



Prevalence of Endo-parasites in Peafowls (*Pavo cristatus*) under Captivity Stress and Ameliorative Effect of Anti-Helminthic Drugs

Arnab Tanveer, Shabana Naz*, Azhar Rafique and Asma Ashraf

Department of Zoology, Government College University, Faisalabad, Pakistan

ABSTRACT

A study was conducted to find the prevalence of endo-parasites in *Pavo cristatus* and the comparative efficacy of Albendazole and Levamisole against the endo parasites at three different locations viz. Jallo Wildlife Park Lahore, Wildlife Park Murree and Wildlife Park Bahawalnagar. Freshly dropped fecal sample were collected once before treatment with anthelmintic drug and twice after treatment and brought to laboratory for qualitative and quantitative analysis. Direct smear method and fecal floatation technique was used for isolation and identification of endo-parasites. Modified McMaster technique was used to calculate Eggs per gram. The data was subjected to ANOVA and Tukey-HSD (post hoc test). Six species of endo-parasites were identified from Jallo Wildlife Park. *Eimeria* (7500) and *Ascaris* (1100) were the most abundant. Murree Wildlife park had only two species i.e. *Eimeria* (3850) and *Strongyloides* (350). In Bahawalnagar Wildlife Park, 4 species of endo-parasites were found (*Eimeria* = 1450, *Strongyloides* = 550, *Heterakis* = 350 and *Hymenolepis* = 550). Levamisole was more efficient against endo-parasites as compared to Albendazole. Efficacy of Levamisole at Lahore, Murree and Bahawalnagar wildlife parks after 15 days was 98.36%, 92.85% and 84.48% respectively as compared to Albendazole whose efficacy was 94.53%, 78.57% and 53.44%, respectively.

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Authors' Contribution

AT collected the data and wrote the manuscript. SN designed the study. AR and AA analyzed the data.

Key words

Peafowls, Captivity stress, Endo-parasites, Albendazole, Levamisole, Anti-helminthic drug

INTRODUCTION

A large number of wild animals and birds are kept in captivity. Captivity can be a zoo, wildlife park, sanctuary, breeding farm and privately owned farm. The purpose of keeping animals and birds in captivity can be conservation, recreation, economic benefits and research (Varadharajan and Kandasamy, 2000). Captivity produces stress in the birds which make them more susceptible to variety of infections as compared to their counterparts in the wild. Captivity stress could be limited space in the enclosures, overcrowding, unnatural habitat, unfriendly environmental conditions and suboptimal management (Athar *et al.*, 2001; Parsani *et al.*, 2003). Under captivity stress, birds also suffer from a number of behavioral problems, improper body functioning, reduced immunity, parasitism and stress-related health.

One of the major stress factors that reduce the performance of the birds is intestinal parasitism (Badran and Lukesova, 2006). Captive birds become susceptible to endo-parasites because of low space, over-crowded enclosures, poor hygiene, improper use of anti-helminthic drugs and finally poor and late health assessments.

All these factors become the cause of easy transmission of endo-parasites from one bird to another (Pradeep *et al.*, 2017). Consequently, the birds may suffer from subclinical conditions of anorexia, depression, emaciation, enteritis, and anemia (Forrester *et al.*, 1978). The endo-parasites also damage the health of birds by consuming their nutrients leading to decreased feed utilization, intestinal obstruction and production of toxins and ultimately death may occur (Pradeep *et al.*, 2017).

A broad range of drugs have been used against helminthes, however, resistance has been reported in small ruminants and other livestock animals (Saddiqi *et al.*, 2006; Jabbar *et al.*, 2008), probably affected by the intrinsic efficacy of the drug itself, pharmacokinetic properties, and susceptibility of the host animal and specificity of the parasite (Basit *et al.*, 2014). The present study was conducted to determine the occurrence of endo-parasites in Indian peafowl kept in captivity and to find the efficacy of Albendazole and Levamisole against these endo-parasites at three different Wildlife Parks of Punjab.

