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Prevalence and chemo-therapeutical investigations of gastrointestinal nematodes in domestic pigeons in Lahore, Pakistan

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Abstract. The prevalence of gastrointestinal nematodes was studied in 143 (80 male and 63 female) domestic pigeons. Faecal samples were collected to determine the gastrointestinal nematodes of domestic pigeons through qualitative and quantitative faecal examinations. A total of 48 (male 33 and 25 female) naturally infected domestic pigeons were divided into G_1 (albendzdole) and G_2 (fenbendazole) treatment-groups along with one control group (C). The overall prevalence of gastrointestinal nematodes was 40.5% (58/143) in domestic pigeons. Likewise, the prevalence of gastrointestinal nematodes in males and females was found 41.3% (33/58) and 39.7% (25/58) respectively. The overall prevalence of *Capillaria obsignata* and *Ascaridia columbae* was found to be 67.2% and 32.8%, respectively. The prevalence of *C. obsignata* and *A. columbae* in males was 72.7% (24/33) and 27.8% (9/33) and in females was 60% (15/25) and 40% (10/25), respectively. There was no significant sex related difference seen in the prevalence of *C. obsignata* ($p>0.56$) *and A. columbae* ($p>0.40$) in domestic pigeons, respectively. The overall efficacy of albendazole and fenbendazole was calculated to be 66% and 71%. A remarkable significant difference ($p<0.05$) was observed in eggs per gram before and after treatment in both G_1 and G_2 treated-groups. The efficacy of fenbendazole was found to be more significant ($p<0.02$) than albendazole.

INTRODUCTION

Pigeons have been traditionally associated with humans and are found commonly around the globe. This affiliation of pigeons with humans has been reported from 3000-5000 B.C according to Sari *et al.* (2008). In Pakistan, pigeons are seen as a symbol of culture and bred as a source of food, and trading purposes in rural areas. Hobbyists keep fancy and racing pigeons. Pigeons are cheap and readily available which make them ideal for laboratory and research experiments in Pakistan.

The close contact of pigeons with other domestic birds increases risk of parasitic infestation in birds and carries a possible zoonotic potential for transmission of diseases to human beings (Cooper, 1984; Kaminjolo *et al.*, 1988; Piasecki, 2006; Marques *et al.*, 2007; Sari *et al.*, 2008). The diseases are mainly spread through faecal

dust from cages contaminated with dry droppings and urine (Marques *et al*., 2007). Several problems are associated with pigeon health. In the last few decades, large numbers of pigeon deaths have been reported and necropsy finding revealed the occurrence of parasitic infestation. Wide ranges of helminthes are found in the gastrointestinal tract of pigeons, the majority of which are responsible for clinical and subclinical parasitism. Infection results in weight loss, anemia, retarded growth, fertility disturbance, emaciation, gut epithelium complications and reduction in immune responses of host against various diseases (Urquhart *et al*., 2000). Such complications in young pigeons eventually lead to death (Basit *et al*., 2006).

The high prevalence of parasites in pigeons, mostly nematodes such as *Ascaridia columbae*, *Dispharnyx* sp. and *Capillaria* sp. have been reported from different areas of the world (Dovc *et al*., 2004). To prevent nematode infestations in pigeons, benzimidazole (BZD) anthelmintic drugs *i.e*., albendazole and fenbendazole were recommended in domestic animal species (Bowman *et al*., 1995). Both albendazole and fenbendazole are safe, inexpensive, potent, effective and are broad spectrum compounds (McCracken & Lipkowitz, 1983) used in various non-indigenous species (Grimes *et al*., 1989). In nematodes, BZD acts by combining with β-tubulin instead of dimerization of BZD with α-tubulin, preventing formation of microtubules (Friedman & Platzer 1980; Brittany, 2008). The affinity of BZD for nematode α-tubulin was found to be greater as compared to vertebrate α-tubulin, and thus is safer to use BZD as compared to other anthelmintics (Lacy & Gill, 1994; Gozalo *et al*., 2006).

