0%	2%	1.66%

5. DISCUSSION

According to current fossil and DNA data, dogs were domesticated from wolves as recently as 15000 years ago (Morey, 2006), or possibly as early as 100000 years ago (Savolaian *et al.*,2002, Lindbald-Toh,2005). There are two different kinds of dogs: domestic dogs and wild dogs. Domesticated dogs differ from wolves in both behavior and appearance, being smaller and having shorter muzzles and teeth. Dogs that are kept as pets and strays that live around us are both forms of domesticated animals. Dogs serve as the host for a variety of zoonotic illnesses. They are in charge of spreading a number of bacterial, viral, and parasitic diseases to people. Stray canines walk freely in our neighborhood after being exposed to many hazardous and polluted chemicals. It is crucial that dog owners and the general public become more informed and aware of how diseases are spread. The following intestinal helminth parasites can infect dogs: Ascarid worm (*Toxocara canis, T.leonina*), Hookworm (*Ancyclostoma caninum, Uncinaria stenocephala),* Whipworm (*Trichuris vulpis),* Tapeworm (*Diphyllobothrium latum, Dipylidium caninum, Echinococcus granulosus, Taenia hydatigena, T.psisiformis, T.taeniaeformis, epirometra* sp.etc (chakrabotry, 2006).

Regarding the prevalence of canine gastrointestinal helminth parasites in the Kathmandu Valley of Nepal, no more information is documented. In order to ascertain the incidence of helminth parasites in dogs in the Kathmandu Valley, 120 fecal samples (60 from strays and 60 from pets) were analyzed.The overall prevalence of 27.5% was found. This prevalence is lower than the result of Ghimire, 2002, Karki, 2003, Giri, 2009, and Satyal (2013). According to Davoust *et al.* (2009) in north-east Gabon, the prevalence was 94.1%; Umar (2009) in Kaduna State, Nigeria, the prevalence was 93.8%; Egua-Aguilar *et al.* (2005) from Mexico, the prevalence was 85%; and Lavallen *et al.* (2005) from Argentina, the prevalence was 89.13%. High prevalence was stated by Martnez-Moreno et al. (2007) from Spain and Minnaar et al. (2008) from South Africa, 71% and 76%, respectively. In contrast to what was found in this study, Sowemimo & Asaolu, 2008 from Ibadan reported a lower prevalence of Duch (Nobel *et al.*, 2004) in Santiago, Chile (Lopez *et al.*, 2006). Climate, geographic location, sample methodology, management and healthcare practices, diagnostic methods, prevention and control measures, and public awareness are some of the factors that determine this diversity.

In the Kathmandu Valley, stray dogs have a greater prevalence of helminth parasites (41.66%) than pet dogs (13.33%). This outcome is consistent to past research from Nepal 46.7% (Satyal *et al;* 2013). and Cuba (Puebla *et al.*, 2015). In comparison to all other research, lower prevalence were found in Pakistan (Khan *et al.,* 2018), Mexico (Chable *et al.,* 2015), Bangladesh (Mahmud *et al.,* 2014), and Iran (Kohansal *et al.,* 2017). The difference in helminth parasites may result from a variety of factors, such as the lack of deworming for dogs, the scavenging of meat and fish offal, repeated contact with contaminated soil and dirt, consumption of raw vegetables washed down with water tainted with human waste, and/or the consumption of fecally contaminated soil (Adanir and Tascl, 2013).

Ancylostoma sp. (39.39%), *Taenia* sp. (24.24%), *Ascaris* sp. *(*15.15%), *Toxocara* sp. (12.12%), and *Trichostrongylus* sp. (9.09%) were the most prevalent parasites found in the current investigation. In the present investigation, *Taenia* sp. and *Ancylostoma* sp. were the most common parasites found. The most prevalent and important canine hookworm is *Ancylostoma* sp. According to Jenkins and Andrew (1993), Australia has a 100% prevalence of this nematode, followed by Tanzania with 57% prevalence and the Galapagos with 57.7% prevalence (Gingrich *et al.,* 2010). Spain had a 6.2% prevalence rate while Hungary had an 8.1% one (Fok *et al.,* 2001). (Causape *et al.,*1996). Nigeria is ranked 17.9% (Sowemimo and Asaolu, 2008), and Australia is 22.1% (Bugg *et al.,* 1999).