MATERIALS AND METHODS

Study site

The present study was conducted at Jallo Wildlife Park, Lahore, Murree Wildlife Park and Bahawalnagar Wildlife Park to determine the rate of infestation by endo-parasites

* Corresponding author: drshabanaz@gcuf.edu.pk
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Table I. Comparative pre-treatment EPG (mean±SE) and prevalence percentage of endo-parasites at Jallo Wildlife Park Lahore, Wildlife Park Murree and Wildlife Park Bahawalnagar.

| Identified Endo-parasitic species | Quantitative analysis | Jallo Wildlife Park Lahore | Wildlife Park Murree | Wildlife Park Bahawalnagar | N | F | Level of significance |
|-----------------------------------|-----------------------|----------------------------|--------------------------|----------------------------|----|-------|-----------------------|
| <i>Eimeria</i> | EPG | 250±71.99 ^a | 128.3±53.37 ^b | 48.33±22.9 ^c | 30 | 3.615 | 0.031 |
| | EPG | 7500 | 3850 | 1450 | | | |
| | Prevalence% | 63.33 | 46.67 | 20 | | | |
| <i>Ascaridia</i> | EPG | 3.33±3.33 | 00±00 | 00±00 | 30 | 1.00 | 0.372 |
| | EPG | 100 | 0 | 0 | | | |
| | Prevalence% | 3.33 | 0 | 0 | | | |
| <i>Strongyloides</i> | EPG | 1.67±1.66 | 11.67±5.71 | 18.97±8.38 | 30 | 2.180 | 0.119 |
| | EPG | 50 | 350 | 550 | | | |
| | Prevalence% | 3.33 | 16.67 | 20 | | | |
| <i>Ascaris</i> | EPG | 75±50.95 | 00±00 | 00±00 | 30 | 2.167 | 0.121 |
| | EPG | 1100 | 0 | 0 | | | |
| | Prevalence% | 6.67 | 0 | 0 | | | |
| <i>Heterakis</i> | EPG | 1.67±1.66 | 00±00 | 11.67±8.194 | 30 | 1.708 | 0.187 |
| | EPG | 50 | 0 | 350 | | | |
| | Prevalence% | 3.33 | 0 | 6.67 | | | |
| <i>Hymenolepis</i> | EPG | 15±11.781 | 00±00 | 18.33±8.80 | 30 | 1.323 | 0.272 |
| | EPG | 350 | 0 | 550 | | | |
| | Prevalence% | 10 | 0 | 13.33 | | | |
| Overall EPG | | 9150 ^a | 4200 ^b | 2900 ^c | 90 | 5.666 | 0.005 |
| Overall prevalence% | | 70 | 56.67 | 46.67 | | | |

EPG, Eggs per gram; SE, Standard Error; N, No. of birds; Means having different letters in a row are statistically significant ($P < 0.05$).

at three different temperature ranges in the same season.

Treatment groups

For treatment of the peafowls with anthelmintic drugs, birds were randomly divided into two groups with 15 birds each. One group was given Albendazole (Group A) while other group was given Levamisole (Group L). Data was collected three times from the same site. Three treatment levels were set. Level 1 pre-treatment (Day 1), Level 2 post-treatment 1st (Day 7) and level 3 post-treatment 2nd (Day 15). Each post-treatment group was further divided into sub-groups for the treatment of Albendazole and Levamisole. Deworming was done after first sampling from all sites. Water was removed from peafowl cages one day before the administration of anthelmintic drugs. Drug was added to drinking water as per prescription on the label. It was given for three days. Water was changed daily and same process repeated again.

Collection of fecal samples

Freshly dropped fecal sample were collected in fecal

cups and labeled properly (Keatts *et al.*, 2016). Color, consistency, blood and mucus was noted for each fecal sample. Samples were stored in pack of handling bags with ice packs for safe transport. All samples were analyzed within 24 h of collection (Kathiravan *et al.*, 2017).