This research aims to show the prevalence of gastro-intestinal nematodes in domestic pigeons and the possible implementation of control measures for these parasites through the use of chemotherapy. Furthermore the efficacy of different anthelmintic (albendazole and fenbendazole) against gastro-intestinal nematodes of domestic pigeons based on mean reduction in eggs per gram (EPG) will be evaluated, to help in selecting the most effective drug against parasitism.

MATERIALS AND METHODS

Study area

This study was carried out in the city of Lahore, a capital of the Punjab province, which has the second largest population and is one of the oldest cities in Pakistan. The climate condition of Lahore is subtropical, in summer temperatures reach over 40ºC and falls to below 5ºC during winter.

Collection of faecal samples

A total of 143 (80 male and 63 female) apparently healthy, domestic pigeons were purchased from pigeon fanciers as well as from the local market. These were tagged according to the owner's name along with location of the shop/fancier. All pigeons were kept in separate cages. Food and water was provided *ad libitum*. Fresh faecal samples were collected at day 0 (pre-treatment) and at day 4, 7, 14, 21 and 30 (post-treatment) from floor of the cage. To avoid the risk of faecal contamination, the upper layer of faeces was removed. All the faecal samples were collected in Petri dishes. After collection, all faecal samples were checked immediately or preserved in 10% formalin solution for future studies and transported to parasitology laboratory, University of Veterinary and Animal Sciences, Lahore.

Faecal examination

Faecal samples were processed for qualitative and quantitative examinations. The qualitative examination of faecal samples was done by direct microscopy examination and flotation methods. The McMaster technique was done for quantitative examination.

Direct microscope examination

Direct microscopy was performed as suggested by William (2001). A small

amount of faecal sample was taken by toothpick and placed on a clean grease-free glass slide. The faecal sample was mixed thoroughly with 1-2 drops of water and debris were removed. A cover slip was placed carefully on the faeces to avoid bubble formation and slide was examined under microscope at 10x objective.

Flotation method

Flotation technique was carried out for detection of nematode eggs as described by Dranzoa (1999) with some modifications. Faecal sample was weighed (3g) and mixed with sodium chloride (saturated) solution. The mixture was passed through tea strainer (nylon) into glass tube. Glass tube was placed in rack. More saline solution was transferred into glass tube to attain positive meniscus and a cover slip was placed immediately on top of glass tube. The glass tube was left standing for 10-15 minutes. Cover slip was then removed and placed on clean microscope glass slide. The microscope glass slide was examined under microscope at X10 objective.

Faecal egg count

The McMaster egg counting technique was performed for counting of eggs as suggested by Soulsby (1982) with minor changes. A glass beaker (100ml) was filled with saturated salt (NaCl) solution up to 20ml. Nearly 25 glass beads was added into glass beaker. One gram of faecal sample was weighed and mixed thoroughly with salt solution. The solution was passed through tea strainer into new clean glass beaker. The debris was discarded and after thoroughly mixing, faecal sample was taken in to Pasteur pipette and both counting chambers were run one by one. The total number of eggs per gram (EPG) was estimated according to the following formula.

$$= \frac{\text{No of counted eggs x Total volume of salt solution (ml)}}{\text{Volume of counting chamber (ml) x Total weight of faeces (g)}}$$

Chemotherapeutical trials

The infected (positive) birds were separated and kept for chemotherapeutics trials. A total of 48 positive pigeons were divided in to two treatment groups (G_1 and G_2) and one untreated group used as control (C). Each group consisted of 16 pigeons and an equal number of male and female pigeons. The treated-groups, G_1 and G_2 were administrated with 10% w/v albendazole (Albazol, Special-T-Products Ltd) and fenbendazole 10% w/v (Panacur®, Hoescht Ltd) with dose rate of 1ml/3kg b.w and 1ml/16 birds respectively in drinking water for three days continuously. The pigeons of group C were left untreated.

