Prevalence and Management of Endoparasitic Worm load in Ostriches of Punjab

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ABSTRACT: Ostrich farming has attained a status of a fast-growing agribusiness in the livestock industry due to the wide range of associated benefits attached to it. This study was designed to assess the prevalence of endoparasites in this economically important bird as well as their treatment to provide better guidelines for successful ostrich farming. The study involved 385 fecal samples of ostriches taken from 55 commercial farms and captive sites in Punjab during the period of January 2020 to December 2020. The relevant data and samples were collected from 15 districts of Punjab. Fecal samples were tested against gastrointestinal worm load by using floatation and sedimentation techniques. Ostriches of 11 commercial farms and Lahore Zoo, Jallo Wildlife Park Lahore, UVAS Ostrich farm Pattoki and Bahawalnagar Wildlife Park were found to harbor protozoan parasites such as Eimeria spp., Balantidium coli, and Amoeboid cyst. It was noteworthy that the use of herbal dewormers showed much better results than chemical anthelmintics. Similarly, those wildlife parks where ostriches were given a natural environment showed negative results for endoparasites. At some farms combination of chemical and herbal anthelmintics was also used. The absence of a reliable nutritional management system caused the mortality of ostriches at 40 farms due to gastric problems and choking. The mortality rate at the age of 2-4 months was 73% while mortality of adult birds from 1-7 years was 27%. Among selected farms, 50% of ostriches were facing lameness, leg deformities, and retarded growth due to improper space, a congested environment, and poor feeding systems. Lastly, more research is needed to make this agribusiness flourished.

Keyword: Endoparasites, Ostrich, Prevalence, Anthelmintics, Management
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INTRODUCTION

Ostrich (Struthio camelus) is the world’s largest flightless carinate bird which is the only living member of the genus Struthio, family ratite, and has the fastest speed among all the birds on land due to its long strong legs. Almost 150 years ago commercial ostrich farming started only for feathers and somewhere for leather (Huchzermeyer, 2002). Nowadays Ostrich farming is emerging in the world as a new horizon in the livestock industry and it is producing meat, skin, oil, and feathers as major products (Abbas et al, 2018). The brain, eyes, and tendons of ostrich leg are also used in the treatment of human diseases (Shanawany, 1995).

In Pakistan, ostrich farming was gaining popularity because of its valuable products. Especially the climatic conditions of Punjab are very favourable for ostrich farming, keeping this in view Livestock and Dairy Development Department (L&DD) was playing a significant role in the promotion of the ostrich industry in Punjab. According to collected data the Government of Punjab registered almost 10,000 ostriches and gave a subsidy of Rs10,000 to farmers for each bird for promotion of the ostrich farms in Punjab (Abbas et al., 2018).

As in all production system parasites are a problem, the results obtained from a study showed the presence of various protozoa (Martínez-Díaz et al., 2000) flagellates, ciliates and coccidian (Martínez-Díaz et al, 2000). In Africa problems found in ostrich framing were tapeworms, nematodes, anthrax, ticks, lice, and ophthalmia (Barton and Seward, 1993; Davis, 1998). In ostriches, no specific infectious or contagious diseases are seen except Libiyostrongylus (wire-worm), which is the only true specific infectious pathogen of ostrich, found in the stomach (Huchzermeyer, 2002). Ostrich shows varying degrees of immunity regarding parasites and the females that select their mate on the bases of ornamentation could acquire males with better resistance to parasites (Bonato et al., 2013). According to previous research reported by Lozano et al., 2021 in ostriches from South America (Mario-González et al., 2017), Asia (Eslami, 2007), Polynesia (More, 1996), Africa (Mukaratirwa, 2004), and Europe, L. douglassii was found infecting both ostriches and emus (Jansson and Christensson, 2000). In another study of ostrich endoparasites, Forty-three stool samples out of a total of eighty from ostriches (N=80) tested positive for the presence of eggs per gram (epg) from the parasites Cappilaria, Ascaridia, and Eimeria spp. Out of 43
Prevalence and Management of Endoparasitic Worm load in Ostriches of Punjab birds, 19 had mature parasites like Cappilaria, Ascaridia, and Eimeria. (Ambreen et al., 2021). But still, these findings are not enough therefore superficial knowledge is not sufficient therefore benefits from the ostrich industry can only be derived after gaining thorough knowledge of ostrich behavior and health-related issues (Mshelia et al., 2010). Ostrich farming is still at its dawn and a lot of research and growth is a requisite to reach the level of success for which the poultry industry is known (Nemejc and Lukesova, 2012).

This study was designed to find the prevalence of endoparasites in ostriches raised in commercial farms and wildlife parks of Punjab at a large scale to provide better insight into health issues and management of these important big birds with big health benefits.