Parasitological examination

Direct smear method and fecal floatation technique was used for qualitative analysis of gastrointestinal parasites (Hodgson, 1970; Fowler, 1978; Soulsby, 1982). Endo-Parasites were identified using identification keys by Jaiswal *et al.* (2013). Modified McMaster technique was used for quantitative analysis (Titilincu *et al.*, 2009). Eggs per gram (EPG) was calculated by using formula: $EPG = (\text{Chamber 1} + \text{Chamber 2}) \times 50$ (Titilincu *et al.*, 2009).

Drug efficacy

Drug efficacy was calculated using the following formula (Basit *et al.*, 2014):

$$\text{Drug efficacy} = \frac{\text{Pretreatment EPG} - \text{Posttreatment EPG}}{\text{Pretreatment EPG}} \times 100$$

Table II. Comparative EPG and prevalence percentage of endo-parasitic species in Indian Peafowl at Wildlife Park of Lahore, Murree and Bahawalnagar.

| Site | Species | | Control | Albandazole | | Levamisole | |
|-----------------------------|----------------------|-------------|---------|-------------|--------|------------|--------|
| | | | | Day 7 | Day 15 | Day 7 | Day 15 |
| Jallo Wildlife Park, Lahore | <i>Eimeria</i> | EPG | 7500 | 500 | 250 | 250 | 150 |
| | | Prevalence% | 63.33 | 53.33 | 20 | 33.33 | 20 |
| | <i>Ascaridia</i> | EPG | 100 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 3.33 | 0 | 0 | 0 | 0 |
| | <i>Strongyloides</i> | EPG | 50 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 3.33 | 0 | 0 | 0 | 0 |
| | <i>Ascaris</i> | EPG | 1100 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 6.67 | 0 | 0 | 0 | 0 |
| | <i>Heterakis</i> | EPG | 50 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 3.33 | 0 | 0 | 0 | 0 |
| | <i>Hymenolepsis</i> | EPG | 350 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 10 | 0 | 0 | 0 | 0 |
| Wildlife Park Murree | <i>Eimeria</i> | EPG | 3850 | 900 | 350 | 600 | 300 |
| | | Prevalence% | 46.67 | 33.33 | 33.33 | 33.33 | 26.66 |
| | <i>Ascaridia</i> | EPG | 0 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 0 | 0 | 0 | 0 | 0 |
| | <i>Strongyloides</i> | EPG | 350 | 0 | 0 | 100 | 0 |
| | | Prevalence% | 16.67 | 0 | 0 | 33.33 | 0 |
| | <i>Ascaris</i> | EPG | 0 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 0 | 0 | 0 | 0 | 0 |
| | <i>Heterakis</i> | EPG | 0 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 0 | 0 | 0 | 0 | 0 |
| | <i>Hymenolepsis</i> | EPG | 0 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 0 | 0 | 0 | 0 | 0 |
| Wildlife Park Bahawalnagar | <i>Eimeria</i> | EPG | 1450 | 950 | 300 | 850 | 450 |
| | | Prevalence% | 20 | 20 | 33.33 | 20 | 20 |
| | <i>Ascaridia</i> | EPG | 0 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 0 | 0 | 0 | 0 | 0 |
| | <i>Strongyloides</i> | EPG | 550 | 400 | 200 | 350 | 0 |
| | | Prevalence% | 20 | 13.33 | 20 | 20 | 0 |
| | <i>Ascaris</i> | EPG | 0 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 0 | 0 | 0 | 0 | 0 |
| | <i>Heterakis</i> | EPG | 350 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 6.67 | 0 | 0 | 0 | 0 |
| | <i>Hymenolepsis</i> | EPG | 550 | 0 | 0 | 0 | 0 |
| | | Prevalence% | 13.33 | 0 | 0 | 0 | 0 |