Drug efficacy

The efficacy of anthelmintic (albendazole and fenbendazole) was determined in treated (G_1 and G_2) and untreated control (C) groups on the basis of percent reduction in EPG, as described by Monskey & Harwood (1941) and Soulsby (1982).

$$\text{Efficacy \%} = \frac{\text{EPG (Pre-medication)} - \text{EPG (Post-medication)}}{\text{EPG (Pre-medication)}} \times 100$$

Data analysis

Chi-squared test was used to analyze the variations in prevalence of gastro-intestinal nematodes of male and female pigeons. Analysis of variance in EPG before and after treatment with anthelmintic drugs on various days was determined using the paired *t*-test. Similarly, ANOVA (two-factor with replication) was applied to analyze the difference in variance in EPG between treated groups G_1 and G_2 (albendazole and fenbendazole respectively) on various days. In all statistical analysis, $p<0.05$ (alpha error less than 5%) was considered as significant.

RESULTS

Prevalence of nematodes

A total of 143 domestic pigeons *i.e.* 80 (male) and 63 (female) were selected for

faecal examination. According to the faecal examination results a total of 58 pigeons were found positive for gastrointestinal nematodes and overall prevalence was 40.5% (Table 1). A higher prevalence of nematodes was seen in males (41.3%) than in females (39.7%) as shown in Table 1. The prevalence of nematodes species (*Capillaria obsignata* and *A. columbae*) in male and female pigeons is incorporated in Table 2. The highest prevalence of *C. obsignata* (Figure 1) was seen in males (72.7%) compared to females (60%). The prevalence of *A. columbae* was higher in females (40%) than males (27.8%). On the basis of sex, however, the difference was not significant in the prevalence of *C. obsignata* (p>0.56) *and A. columbae* (p>0.40) in domestic pigeons, respectively. Overall, prevalence of *C. obsignata* was higher in pigeons compared to *A. columbae*.

Comparative efficacy of anthelmintic drugs

The chemotherapy of infected pigeons was done with two anthelmintic drugs (albendazole and fenbendazole). The decrease in mean of EPG in faecal samples was calculated (Table 3) before and after treatment. The comparative efficacy results of both (albendazole and fenbendazole) are summarized in Table 3.

All the pigeons in group G_1 were treated with albendazole and mean EPG was 1006 at day 0 (before treatment). The reduction in mean EPG after treatment was observed (291, 223, 269, 370 and 552) on days 4, 7, 14, 21 and 30, respectively and albendazole efficacy was 71%, 79%, 73%, 63% and 45%. The lowest (45%) albendazole efficacy was calculated after 14 days, likewise highest (71%) on day 4 after treatment. The overall percentage efficacy of albendazole was calculated 66% during this drug trial. On the other hand, in group (G_2), the mean EPG was 962 before treatment (on day 0). The mean EPG was calculated 228, 197, 235, 333 and 407 on days 4, 7, 14, 21 and 30 respectively after fenbendazole treatment, indicating the egg load reduction 76%, 80%, 76%, 65% and 58%, respectively. In group (G_2), the highest reduction load (80%) of eggs was calculated on day 7 after medication, on contrary 58% on day 30 (post-treatment). The overall efficacy percentage of fenbendazole was found 71% in fenbendazole-treated group (G_2).

Table 1. Overall prevalence of nematodes identified on the basis of microscopic faecal examination

Sex	No of pigeons examined	No of pigeons positive	Prevalence (%)
Male	80	33	41.3
Female	63	25	39.7
Total	143	58	40.5

Table 2. Identified species of nematodes and their prevalence in male and female domestic pigeons

Species	Male (n*=33) Total samples = 80		Female (n*= 25) Total samples = 63		Overall prevalence (%)	P value
	Positive	Prevalence (%*)	Positive	Prevalence (%*)		
C. obsignata	24	72.7	15	60	67.2	>0.56, NS†
A. columbae	9	27.8	10	40	32.8	>0.40, NS†
Total	33	100	25	100	100	

n* and prevalence (%*): shows the number of positive pigeons and prevalence percentage of positive examined pigeons, respectively. † Chi-squared test; N.S describes Non-significant