**MATERIALS AND METHODS**

**STUDY AREA AND SAMPLE SIZE**

A total of 385 feacal samples were collected over a period of year (January 2020 to December 2020) from Lahore Zoo, Zoo Safari Lahore, Jallo Wildlife Park Lahore, UVAS Ostrich Farm Pattoki, Bahawalnagar Wildlife Park, and 50 commercial farms located at 15 different districts of Punjab and ostriches presented by L&DD at different exhibits. Ostriches of all age groups and sex were included in the sampling. For ≤50 birds 4 samples were taken and for ≥51 birds 7 samples were taken.

**SAMPLE COLLECTION**

Samples were collected from the ground by a gloved hands and transferred into coprological sample pots which were placed in a cooler bag with an icepack. Each sample pot was labelled with the date, the owner's name, the address of the sampling area, species of bird, and its sex. Once transported to the laboratory, they were stored for a maximum of five days at 4 °C at the Conservation Biology Lab Institute of Zoology, University of Punjab, Lahore. In three holdings, repeated sampling was planned at different times of the year, to establish whether seasonal conditions influenced the parasite found. In order to view parasitic eggs/oocysts of nematodes, cestodes, and protozoa, both qualitative (flotation, sedimentation, and micrometry) and McMaster quantitative techniques were applied (Papini et al., 2012)

**QUALITATIVE TECHNIQUES**

**i. Flotation Technique**

A total of 1 gram feacal samples were taken in a beaker and 15 ml flotation solution added to it and stirred well. The solution was strained by using a tea strainer and poured into a 15ml tube. Tube was placed in a test tube
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rack, leaving a convex meniscus and gently a cover slip was placed on the top of the test tube. Let the test tube stand for 15 to 20 minutes. Then the coverslip was placed on a clean glass slide and observed under a microscope (4X, 10X, 40X) (Deplazes et al., 2013).

ii. Sedimentation Technique

It is a method for detecting trematodes eggs in which heavier nematode eggs were settled in sediments. Approximately 3 grams of feaces were added in 40-50 ml of tap water taken in a beaker and mixed it well with a stirrer and the solution was strained by using a tea strainer. The solution was poured into a test tube and let stand for 5 minutes. Decant the supernatant carefully and the solution was re-suspended with 5ml water. This step was repeated 3 to 5 times until the supernatant became clear. Then a drop of sediment was poured on a clean glass slide. A cover slip was placed on a glass slide and observed under a microscope (Deplazes et al., 2013).

MICROMETRY OF SAMPLES

Microslides were made from floatation and sedimentation samples and examined under the microscope to spot endoparasites.

Mcmaster Quantitative Technique

Each feaces sample weighed 2 grams, and 28 milliliters of a saturated sugar solution (specific gravity 1.2) was added to the mixture before being filtered and placed on a McMaster slide. Under a light microscope (100X), parasites were identified and enumerated down to a detection limit of 50 OPG and 50 EPG (Zajac and Conboy, 2012).

STATISTICAL ANALYSIS

Using SPSS version 20, we performed statistical analyses on the data collected during the study trials, including the T-test and repeated measures multiple ANOVA (Galen et al., 2022)

RESULTS

A total of 385 feacal samples from 55 locations were examined in this study for the presence of endoparasites and ostriches at 15 locations were found to be positive for endoparasites. Among positive samples *Eimeria spp.* was 66%, *Amoeboid cyst* was 26% and *Balantidium* was 6.6%. Identification of helminths ova and oocyst was done according to the shape and dimensions of eggs, by using standard parasitological techniques. It was seen that younger ostriches less than 1 years of age are more likely to have the infection as compared to those adult ostriches. The effect of sex on the prevalence of endoparasites in both groups (ostriches
Prevalence and Management of Endoparasitic Worm load in Ostriches of Punjab in farms and wildlife parks) did not show any significant effect. During study commercial farms and wildlife parks were visited during this study and findings from questionnaires revealed that extensive deworming was observed for parasites. Both chemical and natural dewormers were used during this period. Parasites found and their ova are presented in the table 1.

Table 1: Endoparasitic worm load of captured farmed ostriches in Punjab

<table>
<thead>
<tr>
<th>Age Group</th>
<th>No. of positive samples</th>
<th>Species of Endoparasites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Eimeria tanella (23×19µm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Young Ostrich</td>
<td>65</td>
<td>15</td>
</tr>
<tr>
<td>Adult Ostrich</td>
<td>16</td>
<td>-</td>
</tr>
</tbody>
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The Table 1 and Fig. 1 represent different species of gastrointestinal parasites in the ostrich of Punjab. Out of 81 positive samples, 65(80.2%) and 16(19.7%) were found in young and adult ostriches respectively. Among Eimeria spp, Eimeria tanella, Eimeria maxima, and Eimeria mitis were identified by using micrometry. Clinical signs like weight loss, emaciation, and bloody diarrhea due to Eimeria tanella were seen in ostriches of a farm in Gujranwala.
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![Graph showing the percentage of different species of endoparasites found in Ostriches.](image1.png)