Prevalence percentage

Prevalence percentage was calculated by using formula:

$$\text{Prevalence\%} = \frac{\text{No. of infected birds}}{\text{No. of examined birds}} \times 100$$

Data analysis

Results were analyzed using SPSS software. Normality and homogeneity of variances were checked for all variables. The data was subjected to one-way ANOVA. Tukey-HSD test was performed as a post hoc test for

pairwise comparisons of means. Significance level was set at $P < 0.05$. Standard Deviation (SD) in post treatment analysis (Table IV) was greater than mean because data was widely distributed about mean. The SD is a description of data's spread. Large SD indicates the data are more spread out while smaller SD shows that data clustered around the mean value. After treatment number of eggs per sample varied from zero (no eggs) to few hundreds (e.g. 200, 500 etc.). Since data had very vast spread about, it had larger SD than mean (Zar, 1999; Isotalo, 2001).

RESULTS

The results of parasitic egg count of different gastrointestinal parasites in Jallo Wildlife Park Lahore, Wildlife Park Murree and Wildlife Park Bahawalnagar are given in Table I. *Eimeria* (Fig. 1A) was significantly high ($P < 0.05$) in Jallo Wildlife Park compared to Wildlife Park Murree and Wildlife Park Bahawalnagar. Similarly, overall egg count was also significantly high at Jallo Wildlife Park Lahore as compared to the other two parks. There was no significant difference in *Ascaridia* (Fig. 1B), *Strongyloides* (Fig. 1C), *Ascaris* (Fig. 1D), *Heterakis* (Fig. 1E) and *Hymenolepis* (Fig. 1F) in the three locations.

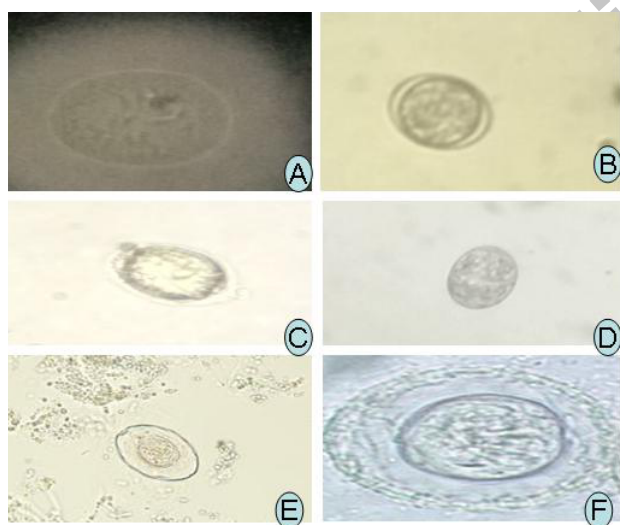


Fig. 1. Morphological identification of the ova of different species. *Eimeria* (A); *Ascaridia* (B); *Strongyloides* (C); *Ascaris* (D); *Heterakis* (E); *Hymenolepis* (F).

As shown in Table II and III, Levamisole was more effective than Albendazole at all three sites. Efficacy of Levamisole at Lahore, Murree and Bahawalnagar after 15 days was 98.36%, 92.85% and 84.48%, respectively as compared to Albendazole whose efficacy was 94.53%, 78.57% and 53.44%, respectively.

In post-treatment fecal analysis, only two species were found. That is why all null results were excluded from statistical analysis (Table IV). *Eimeria* and *Strongyloides* EPG varied significantly in post treatment levels ($F=3.749$, $P=0.054$; $F=2.323$, $P=0.017$) and at each site ($F=3.141$, $P=0.046$; $F=5.086$ $P=0.07$).