In the current study, *Taenia* sp. (24.24 %%) is the second most common parasite. This figure is rather greater than that of research like the 11% study from Spain (Martinez-Moreno). However, this cestode is not as common elsewhere in the world, with prevalence rates of 0.4% in Tanzania (Swai *et al.,* 2010), 2.8% in Hungary (Fok *et al.,* 2001), 0.2% in Belgium (Claerebout *et al.,* 2009), and 2.5% in Spain (Causape *et al.,* 1996). One of the parasitic pathogenic helminths with human origins is *Taenia* sp. *Taenia saginata* infection has also been documented in humans from Pakistan (Arshad *et al.,* 2019); Karachi, 9.70% (Khan *et al.,* 2019). In endemic populations around the world, this illness has a significant economic impact as well as significant health and social ramifications.

Toxocara sp is also called the dog round worm has worldwide in distribution with great zoonotic importance. *Toxocara* sp (12.12%) and *Ascaris* sp *(*15.15%) have

almost similar prevalence in the current study. The prevalence was also almost similar with studies conducted in Tanzania (Swai *et al.,* 2010); and Galapagos Islands (Gingrich *et al.,* 2010).The highest prevalence of this nematode was reported from Canada (Seah *et al.,* 1975); Hungary (Fok *et al.,* 2001); and Spain 29.2% (Martinez-moreno *et al.,* 2007);This nematode was reported in low rate of prevalence from Australia 1.7% (Bugg *et al.,* 1999); Belgium 4.6% (Claerebout *et al.,* 2009); Spain 3.7% (Causape *et al.,* 1996) and Nigeria 9.6% (Sowemimo and Asaolu, 2008). Tropical and underdeveloped nations around the world have high toxocariasis prevalence (Glickman and Magnaval, 1993). In dogs, this nematode's life cycle is finished. Humans serve as unintentional hosts for infections. Young children are most commonly affected by toxocariasis, which is contracted through contact with dirt tainted by the parasite-carrying dogs' feces.

The prevalence of helminth parasites was greater in puppies and adults (10%) in pet dogs under the age of 2 years than in Senior (6.66%) dogs over the age of 5. Results appear to support Giri's results (2009). According to Swai *et al.* (2010), the prevalence of parasites is closely correlated with age and is higher in puppies than in adults. Additionally, a recent study suggests that young people seem to be more infected than adults. Young dogs' immature immune systems and the impact of nursing, which is the primary method of parasite transmission to young pups, serve to underline this. The freely moving nature of the animal and/or the lower degree of immunity in older canines compared to mature dogs could both contribute to the higher prevalence rate (21.66%) in older stray dogs.

In current study, male stray dogs had a higher prevalence of zoonotic helminth parasites (23.24%) than female stray dogs (18.33%), whereas pet dogs had a lower prevalence (5%, male versus 8.66%, female). Zelalem and Mekonnen discovered that male dogs had a greater frequency of gastrointestinal helminths (79.2%) than female dogs (76.8%). Additionally, Biu *et al.* (2012) discovered that male dogs were more affected than female canines (57.1% vs. 52.5%).

Location wise prevalence of helminth parasites is almost similar in Kathmandu district (10%) and Lalitpur district (10.18%) but comparatively less in Bhaktapur district (6.66%).There is similar type of climate and environment within these three districts of Kathmandu valley. This resulted similar results. However, habitat

differences among districts, which can affect the free-living stages of nematodes, differences in the proportion of fecal samples of dogs among districts might be another major factor for the differences.

In this study, local stray dogs were more prevalent than mixed breed dogs (8.33%). In a study that is comparable to the study of Ethiopia, Kush Kumar Yadav (2017) discovered that there was a larger prevalence of crossbred pet dogs (44.16%) than purebred pet dogs (35.77%) in the Rupendi district. However, this difference was not statistically significant (2001). Low-income people typically raise native breed dogs and leave them unattended, as seen by the higher number of stray dogs in the area. They might not have the money to pay for routine deworming, biosecurity precautions, etc. Free roaming results in drinking contaminated water from the streets and dining on toxic rubbish. However, because they are more frequently kept as pets, dogs get superior veterinary care overall, including deworming and access to clean food and water.

