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Prevalence and Chemotherapeutical Investigation of Gastro-Intestinal Nematodes Infestation in *Columba livia* in Faisalabad City

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ABSTRACT

A total of 120 domestic pigeon samples of different ages, both male and female were collected from an urban area. Both dead and alive larvae and eggs (hatched and unhatched) were observed using direct microscopy. Out of 120 samples, 73 samples were infested, 20 samples were infested with *Capillaria longicollis*, 13 samples with *Ascaridia galli*, 17 samples with *A. columbae*, 11 samples with *Capillaria obsignata*, 5 samples with *C. columbae*, and 7 samples with *C. caudinflata* in *C. livia domestica*. Anthelmintic drugs were tested at different concentrations, but the maximum lethal concentration (LC50) effects were showed on 20mg/mL dose. The determined LC50 values were 4.966mg/mL for oxfendazole, 4.789mg/mL for fenbendazole and 5.102mg/mL for levamisole. The EPG level was higher on day 7 of oxfendazole and fenbendazole treatment in *C. livia domestica*. However, in response to Levamisole on days 7 and 14, EPG was lower showing the lethal effects against the gastrointestinal parasites. The results indicated that oxfendazole and fenbendazole had minimal effects on gastrointestinal nematodes, whereas levamisole exhibited significant efficacy against gastrointestinal nematodes.

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INTRODUCTION

Domestic pigeons, scientifically known as *Columba livia domestica*, coexist with humans, being raised for purposes such as food, recreational activities, symbolic representation, and experimentation (Sari et al. 2008; Mansur et al. 2019). The reproduction and roosting locations of domestic pigeons, such as windows of houses and rooftops of buildings, lead to significant health issues (Wackernagel and Bircher, 2010; Boxler et al. 2016). Humans keep pigeons as pets and utilize them for social, religious, ceremonial, and ritualistic purposes, as well as employing them as laboratory animal models. In Nigeria, where they are domesticated, pigeons live as free-range birds, primarily scavenging for seeds, grains, vegetation, and invertebrates like insects (Adang et al. 2007). This makes pigeons susceptible to various disease-causing agents, including helminthes (Adang et al. 2009; Alam et al. 2014). Crowded cages, unnatural living environments, suboptimal management practices, inadequate hygiene, improper use of anti-helminthic drugs, and unfavorable environmental conditions are stressors that lead to altered behavioral patterns and diminished immunity (Khursheed et al. 2014). These factors diminish the birds' immune defenses, making them more prone to gastrointestinal parasites (Khursheed et al. 2014; Pradeep et al. 2017).
Parasites engage in nutrient competition within the digestive tract (Colditz 2008; Maizels et al. 2012) and leading to lethargy and sluggishness in birds due to nutritional deprivation (Badran and Lukesova, 2006). Additionally, the defense mechanisms and immune responses activated by birds against parasitic infections contribute to a higher metabolic cost, resulting in an increased metabolic rate and immunopathology (Maizels et al. 2012).
Infections caused by nematodes such as *Capillaria spp* and *A. galli* are linked to slowed growth, weight loss, diarrhea, impaired nutrient absorption, and the transmission of pathogenic infections (Das et al. 2010; Gauly et al. 2007; Kilpinen et al. 2005). An elevated burden of worms negatively impacts feed conversion, egg production (Stehr et al. 2019; Tarbiat et al. 2020).and the liver lipid reserve in laying birds (Sharma et al. 2018). *A. galli* exhibits a substantial reproductive capacity, releasing a considerable number of eggs into the environment to support transmission through its direct life cycle (Taylor et al. 2007; Wongrak et al. 2015).