Table III. Drug efficacy of Albendazole and Levamisole at Wildlife Park of Lahore, Murree and Bahawalnagar.

| Anthelmintic drugs | Post treatment(7) | | Post treatment(15) | |
|---------------------|-------------------|------------|--------------------|------------|
| | EPG | Efficacy % | EPG | Efficacy % |
| Lahore | | | | |
| Albendazole | 500 | 94.53 | 250 | 97.26 |
| Levamisole | 250 | 97.26 | 150 | 98.36 |
| Murree | | | | |
| Albendazole | 900 | 78.57 | 350 | 91.66 |
| Levamisole | 700 | 83.33 | 300 | 92.85 |
| Bahawalnagar | | | | |
| Albendazole | 1350 | 53.44 | 600 | 79.31 |
| Levamisole | 1200 | 58.62 | 450 | 84.48 |

EPG, Eggs per gram.

DISCUSSION

Qualitative and quantitative analysis of end parasites

It was observed that there is a strong correlation between the prevalence of end parasites and temperature of the areas of the study. A total of 30 samples were analyzed before treatment from each site. Overall prevalence of endo-parasites in Jallo Wildlife Park, Wildlife Park Murree and Bahawalnagar was 70%, 56.6% and 46.6%, respectively. Zamora-Vilchis *et al.* (2012) conducted a study on the prevalence of end parasites in lowland and upland areas and found that the parasites were more prevalent in the areas with higher temperature as compared to upland areas. Prevalence of End parasites in peafowls kept at Bhawalnager wildlife Park is less than that of Lahore which can be explained as the moist conditions favor the outbreak of end parasites (Card and Nesheim, 1972; Matter and Oester, 1989). These results are in accordance with results (56.32%) of Basit *et al.* (2014). Results of current study also related to Lierz *et al.* (2002). He studied endo-parasites in 84 birds of prey and owls in Germany with overall prevalence of 58.3%. Similar trends can be seen in studies of Patel *et al.* (2000) who reported 48% overall prevalence, 68% was reported by Wojcik *et al.* (1999) and Varghese (1987) concluded 67.3%. Pal and Ahmed (1985) reported 69% overall prevalence whereas Kurt and Acici (2008) found prevalence percentage of 88%. Phiri *et al.* (2007) found that 95% of the study birds were infected. Eshetu *et al.* (2001) reported prevalence of 91.01% while Poulsen *et al.* (2000) reported 100% prevalence of endo-parasites.

Table IV. Comparative analysis of post-treatment EPG mean±SE of *Eimeria* and *Strongyloides* at Wildlife Park of Lahore, Murree and Bahawalnagar (n=45).

| | Eimeria | | | | Strongyloides | | | |
|-------|--------------------------|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|----------------------|
| | PostA1 | PostL1 | PostA2 | PostL2 | PostA1 | PostL1 | PostA2 | PostL2 |
| L | 33.3±1.1 ^{a-a} | 10± 0.7 ^{b-a} | 16.6±3.1 ^{c-a} | 10± 0.7 ^{b-a} | 00±00 | 00±00 | 00±00 | 00±00 |
| M | 60± 1.29 ^{a-b} | 40±1.6 ^{b-b} | 23.3±1.6 ^{c-b} | 20±1.4 ^{d-b} | 00±00 ^{a-a} | 6.6±1.5 ^{b-a} | 00±00 ^{a-a} | 00±00 ^{a-a} |
| B | 63.3±1.87 ^{a-c} | 56.6±1.7 ^{b-c} | 20±1.6 ^{c-c} | 30±1.9 ^{d-c} | 26.6±0.3 ^{a-b} | 23.3±1.5 ^{b-b} | 13.3±2.6 ^{c-b} | 00±00 ^{d-b} |
| Total | 52.2±1.8 ^{a-c} | 35.5±1.5 ^{b-d} | 20±3.9 ^{c-c} | 20±4.7 ^{c-d} | 8.8±2.5 ^{a-c} | 10±1.2 ^{b-c} | 4.4±1.9 ^{c-c} | 00±00 ^{d-c} |

N, Total no. of samples examined; Means having different letters in a row or in a column are statistically significant (P<0.05). PostA1, Post-treatment First, subgroup Albandazole; PostL1, Post-treatment First, subgroup Levamisole; PostA2, Post-treatment Second, subgroup Albandazole; PostL2, Post-treatment Second, subgroup Levamisole; L, Lahore; M, Murree; B, Bahawalnagar.