In conclusion, dog represent a potential public health risk; transmit infective form of parasites to humans. This study calls for the fact that stray dogs play a key role in environmental contamination than house hold dogs. Close contact of humans with untreated dogs, no deworming practices and the favorable climatic conditions are the survival factors of infective stages of dog helminth parasites. The general public and dog owners should be aware on the presence of dog parasites in their surroundings.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

A cross-sectional study was conducted to determine the prevalence of gastrointestinal helminth parasites in dogs of Kathmandu valley. The study showed the overall prevalence of gastrointestinal helminth parasites to be 27.5% with high prevalence in stray dogs (41.66%) than pet dogs (13.33%).

In Kathmandu valley dogs were found to be infected by five different helminth parasites. They were *Ancylostoma* sp ,*Ascaris* sp ,*Taenia* sp ,*Toxocara* sp and *trichostrongylus* sp .Among them pet dog were found to be infected with only three species, i.e. Ancylostoma sp, *Ascaris sp* and *Toxocara* sp. The prevalence showed *Ancylostoma* sp. infection is the most prevalent followed by *Taenia* sp, *Ascaris* sp,*Toxocara* sp. and *Trichostrongylus* sp. Age wise prevalence showed that dogs of senior stage (above 5 years age) were infected more by helminth parasites in both pet and stray dogs. Comparatively, helminth parasitic infection is higher in stray dogs in all the stages of age than that of pet dogs. Female dogs of Kathmandu valley were infected more by helminth parasites than male dogs. In case of stray dogs, helminth infection was recorded more in male dogs but in case of pet dogs this infection was higher in female dogs. Likewise, the stray dogs of Bhaktapur district were less infected by helminth parasites than that of Kathmandu and Lalitpur districts. The breed wise prevalence showed that local breed stray dogs were more infected than mixed breed. In case of pet dogs, two breed among the studied breed i.e. German sephard and German retriever seems to be not infected by any helminth parasites.

Hence, it is concluded that helminth parasitic infection is still a significant problem in the dogs of Kathmandu valley.

6.2 Recommendations

1. Deworming of dogs with effective anthelmintic should be enforced on the basis of periodic mass treatment in the endemic areas.
2. The uncontrolled population of stray dogs along with semi-domesticated dogs exist a close proximity to increasing densities of human population need to be focused while implementing controlled strategies by local government.

3. The general public and dog owners should be aware on the presence of dog parasites in their surroundings.

4. German shepherd and German retriever breeds of dogs seem to be resistance to helminth parasite and recommended to rear as pet dog at home.

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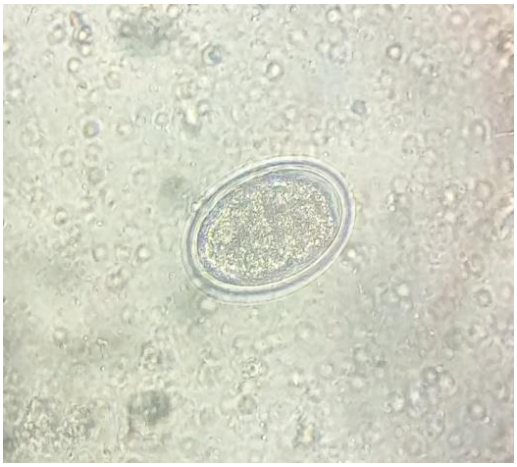
ANNEXES



Ancylostoma sp(at 40X)

Ascaris sp(at 40X)

Toxocara sp(at 40X)

Taenia sp(at 40X)