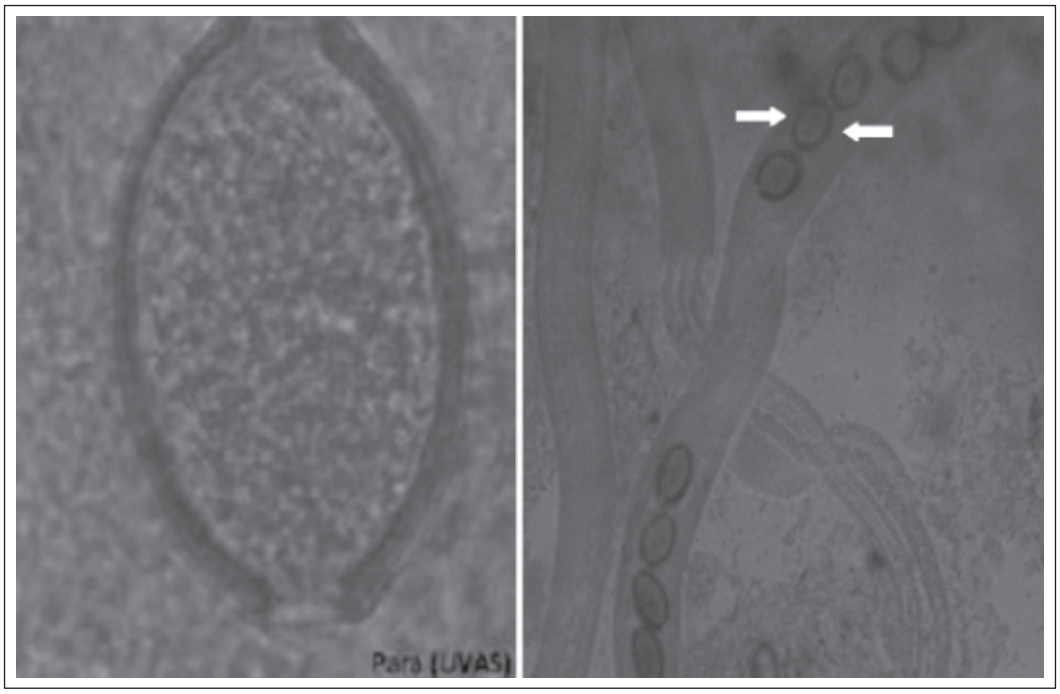

Figure 1. (A) *C. obsignata* egg (40X), (B) Barrel shaped *C. obsignata* eggs in female uterus, shown between arrows (10X)

Table 3. Comparative efficacy of anthlementic drugs in pigeons

Groups	No of birds	Drugs	EPG (mean) and drug efficacy (%) Days						Overall efficacy (%)
			0	4	7	14	21	30	
G_1	16	Albendazole	1006	291 71%	223 79%	269 73%	370 63%	552 45%	66
G_2	16	Fenbendazole	962	228 76%	197 80%	235 76%	333 65%	407 58%	71
C	16	Nil	845	900	879	1130	1323	1520	Nil

Statistical analysis

On day zero (before treatment) mean faecal egg counts (EPG) were 1006, 962 and 845 in group G_1, G_2 and C with standard deviation of 256, 314 and 270, respectively.

The mean EPG on day 4 in group G_1, G_2 and C was 291, 228, and 900 with standard deviation of 83, 79 and 359 respectively. This indicated reduction in variation between treated and non-control group. The paired t-test showed highly significant difference ($p<0.05$) in EPG before and after treatment in group G_1 and G_2 (Table 4).

On 7th day mean EPG in group G_1, G_2 and C was 79, 197 and 879 with standard deviation of 70, 71 and 325 respectively. There was a significant difference ($p<0.05$) in EPG before and after treatment in group G_1 and G_2 shown in Table 4.