In the present study prevalence of *Heterakis* in two sites was very low compared to other studies. *Heterakis* prevalence was 3.33% in Jallo, and 6.67% in Bahawalnagar while in fecal samples from Murree Wildlife Park no *Heterakis* was found. On the contrary in a study by Basit *et al.* (2014), relative prevalence of *Heterakis* was 36.73% and 29% was reported by Kurt and Acici (2008) in a study on chicken. Prevalence of *Ascaridia* in current study was 3.33% and it was found only in Jallo Wildlife Park. Contradicting results are seen in study by Basit *et al.* (2014) who found 26.53% prevalence of *Ascaridia* and 28.8% was reported by Phiri *et al.* (2007).

Relative prevalence of endo-parasites in Indian Peafowl at Jallo Wildlife Park was maximum i.e. 70% followed by 56.67% in Murree and 46.67% in Bahawalnagar Wildlife Park (Table I). Number of infected birds were more at Jallo Wildlife Park (21) and Wildlife Park of Murree (21) as compared to Bahawalnagar (14). It is established fact that better management conditions lessen the chances of parasitic infections. This claim is also supported by Sascnyanga (1982) where he reported the infestation by *Ascaridia galli* was more (49%) in birds kept under ordinary conditions and only 8% *A. galli* were found in birds under good conditions. He further added that changes in prevalence of endo-parasites at various localities might be due to changed climatic conditions and management practices. Higher prevalence rate like 88%, 95.2% 91.01% and even 100% is reported by Kurt and Acici (2008), Phiri *et al.* (2007), Eshetu *et al.* (2001) and Poulsen *et al.* (2000) respectively.

Drug efficacy

In present study it was found that Levamisole was more effective than Albandazole at all the three sites. Efficacy of Levamisole at Jallo Wildlife Park Lahore, Murree Wildlife Park and Bahawalnagar Wildlife Park after 15 days was 98.36%, 92.85% and 84.48% respectively compared to Albandazole with efficacy of 94.53%, 78.57% and 53.44%

in respective Wildlife Parks and are in accordance with Basit *et al.* (2014) who found the efficacy of Albandazole being 94.92% at 10th day. Ashraf *et al.* (2002) observed 95.79% efficacy of Albandazole against endo-parasitic nematodes. Similar trend was observed by Villanua *et al.* (2007). In a study on Red Legged Partridges, efficacy of Albandazole against endo-parasites was only 38.8%. Khan *et al.* (2010) concluded that Albandazole was more effective (96.33%) than Levamisole (84.90%). However, a study by Sharma *et al.* (1989) and Clarkson and Beg (1970) reported results similar to current study. They stated that Levamisole was 100% effective against endo-parasites as it inhibits activity of malate dehydrogenase.

In present study Albandazole and Levamisole were 100% efficient against all endo-parasites except *Eimeria*. Jiang and Li (1985) found 100% efficacy of Albandazole against *A. galli*. Albandazole was 100% effective against *Strongyloides* in both sites except in Wildlife Park Bahawalnagar where after 15 days prevalence of *Strongyloides* was 20% with EPG of 200. This anomaly in efficacy may be attributed to genetic resistant of nematodes against repetitive use of Albandazole. Schou (2003) also observed a similar trend. He reported that nematodes in cattle showed resistance to use of Albandazole as dewormer especially *A. galli*.

CONCLUSION

Levamisole has better effect against gastrointestinal parasites as compared to Albandazole. Albandazole is a commonly used dewormer and endo-parasitic species are developing resistance against it. So it is recommending that with proper hygiene measures, good management practices and use of different dewormers, especially with Levamisole, we may be able to reduce the infestation of animals and birds in captivity by endo-parasites and reduce economic loss.

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Statement of conflict of interest

Authors have declared no conflict of interest

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