The use of anti-helminthic drugs is common for the eradication of gastrointestinal parasites. While these drugs are effective in eliminating parasites, they also impact the health of birds. Fenbendazole, oxfendazole, albendazole, levamisole, and pyrantel pamoate are among the commonly employed anti-helminthic drugs (Khan et al. 2010; Ashraf et al. 2011; Basit et al. 2014; Naz et al. 2022).
Hence, effective management in non-caged birds typically involves the routine administration of anthelmintics to eliminate worms and minimize environmental contamination. The chemical control of helminth infections in poultry relies on a restricted range of anthelmintic products available globally (Tarbiat et al. 2020). In Australia, the sole registered chemicals for treating nematode infections in poultry have been levamisole (LEV), which is commonly administered through water as an anthelmintic (Ruhnke, 2015).
The various anthelmintic classes exert antiparasitic effects on nematodes through distinct mechanisms. Fenbendazole, known for its broad-spectrum activity against nematodes, has been approved for in-feed use in Australia. On the other hand, levamisole operates as a cholinergic receptor agonist within the neuromuscular system of parasites. It specifically stimulates excitatory nicotinic acetylcholine receptors located on muscle cells of nematodes, leading to spastic paralysis in susceptible individuals (Martin and Robertson, 2010).

MATERIALS AND METHODS

Sampling site

C. livia domestica fecal samples were collected from urban areas in Faisalabad, Pakistan. A semi-arid climate characterized by extremely hot and humid summers, as well as dry and cool winters. In June, the average maximum and minimum temperatures reach 45.5°C (113.9°F) and 26.9°C (80.4°F), respectively. Conversely, January sees average minimum and maximum temperatures of 19.4°C (66.9°F) and 4.1°C (39.4°F).

Collection of fecal samples

To assess the occurrence of gastrointestinal parasites, an examination of 120 fecal samples was conducted. Newly expelled feces were gathered in cups with a sterilized spatula. To prevent contamination, individual cups and spatulas were employed for each sample, and the cup lids were securely sealed and transportation to the laboratory. The analysis of fecal samples under direct microscope was completed within a 24-hour timeframe.

Drug Efficacy

Controlled test was conducted for efficacy of drugs on the basis of reduction in EPG (egg per gram) pre and post medication using the following formula;
Percent efficacy = a - b ⁄ a × 100
Where a =EPG- pre-medication.
b =EPG- post-medication

Sample strategy and drug trials

Out of 73 infected *C. livia domestica* , 45 randomly divided into 3 groups A, B and C, each comprising of 15 birds, While 28 uninfected birds were kept in group D. Each group was administered a different anthelmintic: group A (15 pigeons) treated with oxfendazole, group B(15 pigeons) treated with fenbendazole, group C (15 pigeons) treated with levamisole and treatment orally. To facilitate convenience, the pigeons were housed in separate cages. Because of the weight of infected birds ranging from 250 to 400 grams, the dose was determined between 0.5, 1, 2, 5, 10, 20mg/mL. Single doses of fenbendazole, oxfendazole and levamisole (20mg/mL) were administered through the drinking water as per the prescribed label instructions. To induce thirst, water was removed from the cages one day before drug administration. The drug-containing water was changed twice daily to ensure the effective consumption of the drugs. Fecal samples were collected three times: initially from the control group on Day 1 before division into treatment groups and subsequently on Day 7 and Day 14 after the administration of anthelmintic drugs.

Statistical analysis

The findings were organized into tables and subjected to statistical analysis using the SPSS software. Probit analysis was conducted to determine the lethal concentration (LC50) and 95% fiducial limits (95% FL) of the medications. The variance among treatment groups was assessed using one-way ANOVA. Results were regarded as statistically significant ($P<0.05$). Mean and standard deviation (S.D.) values were also calculated.

