Occurrence of Intestinal Helminth Parasites in Domestic Dogs (Canis familiaris domesticus) in Arevalo, Iloilo City, Philippines Using the Parasep Fecal Parasite Concentration Tenchnique

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Abstract – This study tested domestic dogs in Arevalo Iloilo City Philippines for the prevalence and intensity of helminth parasites using the parasep fecal parasite concentration technique.The parasites were identified along with the risk factors that accompanied it. Results showed that 21 out 30 dogs were positive for infection with Ancylostoma sp. as the most prevalent species with a prevalence of 30 percent and intensity of 93 eggs per gram. This study concluded that there is an occurrence of intestinal parasitic infection of domestic dogs in Arevalo, Iloilo City, Philippines.

Introduction. – The domestic dog (Canis familiaris domesticus) is generally considered as the first domesticated animal and has been mans companion since the start of animal domestication (Gharekhani 2014). Furthermore, it is also considered as one of the most common household pets in the world. Its constant interaction with human beings made it one of the few organisms who are adapted to the human niche, thus dogs can also be affected by the diseases human beings may acquire. However government actions such as providing information to the citizens about the risks of disease transmission, control of zoonoses transmitted by domestic animals and control of stray dogs are practically non-existent here in the Philippines, resulting in an increasing risk of exposure to zoonoses transmitted by these animals. Dogs serve as reservoir for an array of protozoan and helminth parasites shedding off oocysts/ cysts and eggs in their feces, which could be a cause of soil and water contamination thus increasing the spread of diseases (Santarm et al 2004). There are approximately 60 species of parasites known to infest dogs, some of which are Taenia sp., Echinococcus sp. (hydatidosis), Dipylidium caninum, Toxocara canis (visceral larval migrans), Ancylostoma sp., (cutaneous larval migrans), Giardia sp., and Cryptosporidium sp.. A majority are considered zoonotic, which means that it is capable of human infestation. Additionally, humans are usually the final or definitive host of these parasites where it reaches sexual maturity (Gharekhani 2014). Some of the main causes of morbidity in dogs are intestinal parasites such as Ancylostoma sp., Toxocara sp., Trichuris sp., especially for newly whelped or for puppies (Martinez-Moreno et al.2007). Stray dogs, which are prevalent in almost every industrial area in the Philippines, could be considered as vulnerable targets of parasitic infection. Taking account that helminth eggs remain viable for months in contaminated soil where these dogs scavenge for food, it is most likely that they would ingest these eggs thus becoming the reservoir hosts for future infestations. Symptoms of the presence of parasites in dogs include vomiting, diarrhea, dermatitis, and may sometimes be asymptomatic (Getahun 2012). Specifically, hookworm infestations (Ancylostoma sp.) could develop a severe, sometimes fatal anemia (Bowman 1999). Studies have shown that the prevalence of intestinal parasites in dogs ranges from 4-40 percent both in Japan and Canada, with a higher prevalence in developing countries such as Nigeria and Ethiopia, which reaches to 60 percent (Amissah-Reynolds et al. 2016). Additionally, since dogs live in close association to humans, zoonotic transmission or the transfer of a disease directly from animals to humans could lead to infestation by accidental ingestion of the oocysts. Hookworm larvae (Ancylostoma duodenale) could also penetrate the skin and may travel to organs like the respiratory tract before proceeding to the intestines to develop into maturity (Institute for International Cooperation in Animal Biologics 2013). If not treated, it may cause malnutrition, diarrhea and/or excessive weight loss. Fatal cases include morbidity and mortality particularly in undeveloped areas where autoinfection is rampant

(Kuciket al. 2004). The awareness whether a certain community contains a population of infected dogs is important given the fact that parasitic infection covers a much larger scope than the regular household. Since soil and water contamination could take place through improper fecal management and open defecation of dogs, the prevalence of parasitic infestation in stray and leashed dogs as well as its intensity could provide an image of how rampant the infestation is. Being able to determine the presence and the scope of infection could offer help especially in looking for possible solutions to this problem especially when the Philippines doesnt have a any data or statistics that shows this.

Methods. – *Selection of Study Site.* Iloilo is one of the four (4) provinces in Panay Island located in Region-VI Western Visayas of the Philippines to which its capital is Iloilo City. Currently, there are seven (7) districts in Iloilo City which are Arevalo, Iloilo City Proper, Jaro, La Paz, La Puz, Mandurriao, and Molo. Based on the 2010 Census on Population and Housing (CPH), the current population of Iloilo City is 424,169. According to a study conducted by Robinson et al. 1996, the human to dog ratio in the Philippines is one is to three (1:3). This means that for every dog, there are three (3) humans accompanying it. Based on this data, there should be about 142,000 dogs in Iloilo City. Among these districts only one (1) district was selected. The number of barangays was considered due to the limited time frame given for the study. Added with safety reasons, the district of Arevalo was selected as the district to be the study site. It is located 10.6859 N, 122.5118 E having 13 barangays which are Bonifacio, Calaparan, Dulonan, Mohon, Quezon, San Jose, Santa Cruz, Santa Filomina, Santo Domingo, Santo Nio Norte, Santo Nio Sur, Sooc, and Yulo Drive.