Trichostrongylus sp

Pet dogs sample at lab

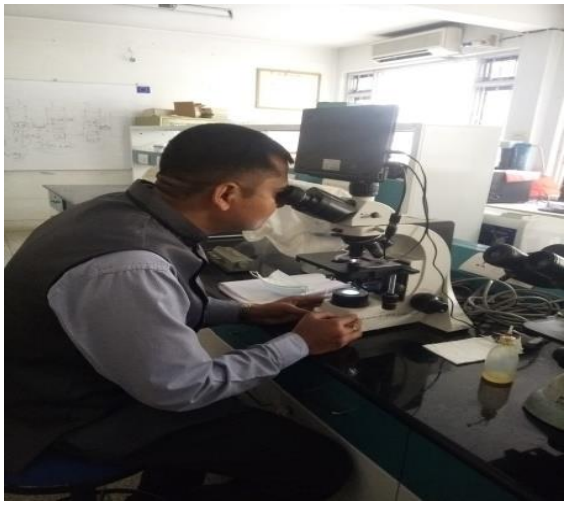
Stray dogs samples at lab

Centrifuging Technique

Sample Collection

Sample Collecting Site

Sedimentation Technique



Microscopic Observition

Government of Nepal

Nepal Veterinary Council

Ref. no. Ethical. 200/2077.78

Date: March 26, 2021

Subject: **Ethical clearance for a research entitled "Gastrointestinal Parasites of Stray Dogs in Kathmandu"**

To

Mr. Govinda Prasad Bhattarai

M.Sc. Student

Central Department of Zoology, Tribhuvan University

Dear **Mr. Bhattarai,**

With reference to your application dated 2077.12.02 regarding the ethical approval for a research study described below, I have the pleasure to inform you that an ethical clearance has been approved for the specified research study with the following terms and conditions.

1. Study detail :
   a. Title: **Gastrointestinal Parasites of Stray Dogs in Kathmandu Valley**
   b. Nature of study: **Requirement of M.Sc. course in Tribhuvan University.**
   c. Principal Investigator: **Mr. Govinda Prasad Bhattarai**
   d. Veterinarian : **Dr. Gyanendra Thakur, NVC Regd no. 859**
   e. **Re**sesearch methodology:
      i. Animal used : **Stray dogs from Kathmandu**
      ii. Sample : **Fecal sample collection under the supervision of veterinarian**
      iii. Lab. test : **Coprological test in laboratory.**
   f. Research laboratory:
      i. **Central Department of Zoology, TU, Kirtipur**
      ii. **Central Veterinary Laboratory, Tripureswor, Kathmandu.**

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नेपाल पशु चिकित्सा परिषद्

Government of Nepal

Nepal Veterinary Council

2. The researcher shall satisfy the requirement prescribed in the prevailing law for export (if necessary) of biological material outside of the country.
3. The study described above shall be carried out according to standard protocol under the supervision of the registered veterinarian mentioned above.
4. The welfare aspect of animal will be well taken care during the research. Any activity posing threat to animal welfare shall be dealt in accordance with the prevailing law.
5. NVC shall retain the right to withdraw or amend this Ethical Approval, if
    a. any unethical principal or practices are revealed or suspected
    b. relevant information has been withheld or misrepresented and
    c. regulatory changes of whatsoever nature so require
    d. in case of violation of animal welfare or detection of activities intended cruelty to animal.
6. The Principal Researcher shall report to NVC in the prescribed format, where applicable,
    a. Six-monthly progress report regarding the ethical compliance, status of animal and the completion report at the end of the project.
    b. However, NVC must be informed immediately of
        i. any material change in the conditions or undertakings mentioned in the document,
        ii. any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.
        iii. any change or revision in protocol during the course of the study, and
7. NVC may carry out monitoring of the study as and when required. However, it is the responsibility of researcher to organize periodic monitoring of study by NVC.
8. The validity of this ethical clearance is one year effective from **March 26, 2021 to March 25, 2025 AD**. You will be required to apply for renewal of ethical clearance on an annual basis till the study is not completed.

Wishing you well in your research

26.03.2021

Dr. Narayan Prasad Ghimire

Copy to: Chairperson, Nepal Veterinary Council

Vice Chairperson, Professional Standard and Complaint Assessment Committee, Nepal Veterinary Council