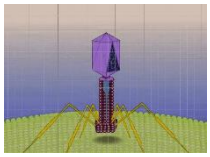

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# Prevalence and Chemotherapy of Feline Ancylostomiasis in and around Lahore, Pakistan

Awais Bokhari[1*], Muhammad Sarwar Khan[2], Muhammad Hassan Saleem[3], Ruheeb Aslam Sandhu[4], Ahmad Yar Qamar[5], Muhammad Azhar[6*], Nazish Iqrar[7], Bushra Anwar[7]

1. Livestock and Dairy Development Department, GOP, Lahore, Punjab, Pakistan
2. Department of Clinical Medicine and Surgery, UVAS, Lahore, Punjab, Pakistan
3. Department of Veterinary Medicine, UVAS, Lahore, Punjab, Pakistan
4. Health and Safety Division, Al Khonji LLC, Muscat, Oman
5. Department of Clinical Sciences, Jhang Campus UVAS, Jhang, Punjab, Pakistan
6. Punjab Wildlife and Parks Department, GOP, Lahore, Punjab, Pakistan
7. Institute of Biochemistry and Biotechnology, UVAS, Lahore, Punjab, Pakistan

**Corresponding Author's Email: azhar.vet11@gmail.com**

**ABSTRACT:** *Ancylostomiasis is a prevailing parasitic disease in cats and trend to keep cats as a pet is increasing in the society. So, the present study was designed to determine the prevalence of Ancylostomiasis in pet cats, its treatment and to see effect of Ancylostomiasis in various blood parameters. In the present study, 300 fecal samples were collected from pet cats from September 2021 to January 2022 and 65 samples (21.67%) were found positive for the Ancylostoma spp. For the chemotherapeutical trials, cats were divided into three groups viz. A, B and C. Group A was treated with Pyrantel Pamoate, Group B was treated with Albendazole. Group C was kept as a positive control. The Pyrantel Pamoate was more effective than Albendazole with an efficacy rate of 94.20% and 86.81% respectively. There was a change in hematological values due to Ancylostomiasis. The values of hemoglobin, packed cell volume, and total erythrocyte count were lower in positive samples, and the value white blood cell count was not affected much in Ancylostoma affected groups. It was concluded that Ancylostomiasis was prevailing in cats and Pyrantel Pamoate is a good remedy to treat it as compared to Albendazole. However, disease mapping and molecular epidemiology are recommended for further detail analysis.*

***Keyword:*** Prevalence, Ancylostomiasis, Pyrantel Pamoat, Albendazole

## INTRODUCTION

The cat is considered an adoptable pet of the modern world due to its affection for humans and can act as potential pest control that can cause harm to the human population. Cats are devoted pets of humans. This link between humans and animals has significant advantages for human physiological health, socialization, and emotional growth (McGlade et al., 2003). However, recent reports have indicated the transmission of certain zoonotic diseases from cats to humans (Hill et al., 2000; Martínez-Moreno et al., 2007). The cats do harbor organisms of zoonotic importance that have detrimental effects on human health (Hill et al., 2000). There is more contact between domestic animals and people due to rise in companion animals, which exposes people to zoonotic organisms (Lorenzini et al., 2007; Robertson et al., 2000). As compared to dogs, cat feces pose a more potent threat towards environmental contamination for zoonotic parasites (Szwabe and Błaszkowska, 2017).

In 1983, Hendrix and Blagburn reported gastrointestinal parasites as one of the major causes of death in cats (Szwabe and Błaszkowska, 2017). Among all the gastrointestinal parasites Ancylostoma is regarded as a major parasite infecting cats. The common species of Ancylostoma in cats are *Ancylostoma tubaeforme*, *Ancylostoma braziliense*, and *Ancylostoma ceylanicum* with different prevalence rates depending upon the geographical region (Knaus et al., 2020). In the infected cats, Ancylostoma sucks the blood from the intestinal walls and releases anticoagulants causing bloody diarrhea (Schaer, 2009) that leads to microcytic hypochromic anemia. Ancylostoma can migrate from the intestine to the lungs and trachea of cats causing vomiting and respiratory issues (Sazalli et al., 2016). The anemia is responsible for the pale mucus membrane which was seen during physical examination.