On day fourteen in group G_1, G_2 and C, the mean EPG was 269, 235 1130 with standard deviation 90, 66 and 416

Table 4. Description of results of *t*-test for paired samples

Treatment groups	Comparison (Days)	t-Stat	t-Critical	*P* value*
Albendazole	0 and 4	9.8	2.13	<0.00000063
	0 and 7	12.3	2.13	<0.00000029
	0 and 14	10.7	2.13	<0.0000019
	0 and 21	6.25	2.13	<0.00013
	0 and 30	6.8	2.13	<0.00062
Fenbendazole	0 and 4	9.3	2.13	<0.0000013
	0 and 7	8.8	2.13	<0.000024
	0 and 14	10.5	2.13	<0.000023
	0 and 21	8.5	2.13	<0.000046
	0 and 30	7.2	2.13	<0.00029

*dependent two sample t-test, P<0.05 considered as significant

respectively. The statistical analysis with paired t-test (Table 4) showed significant difference ($p<0.05$) in EPG before and after treatment in both treated-groups (G_1 and G_2).

The mean EPG on 21 day after treatment in group G_1, G_2 and C was calculated 370, 333 and 1323 with standard deviation 62, 131 and 270 respectively. The significant difference ($p<0.05$) was seen in EPG before and after treatment with anthelmintic in group G1 and G_2 (Table 4).

On 30th day mean EPG in treated-groups (G_1 and G_2) and control (C) was 552, 407 and 1520 with standard deviation 171, 175 and 435 respectively. There was a significant difference ($p<0.05$) in EPG before and after treatment in group G1 and G_2 (Table 4).

The ANOVA (two-factor with replication) test was done to determine the variance in effectiveness of both anthlementic drugs in reduction of EPG during whole experimental period after treatment. The efficacy of fenbendazole was found more significant ($p<0.02$) than albendazole.

DISCUSSION

Parasitic infestations are responsible for severe health problems in domestic pigeons. Few studies have been reported in Pakistan on prevalence and chemotherapy of gastrointestinal nematodes of domestic pigeons. A total of 143 (80 male and 63 female) domestic pigeons were studied. Two methods (quantitative and qualitative) for examination of faecal samples were performed. Fifty eight (58) out of 143 domestic pigeons were infected with gastro-intestinal nematodes. The overall prevalence of gastrointestinal nematodes was found to be 40.5%, while 41.3% and 39.7% were found for male and female domestic pigeons, respectively. This high prevalence (40.5%) of gastro-intestinal nematodes in domestic pigeons corresponded with the studies of Basit *et al.* (2006) and found overall 57% infestation rate in pigeons (60% and 55% in wild and domestic pigeons, respectively). Senlik *et al.* (2005) found helminthes infestation in 74 out of 100 necropsied domestic pigeons. Similarly, the overall prevalence of helminthes was found much higher (74.14%) according to the necropsy findings of Marques *et al.* (2007) in pigeons (*Columbia livia*). In 1987, Begum & Shaikh found 86% overall prevalence of gastro-intestinal parasites in pigeons (*C. livia*) in Bangladesh. Patel *et al.* (2000) reported 48.11% positive cases of gastrointestinal parasites in captive birds of Gujarat Zoo, which is in agreement with results of current study.

The prevalence of *C. obsignata* (67.2%) in domestic pigeons was greater than *A. columbae* (32.8%). This occurrence