Selection of Domestic Dogs. Selection criteria for the dogs included the presence of leashed dogs in households and consent from the owners to collect samples with a criteria of if and only if the fecal sample was approximately one (1) to three (3) days old. Information such as the age, breed, diet, and medical treatment such as vaccination and deworming of the dogs were also taken. After one household was selected we selected the next household approximately 10 households away from the first one to avoid the auto infections of parasites in dogs.

Collection of Fecal Sample. One mini parasep fecal sample container was given to each of the chosen households. Instructions on how to collect the samples were given and told to collect one gram of stool. They were visited again one (1) day after the distribution in order to obtain the fecal samples. One (1) gram of stool samples from each dog was obtained and was placed in the fecal container and then placed in a storage box until the collection of samples was completed. The obtained specimens were then brought to the Philippine Science High School Western Visayas Campus Laboratory for processing.

Preparation of Fecal Sample. The Parasep Fecal Parasite Concentration Technique (PFPCT) was used in order to process the fecal samples and to detect the presence of helminth eggs. Using the scoop of the Mini Parasep, one (1) gram of stool sample was collected and placed inside the container that contained the mixture of 10 percent Formalin and Triton X.

Microscopy. When all of the samples have undergone processing, each sample was examined using a microscope. Using a pipette, about one (1) ml from each test tube was dropped on a glass slide and then covered using a glass slip. Under the low power objective, each slide was examined using the traditional S-direction scanning. All eggs found were counted and recorded. Parasite Identification In order to identify the genus of the parasite, the morphology of each suspected egg was compared to actual parasitic eggs. See Fig.1

Parasitic Egg	Morphology
Dipylidium spp.	Refer to Appendix B.1
Taenia spp.	Refer to Appendix B.2
Toxocara spp.	Refer to Appendix B.3
Trichuris spp.	Refer to Appendix B.4
Ancylostoma spp.	Refer to Appendix B.5

Figure 1: Standard Morphology of Eggs

Data Analysis. The terms prevalence and mean intensity of infection were used to denote the percentage of infected hosts in the sample and the number of parasite that was recovered from each infected host. The intensity of helminth eggs present was in eggs/g. The prevalence and mean intensity was be determined using the following. See Fig 2.

$$Prevalence = \frac{Number\ of\ Samples\ Positive}{Total\ Number\ of\ Samples}$$

Figure 3.1

$$Mean\ Intensity = \frac{Number\ of\ Eggs\ per\ Sample}{Number\ of\ Positive\ Samples}$$

Figure 3.2

Figure 2: Prevalence and Intensity

Safety and Precautions. Segregation of waste materials were followed thoroughly. All materials that were used were labelled. Ideal proper laboratory attire were worn. Laboratory gown, pair of gloves, and masks were used during the entire duration of the study. All glassware were handled with care. All glassware were rinsed three times into the waste container before they were cleaned. Extreme caution was followed when handling the chemicals.

Glassware were stored in a safe place to avoid scratches and breakage. After every experiment, the glassware were cleaned and dried as soon as possible prior to storing. All the glassware used were placed on a pot full of water and were left for boiling after using them. The glassware were

then rinsed with tap water twice, they were first rinsed with liquid detergent and then rinsed for the second time with distilled water. Glassware were then placed upside down in a tray for drying. Uncontaminated materials and contaminated materials were labeled. Then when dry, the materials were ready for storage or for disposal. Glasswares contaminated with biological material were emptied and decontaminated by boiling. They were rinsed thoroughly with tap water, drained and air dried.

PARASITE SPECIES	EXAMINED	INFECTED	PREVALENCE [%]	MEAN INTENSITY [epg]
Ancylostoma sp.	30	9	30.0	93
Trichuris sp.		8	26.7	91
Toxocara sp.		3	10.0	21
Larvae		1	3.3	39

Figure 3: Prevalence and mean intensity of gastrointestinal helminth in Domestic Dogs in Arevalo, Iloilo City, Philippines

Comparison of Dog Ages. Higher prevalence of Ancylostoma sp. is associated with puppies with a prevalence of 80 percent compared to those of adult dogs which is around 65 percent. This result is also similar to the study of Esquivel et al. (2015), Savilla et al. (2011), and Little et al. (2009) to which their studies showed that puppies whose age were below 12 months were more susceptible to parasitic infection. A possible inference to this is that since puppies tend to stay where their mother stays, especially leashed dogs, the tendency is that the puppies will acquire the parasites through auto-infection. This is possible because eggs can thrive on soil or a certain area for a long time which is why proper cleaning of a dogs home is encouraged. Another reason is that some parasitic worms such as Trichuris canis can be transferred directly from parent to offspring during birth. Comparison of Diet. A higher prevalence in dogs who ate table food was also associated with a prevalence of 85.7 percent compared to those who were fed with dog food and left-over food with prevalences of 60 percent and 69.2 percent respectively. However, this result is in contrast with the study conducted by Coman (1974) where wild dogs who ate unclean food or those found in the wild had a higher prevalence in terms of occurrence of parasites. The reason behind the relationship between dogs who ate table food having a higher prevalence compared with those who did not is deemed unclear.