Ancylostoma also poses a threat to the human population (Edosomwan and Chinweuba, 2012) as it causes cutaneous larval migran in humans (Supplee et al., 2013). Humans can get the infection either via direct contact with the infective larvae present in the feces of cats (Heukelbach and Feldmeier, 2008) or via a contaminated environment (Coelho et al., 2011). The larva penetrates the dermal tissue causing a local inflammatory reaction that is characterized by a single linear, pruritic, and slow-growing lesion (Feldmeier et al., 2006; Torres-Chablé et al., 2015). Infected humans suffer from intense itching especially during

the night as compared to day (Pessoa and Martins, 1963). The condition of the patient becomes more severe if several creeping eruptions occur simultaneously (Heukelbach et al., 2002). Furthermore, Ancylostoma infection can lead to anemia, diarrhea, and epigastric pain.

Treatment approach in this case involved therapy and supportive care. Supportive care may include fluid therapy, iron supplements and blood transfusion. For fluid therapy, it is important to replace the fluid that was lost due to vomiting and diarrhea and also to stabilize the cat. Iron supplement and blood transfusion should be considered in chronically ill cats where the anemia is very severe and they are recumbent. Tetrahydropyrimidines, Benzimidazoles, and Macrolides are pharmacological classes that can be used to treat Ancylostoma. Within an hour of injection, adult and larval forms of hookworms and whipworms can control nematode parasites. Tetrahydropyrimidines, such as Pyrantel, are the preferred method of treatment for hookworm.

In recent years, the popularity of cats as a pet has increased in Lahore, Pakistan. Therefore, the risk of transmission of Ancylostomiasis from cats to the human population has increased. Keeping in view the zoonotic importance of Ancylostoma and the absence of data regarding its prevalence in the cat population of Lahore, the present study was designed. The objective of the present study was to examine the prevalence of Ancylostoma and its effects on the different blood parameters of the infected cats. Moreover, the therapeutic efficacy of Pyrantel Pamoate and Albendazole against Ancylostomiasis in cats was also compared in the study.

# MATERIALS AND METHODS

## 1. Animal Used

In the present study cats that were brought for treatment to the Pet Center of the University of Veterinary and Animal Sciences (UVAS) and other government and private hospitals located in the urban and peri-urban areas of Lahore. All the samples were collected with the prior approval of animal owners. The sex of cats was determined by the physical examination of cats. The approximate age of cats was calculated by dentition (Adolph and Hoy, 1960) and categorized into 4 groups: <6 months old, 6 months–1.5 years, 1.5–5 years, and 5–10 years.

## 2. Collection and Processing of Fecal Samples

Fecal samples (n=300) were randomly collected from the cats (of different age

groups). Fecal samples of about 5 gm were collected directly from the rectum of each cat with gloved lubricated hands. After collection, the samples were placed in self-sealing polythene bags and were transported to the laboratory in an ice-packed cooler. The fecal samples were stored at 4°C and processed within 24 hours of collection. The samples were further examined in the postgraduate laboratory of Department of Veterinary Medicine, UVAS Lahore. The fecal samples were stored at 4°C and processed within 24 hr. of collection. The examination of samples was performed in the Clinical Medicine Laboratory, UVAS, and Lahore. Different methods used for fecal examination include the direct smear method, flotation technique, and McMaster technique.

### 3. Direct Smear Method

The direct smear method was performed following the procedures of Hendrix and Robinson, (2006). A small amount of fecal material was taken in a petri dish and thoroughly mixed with the saline solution to prepare a uniform suspension. Then, a drop of suspension was placed on the clean glass slide with a cover slip. The detection of parasitic eggs was performed under the microscope at 10X (Olympus CX23). Each sample was examined in triplicates.

### 5. Floatation Methods

The fecal samples that were found negative for Ancylostoma using direct smear were subjected to floatation for further screening by using methods of Zajac and Gary (Jenkins). Briefly, a small quantity of fecal material was mixed with the saturated salt solution in a test tube. The solution was properly mixed and then filtered into another test tube. The test tube was filled with the saturated salt solution till its mouth. A cover slip was placed over the test tube and remained undisturbed for 10–15 min. The cover slip was then taken off, put over the glass slide, and looked at under Microscope with Magnification of 40X.