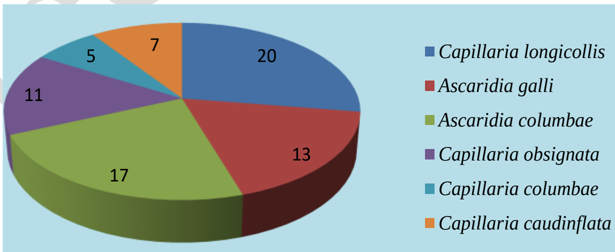

Fig. 1: Pie graph of overall prevalence of gastrointestinal nematodes of *C. livia domestica.*

RESULTS

During this study, two different species of nematodes were identified after examination of 120 pigeon fecal samples. The data collected revealed 73samples positive for nematode infection in overall prevalence rate.
Out of 120 samples, 20 samples were infested with *C. longicollis*, 13 samples with *A. galli*, 17 samples with *A. columbae*, 11 samples with *C. obsignata*, 5 samples with *C. columbae*, 7 samples with *C. caudinflata* in overall Prevalence of gastrointestinal nematodes of *C. livia domestica* (Table 1).
Table 3, 4 and 5 showed that all concentration had influenced the rate of larval development. 20 mg/ml indicated the maximum power of larval development inhibition.
Table 6 showed treatment-wise occurrence of gastrointestinal parasites in *C. livia domestica* in Faisalabad. The EPG level was higher on day 7 of oxfendazole and fenbendazole treatment in *C. livia domestica*. However, in response to Levamisole on days 7

Table 1: Overall prevalence of gastrointestinal nematodes of *C. livia domestica*

Name of nematodes	No. of infected samples	Prevalence %	EPG range	Mean±S.E.M
Capillaria longicollis	20	16.66%	100-200	151±1.65
Ascaridia galli	13	10.83%	100-200	167±2.25
Ascaridia columbae	17	14.16%	100-250	131±1.98
Capillaria obsignata	11	9.16%	100-200	175±1.65
Capillaria columbae	5	4.16%	100-200	189±2.22
Capillaria caudinflata	7	5.83%	100-300	199±2.56

Table 2: Comparative anthelmintic efficacy

Group	Initial day Pre-medication (Fecal egg count)	7 days Post-medication (Fecal egg count)	14 days Post-medication (Fecal egg count)
A: oxfendazole	1800	566	290
B: fenbendazole	1400	520	250
C: levamisole	1100	450	150
D: untreated and infected pigeons	1900	2066	2270
E: uninfected and untreated	-------	------	------

Table 3: The LC50 and percentage of larval development of nematodes at different concentration of oxfendazole in pigeon.

Dose mg/ml	Dose mg/mL	Log dose	Hatch%	Standard Deviation	Probit	Regression
0.5	500	2.698	94	30.10	6.55	6.38
1	1000	3.000	83	26.62	5.95	6.04
2	2000	3.301	71	23.87	5.55	5.68
5	5000	3.698	49	16.56	4.97	5.01
10	10000	4	29	40.45	4.45	4.40
20	20000	4.301	19	41.07	4.12	4.09

LC50 = 4.966mg/ml

Table 4: The LC50 and percentage of larval development of nematodes at different concentration of fenbendazole in *C. livia domestica*

Dose mg/ml	Dose ug/mL	Log dose	Hatch%	Standard Deviation	Probit	Regression
0.5	500	2.698	97	26.71	6.88	6.89
1	1000	3.000	88	24.64	7.05	6.55
2	2000	3.301	79	22.16	5.81	6.21
5	5000	3.698	63	17.16	5.33	5.59
10	10000	4	42	9.19	4.8	4.79
20	20000	4.301	29	45.25	4.45	4.29

LC50 = 4.789mg/ml

Table 5: The LC50 and percentage of larval developmentof nematodes at different concentration of levamisole in pigeon.