of *C. obsignata* and *A. columbae* in pigeons was found according to the research conducted in Turkey (Senlik *et al.*, 2005) in domestic pigeons. The abundance of Ascaridia and Cappilaria spp. was found in Nidge, Turkey (Sari *et al.* 2008) in domestic and wild pigeons and in Ljubljana, Slovenia (Dovc *et al.*, 2004) in free-living pigeons (*Columba livia domestica*) and in southern Brazil (Marques *et al.*, 2007). In captive birds, Patel *et al.* (2000) found *Ascaris* (20.75%) and *Capillaria* (13.2%). This difference in prevalence of gastrointestinal nematodes mostly relies on geo-climate conditions. The prevalence of *C. obsignata* in male and female domestic pigeons was found 72.7% and 60%. Similarly, the prevalence of *A. columbae* was 40% in female pigeons than male (27.8%) pigeons. In this study, the highest prevalence of *C. obsignata* in male pigeons and *A. columbae* in female deviated a little from findings of Senlik *et al.* (2005). Statistically, there was no significant difference (P<0.05) observed in prevalence of gastrointestinal nematodes between male and female domestic pigeons. Similarly, non-significant sex-related difference in prevalence of gastrointestinal nematodes (*C. obsignata* and *A. columbae*) in domestic pigeons was also found according to the findings of Senlik *et al.* (2005). This insignificant difference might be due to close association of male and female domestic pigeons for food and flight, therefore equal possibility of getting infestation of gastrointestinal nematodes from environment. In 2000, a research conducted by Gicik & Burgu concluded that there is no significant difference (P<0.05) between male and female wild pigeons in overall prevalence of helminthes.

The highest efficacy (79%) of albendazole was seen on day 7 (post-treatment), and then gradual decreased in efficacy (73%, 63% and 45% on day 14, 21 and 30 after treatments respectively) of albendazole was calculated. The average efficacy was 66%. These findings were in close association with results of Parsani *et al.* (2007) who found efficacy of albendazole to be 88.9%, 72.7%, 61.6% and 50% on days 7, 14, 21 and 30 after treatment. In both studies, the egg load reduction occurred gradually post treatment. A final study result of Jongsuksuntigul *et al.* (1993) suggested that albendazole is a highly effective benzimidazole derivate against *Ascaridia*, *Trichuris* and hookworm infestations. A significant difference (P<0.05) in EPG reduction was found before when treated with albendazole. Similarly, average efficacy of fenbendazole was determined 71%. The highest efficacy of fenbendazole was 80% on day 7 after treatment. The gradual reduction in efficacy of fenbendazole (76%, 65% and 58%) was found on day 14, 21 and 30 post-treatment, respectively. Parsani *et al.* (2007) reported 90%, 75%, 60% and 55% efficacy of fenbendazole on days 7, 14, 21 and 30 post-treatment respectively. The present findings are also in close agreement with Kirsch (1983) who reported almost 90% and 100% efficacy against *C.obsignata* with administration for 4 days and 5 days respectively in pheasants (*Phasianus colchicus colchicus*) and partridges (*Perdix perdix*). Pavlovici *et al.* (2003) reported that fenbendazole administration with dose rate of 20mg/kg for three days commenced good therapeutic efficacy. Likewise, studies carried out in 1979 (Kirsch & Degenhardt) and 1980 (Scupin & Nannen) confirmed, high efficacy of fenbendazole (90% to 100%) against *Capillaria* and *Ascarida* in pigeons. Ssenyonga (1982) reported almost 100% efficacy of fenbendazole against helminthes parasites of poultry. There was a significant difference (P<0.05) in EPG before and after treatment with fenbendazole.

A substantial decline was found in the efficacy of both drugs *i.e* albendazole and fenbendazole on day 21 and 30 post-treatment. This unpredictable difference in efficacy of both anti-helminthes may be due to improper intake of medicated water, various geo-climate conditions, persistent infection among pigeons and presence of nematodes eggs in cages due to improper

routine cleaning of pigeon cages. Kirsch (1983) and Kulisic *et al.* (1993) suggested that medicated feed therapy was found most effective to control nematodes species in farmed breed pheasants. According to Scupin & Nannen (1980) an increased dose rate of anthelmintics would increase the efficacy without any risk. History of non-prescribed anthelmintics persists in Pakistan in birds as prophylaxis, this inadequate and incomplete treatment results in reduction of efficacy of anthelmintics.

The findings of the present study suggested that gastrointestinal nematodes *i.e C. obsignata* and *A. columbae* are prevalent in domestic pigeons in Pakistan. Moreover, fenbendazole is more effective to control gastrointestinal nematodes infestation than albendazole and should be used routinely to control parasitism in pigeons.

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