### 6. McMaster Technique

Eggs per gram (EPG) of feces in Ancylostoma positive samples was calculated by McMaster technique following the same protocol as previously explained by Taylor et al (Taylor et al., 2007). Briefly, a mixture was prepared using 42 mL of salt-saturated solution with 3 g of feces in a jar. The jar solution was shaken continuously till feces were thoroughly mixed with the solution. The mixture was filtered through the sieve and kept

untouched at room temperature for 5–10 min. The McMaster slide was then charged with the solution one by one and kept uninterrupted for 5 min. Finally, the EPG was calculated by counting the number of eggs in the counting chamber.

**4. Chemotherapeutic Trials**

Eighteen Ancylostoma positive cats and six healthy cats were selected for therapeutic trials and were equally divided into three groups; A, B, and C. Group A was treated with Pyrantel Pamoate (Combantrin, Pfizer Pakistan) @ 20mg/kg orally. Group B was treated with Albendazole (Zentel, GlaxoSmithKline Pakistan) @ 50mg/kg orally. Whereas group C served as the positive control which was treated at the end of trial. The fecal samples of all the treatment groups were observed on day 0 (Pre-medication) and days 7, 14, and 21 (Post-medication) for the Ancylostoma egg. The drug efficacy was calculated based on the reduction of eggs.

**7. Hematological Studies**

For hematological studies, 3 mL blood sample was taken directly from the cephalic vein of each cat. Following collection, the blood sample was put into a sterile tube coated with heparin to measure the percentage of red blood cells (RBCs), hemoglobin (Hb), white blood cells (WBCs), and packed cell volume (PCV) using a hematological analyzer (Abacus Junior Vet, Diatron©).

**STATISTICAL ANALYSIS**

Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA) was used for analyzing the data. All values were expressed as mean ± standard deviation (SD), and a p-value less than 0.05 indicated statistically significant. Data related to the effect of drugs on EPG was analyzed using a repeated measures analysis of variance (ANOVA), data related to the blood parameters was analyzed using an independent sample t-test, and the incident rate was analyzed using the chi-square test.

**Table 1: The Incidence Rate of *Ancylostoma* in Cats**

| Groups | Total Samples Collected | No. of Positive Samples | Prevalence (%) |
|---|---|---|---|
| < 6 Months | 65.00 | 18.00 | 27.70% |
| 6 Months–1.5 Year | 138.00 | 29.00 | 21.00% |
| 1.5 Year–5 Years | 72.00 | 13.00 | 18.00% |
| > 5 Years | 25.00 | 5.00 | 20.00% |
| **TOTAL** | 300.00 | 65.00 | 21.67% |

## RESULTS

According to the age of the cats, 300 samples were sorted into groups. Overall, 21.67% of the cats tested positive for *Ancylostoma*, however the incidence rate varied depending on the age of the cats. The age group 1.5 years to 5 years had the lowest incidence rate of *Ancylostoma* 18.0%, and kittens (6 months) had the highest incidence rate of 27.7% among all age groups. Cats >5 years of age had a 20.0% incidence rate, while cats between 6 months and 1.5 years had a 21.0% incidence rate.

**Table 2: Presences of Ancylostoma Eggs per Gram (EPG) of Cat Feces Using Two Different Drugs Orally**

| Treatment Groups | Days of EPG | | | |
|---|---|---|---|---|
| | Day 1 | Day 7 | Day 14 | Day 21 |
| **Group A Pyrantel Pamoate** | 758.3±58.40 | 650.0±89.40$^{ab}$ | 375.0±61.40$^{b}$ | 66.6±25.80$^{b}$ |
| **Group B Albendazole** | 741.6±205.90 | 525.0±178.20$^{b}$ | 300.0±141.40$^{b}$ | 100.0±44.70$^{b}$ |
| **Positive Control** | 716.6±157.00 | 916.60±271.40$^{a}$ | 1200.0±282.80$^{a}$ | 1500.0±209.70$^{a}$ |

**Note***: EPG, egg per gram.* $^{a, b}$ *Values in a column with different superscripts letter differ significantly (P<0.05).*

The effect of oral treatment of these drugs was observed by counting the EPG at day 1, day 7, Day 14 and Day 21 of trial. The EPG count showed by the McMaster Technique at the end of trial for Group A, B and C was 66.6±25.8, 100.0±44.7 and 1500.0±209.7 respectively. Pyrantel Pamoate and Albendazole produced significant effect ($P<0.05$) on EPG at Day 7, Day 14 and Day 21 as compared to the positive control.