Dose mg/ml	Dose ug/mL	Log dose	Hatch%	Standard deviation	Probit	Regression
0.5	500	2.698	92	29.68	6.41	6.30
1	1000	3.000	77	25.17	5.74	5.84
2	2000	3.301	61	20.17	5.28	5.36
5	5000	3.698	43	14.50	4.82	4.81
10	10000	4	28	43.86	4.42	4.35
20	20000	4.301	14	44.51	3.92	3.93

LC50 = 5.102mg/mL

Table 6: Treatment wise incidence of gastrointestinal parasites

Parameters	Oxfendazole (14 Days)	Fenbendazole (14 Days)	Levamisole (14 Days)
Dose mg/mL	20mg/mL	20mg/mL	20mg/mL
LC50 mg/mL	4.966mg/mL	4.789mg/mL	5.102mg/mL
EPG range	300	200-250	100-150
Prevalence rate	19%	15%	14%

and 14, EPG was lower showing the lethal effects against the gastrointestinal parasites. The determined LC50 values were 4.966 mg/ml for oxfendazole, 4.789 mg/ml for fenbendazole, and 5.102 mg/ml for levamisole. These results suggest that levamisole showed superior effectiveness against gastrointestinal parasites.

DISCUSSION

The investigation encompassed the determination of nematode prevalence in all 120 samples of domestic pigeons, covering various ages and genders. The assessment of nematode prevalence involved counting the number of eggs (EPG - eggs per gram), cysts (CPG - cysts per gram), and oocytes (OPG - oocytes per gram). The McMaster technique was employed to quantify the number of eggs, cysts, or oocytes in each sample. The counting of these entities was carried out using the direct microscopy method. The analysis revealed that EPG and larval counts from fecal cultures were higher in summer compared to winters. Additionally, concerning the genders sampled, the prevalence rate was greater in males than in females. The study revealed the highest prevalence of nematodes, specifically *C. longicollis*, at approximately 16.66%, with an egg count ranging between 100-200 eggs per gram (EPG). It was observed that *Capillaria* infection is more common in young and growing pigeons compared to adults (Biu and Umoru, 2010). In older pigeons, the infection can also manifest, and they typically serve as carriers, acting as a source of infection for other avian hosts (Parsani et al., 2006). Males exhibited a higher rate of helminthic infection (50.83%) compared to females (32.5%). This observation aligns with the findings of Umaru et al. in 2017 in Taraba State, Nigeria, where a prevalence of 55% was reported in male pigeons, contrasting with 23.3% in female pigeons. This pattern is consistent with other data suggesting that intrinsic biological differences between host sexes may contribute to variations in parasite infections. The rationale behind this is that pigeons kept in substandard conditions with poor management and hygiene practices tend to have a higher rate of parasite infections than those kept in well-managed and hygienic conditions. The factors influencing the spread of parasites include environmental conditions, social practices, and individual habits.

Upon examination, it was established that both oxfendazole and levamisole demonstrated optimal outcomes. However, the results indicated that levamisole exhibited greater anthelmintic efficacy compared to oxfendazole. It is noteworthy that levamisole formulations are recognized for their bitter taste, potentially hindering intentional consumption by birds when administered through the drinking water system. Palatability concerns are commonly encountered in the administration of veterinary drugs (Aleo et al., 2018). The Fenbendazole oral solution has low water solubility (Ryu et al., 2013). This discovery implies that when resistance develops in Ascarid populations to one of the constituent medications, a viable control option may be provided by combination anthelmintics. Following the completion of all experiments, the calculated LC50 for oxfendazole was 4.966 mg/ml, for fenbendazole was 4.789 mg/ml, and for levamisole was 5.102 mg/ml, indicating that levamisole exhibited greater effectiveness against gastrointestinal parasites, this was based on the observations. However, in contrast, levamisole demonstrated higher anthelmintic efficacy than both oxfendazole and fenbendazole.

Conclusions

The research findings indicated the presence of nematodes, including *C. longicollis, A. galli, A. columbae, C. obsignata, C. columbae, and C. caudinflata*, in *C. livia domestica*. The use of anthelmintic drugs induced notable alterations in *C. livia domestica*. Among the administered drugs, levamisole exhibited the most effective anthelmintic activity for treating nematode infestation in domestic pigeons, surpassing the efficacy of oxfendazole and fenbendazole.

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