Results and Discussion. – This study aimed to determine the presence of helminthic infection among dogs in Arevalo, Iloilo City, Philippines. The method Parasep Fecal Concentration Technique was used in order to isolate the helminthic eggs from the fecal debris. Each sample was viewed under a light microscope using the High Power Objective. It also aimed to associate some risk factors to the prevalence and intensity of parasitic infection such as age, breed, diet, and previous medications specifically deworming. Data were recorded and analyzed. Domestic dogs are possible reservoir hosts of helminth parasites. This could pose public health hazards as dog parasites usually are zoonotic, or if not, could cause visceral larva migrans and may cause complications to to the human body (Lim 2010). By determining the presence and by identifying the prevalence, intensity, and risk factors associated with helminth parasitic infection, further studies could be made to minimize this (Paul et al. 2010 and Traversa et al. 2014). Originally, this study aimed to have at least 10 samples per barangay, but in the middle of the conduct of the study, a change in the method of isolating parasitic eggs, which was Formalin-Ether Concentration Technique (F.E.C.T.) originally, was made due to a restriction in the distribution of the chemical diethyl ether of the government. The head of the medical laboratory of University of San Agustin Iloilo, advised that PFPCT should be used as an alternative to F.E.C.T. due to the fact that it is easier and faster to conduct compared to the latter. Also, only 40 paraseps were procured and were distributed to the barangays found in Arevalo, Iloilo City because the said laboratory only had one (1) box which contained 40 paraseps. An additional waiting time of four (4) weeks would be needed for the supply to arrive and due to the time restriction during the conduct of the study, it was advised that the data gathering should be continued. Instructions were given to the dog owners on how to use the parasep to obtain one (1) gram of fecal sample, but some did not follow the directions which lead to the destruction of the paraseps filter. Also, some homeowners discarded the paraseps thinking that it would not be taken again by the researchers which finally lead the researchers to obtain 30 fecal samples. A positive occurrence was found for helminth infestation in Arevalo, Iloilo City, Philippines which was around 70 percent of the total sample. Therefore, out of 30 samples examined, 21 were positive for helminth infection while nine (9) were negative. Results also showed that the parasite with the highest mean intensity was Ancylostoma sp. (93 eggs/gram), followed by Trichuris sp. (91 eggs/gram), and Toxocara sp. (21 eggs/gram). See Fig. 3

Comparison of Sex. Male dogs were seen to be more susceptible to parasitic infection compared to females with prevalences 68.8 percent and 57.1 percent respectively.

Literatures in the correlation of dogs sex to its susceptibility to parasitic infection shows no consistency. Studies conducted by Katagiri et al. (2008) showed that there is no significant difference in the vulnerability of both sexes. However, another study showed that parasitic infection was more prominent in female dogs (Sowenimo 2009). In contrast, Venturini et al. (2008) reported that males have a higher prevalence in terms of helminthic infection. *Comparison of Medication In-take.* Certain associations cannot be made in this study especially since the col-

lected fecal samples only came from dogs who were not dewormed. Although studies showed that dogs who were dewormed showed less susceptibility to parasitic infection compared to those who were not (Asano 2004).

RISK FACTORS	CATEGORIES	EXAMINED	INFECTED	PREVALENCE [%]
Age	Puppies	10	8	80.0
	Adults	20	13	65.0
Diet	Dog Food	10	6	60.0
	Leftovers	13	9	69.2
	Table Food	7	6	85.7
Medication	Dewormed	0	0	0
	Not	30	21	76.7
Sex	Male	16	11	68.8
	Female	14	8	57.1

Figure 4: Overall prevalence of gastrointestinal helminths based on different risk factors Common factors can be associated to the high overall prevalence. One of these is autoinfection in dogs due to improper cleaning of cages. The dogs were placed in the same area of the house where they both ate and defecated. Most helminth parasites can be acquired through the fecal-oral route, while some can infect through skin penetration. When areas where feces with parasitic eggs are not cleaned thoroughly, the eggs can hatch into larvae and penetrate the skin of the dogs, or the eggs can stay dormant until they are ingested by the dogs. This is supported by the study conducted by Esquivel et al. (2015) on dogs who lived in a cage in a dog shelter showed that since no disinfectant were used when cleaning the dogs cages, a higher prevalence rate of parasitic infection was seen compared to their counterparts who were taken care of properly and were not leashed. The dogs could have acquired the infection long before the study was

conducted, and since they were not able to receive deworming, this could have highly increased the prevalence

rate. The dogs could have acquired the parasites in other dogs with direct contact it may be during long walks or stray dogs entering the property. It may be also due to ticks, mosquitoes, and other parasites from other dogs.

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