**Table 3: Comparison of Different Blood Parameters between *Ancylostoma* Positive and Negative Cats**

| Groups | Blood parameters | | | |
|---|---|---|---|---|
| | **HGB (g/dL)** | **RBCs /µL** | **WBCs/µL** | **PCV %** |
| *Ancylostoma* negative cats (6) | 12.30±0.10[a] | 7.50±1.20[a] | 12.10±2.00 | 31.40±1.70[a] |
| *Ancylostoma* positive cats (8) | 6.70±0.20[b] | 3.90±0.50[b] | 14.40±2.90 | 20.60±0.70[b] |

**Note***: a, b Values in a column with different superscript letters differ significantly (P<0.05).*

Table 3 compares the blood parameters of 8 Ancylostoma positive and 6 negative samples. The HGB, RBCs and PCV% differs significantly in both groups as potential drop in level of HGB, RBCs and PCV% was seen in Positive samples of Ancylostoma. The WBC count was not significantly affected by Ancylostoma.

## DISCUSSION

In the current study, the prevalence of parasites in feline fecal samples from different areas of Lahore was (21.67%) that indicated cats were infected with parasites that cause Ancylostomiasis. Kittens (6 months) had the highest incidence rate of Ancylostoma, with a rate of 27.7%, while the age group 1.5 years to 5 years had the lowest prevalence (18.0%). Kittens have a significant risk of contracting an infection. The incidence of Ancylostoma in cats varies and may be influenced by factors such as age, population type, geographic location, season, and local animal populations' behaviors and habits. Hookworm infestations can be severely debilitating for cat, and even fatal (Younas et al., 2014).

The primary pathogenic mechanism of the Ancylostomiasis is blood loss, Anemia and Iron deficiency. The findings of comparison of basic blood parameters of positive and negative

samples are in consistent with the chapter (Hall, 2019). A significant drop is seen in HGB, PCV% and RBCs in cats tested positive for Ancylostoma.

The results on egg counts in groups A-C are shown in table 2. Significant ($P<0.05$) decrease in egg count is seen in Group A and B as compared to C. Findings proved that Pyrantel Pamoate and Albendazole were effective against Ancylostomiasis. The comparative efficacy of both drugs against Ancylostoma was non-significantly different throughout the drug trial. The result of present study are similar to findings of (Nolan et al., 1992) who found that Pyrantel Pamoate along with ivermectin is effective against canine hookworms and caused 99.6 % reduction in worms. According to the results of this investigation, Albendazole is beneficial against Ancylostomiasis, which is consistent with the findings of the paper (Colella et al., 2021).

It is important to consider this parasite's zoonotic potential as infection may spread from cats to other animals and humans. Due to poor domestic cat health management and the existence of neighbourhood cats that roam freely and may come into contact with parasite eggs and larvae in stray cat-infested soil, indigenous populations are particularly at risk. This makes an excellent setting for infections to flourish, especially in the warm, humid tropics. Precautions should be taken to prevent exposure to such situations as well as the administration of the appropriate dosage of medication. To ensure that cats can fight off infection without developing drug resistance and risk to human communities could be minimized. A control program is needed to be applied for Ancylostomiasis in pet cats caused by *Ancylostoma* spp. for the animal as well as public health aspect. The findings of this study could possibly be applied to molecular epidemiology and disease mapping.

## CONCLUSION

It was concluded by this study that cats had a significant prevalence of Ancylostoma infection particularly in age group less than 6 months. The study highlights the need for efficient preventative measures and treatment choices and recommends Pyrantel Pamoate as compared to Albendazole. However, further research is required to establish the best treatment regimens for the ill cats.

### Ethical Approval

Ethical approval to work with animals was taken from Ethical Review Committee, University of Veterinary

and Animal Sciences Lahore, Pakistan and no animal was harmed during study.

**Conflict of Interest**

Author's declare there is no conflict of interest.

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