**Fig. 1: Seasonal Distribution of different Species of endoparasites found in Ostrich**

![Graph showing the number of positive cases over months.](image2.png)

**Fig. 2: Seasonal prevalence of endoparasites**

It is very clear from Fig-2 that the maximum number of positive samples in the month of July is the rainy season in Pakistan and facilitates the parasitic population.
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Fig. 3: Comparison of Chemical, Herbal, and No Deworming at Farmed and Captive Sites of Punjab for Anthelmintic Properties

Chemical deworming showed more positive results than herbal deworming as shown in Fig. 3. The herbal medicine, PAK-1 (1Kg - Mentha arvensis, 1Kg - Cichorium intybus, 1Kg - Silybum marianum, 1Kg - Trachyspermum ammi, 1Kg - Azadirachta indica, 500 grams - Pimpinella anisum, 500gms - Curcuma longa, and 500 grams - Cassia ngustifolia) showed good results for ostriches at commercial farms. The farms that were not using any kind of anthelmintic had negative and positive results similar to farms using chemical anthelmintic for their birds. The risk factor analysis of Age and Season with parasitic worm load using the chi-square test gives p-value <0.00, hence the association between age and season with parasitic load is significant.

DISCUSSION

Due to the worldwide importance of ostrich farming, control of parasites in ostrich is now becoming an emerging issue. Unfortunately, despite its great economic potential, the ostrich received little attention from scientists and there are only a few studies focused on its endoparasites. The present study shows very little endoparasitic infection in both, the farmed ostriches and that of wildlife parks. A sampling at 55 farms & captive sites was performed from January 2020 to December 2020.

In the winter season, all samples were found negative, this can be attributed to climatic conditions like low temperature, low humidity and low rainfalls which inhibit the growth and propagation of parasites. A study on the prevalence of gastrointestinal
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parasites in desi fowl in and around Gannavaram, Andhra Pradesh, India also supports this fact. The results showed a significant relationship between seasonality. The data obtained from results indicated a high prevalence in the rainy season (43.41%) followed by the summer (39.91%) and winter (17.68%) seasons (Sreedevi et al., 2016). On the contrary, a study in Sweden showed that nematode (L3-stage larvae of L. douglasi) can survive in the winter season (Jansson et al., 2002).

In summer a few oocysts of *Eimeria*, not enough for sporulation, were seen in the faecal examination of some ostriches. *Isospora struthionis* is reported on zoo ostriches in Russia (Huchzermeyer, 1998). During the summer and rainy season, From May 2020 to September 2020, 15 farms out of 55 were found to be infested with *Eimeria spp*. *Amoeboid cyst* and *Balantidium coli* similar was reported by Priyanka et al., 2021. A similar study was carried out on 7 ostrich farms in three states of northern Nigeria from May to September.

All farms except one had samples positive for *Eimeria* (Mshelia et al, 2010). Whereas in research of Gaborone a decline was found during June and July months, and Coccidia oocyst was not found in any of the ostriches (Binta et al., 2003). At a commercial farm in Faisalabad faecal sample was found to have cysts of (50µm×68µm), and according to the dimensions it was identified as *Balantidium coli* which is not reported yet from ostrich. The infection of this parasite could be due to local environment of farm because ruminants were also present on the farm. A very low pathogenicity in ostriches was attributed to these parasites because eggs and oocyst count were generally very low. However, in two farms, one in Gujranwala and the other in Lahore ostriches were found to have enteritis, faeces with blood, diarrhoea and death due to coccidiosis infection.

The prevalence of very low endoparasitic count can be attributed to the extensive use of dewormers and natural herbs used on farms along with feed. A herbal dewormer named PAK-1 (1Kg- *Mentha arvensis*, 1Kg- *Cichorium intybus*, 1Kg- *Silybum marianum*, 1Kg-*Trachyspermum ammi*, 1Kg- *Azadirachta indica*, *Pimpinella anisum*, *Curcuma longa*, and *Cassia angustifolia*) have been used with positive outcomes, outperforming chemical dewormers commonly used on farms. The recommended dosage of PAK-1 was 10ml/30lit water, twice weekly. There was no statistically significant variation in the prevalence...
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of endoparasites between sex groups of ostriches. These results are consistent with those reported by Jamil et al. in 2022. Herbal medicines are an effective source of prime components for drug detection and the formation of phytopharmaceuticals in the control of devastating parasitic infections. There is a prerequisite to applying traditional medicine information in clinical applications via value addition.

The effect of age on the prevalence of infestation, however, showed chicks (less than 12 months) had a higher prevalence as compared to adult ostriches. This could be due to a lack of immunity (Soulsby, 2015). Commercial farms and wildlife parks were visited during this study and findings from questionnaires revealed that extensive deworming was observed for parasites. This extensive use of chemical dewormers has many negative effects on captive birds' health and funds. According to the literature, urban zoos frequently draw wild animals like rats, herons, vultures, and pigeons. This greatly contributes to the spread of disease among confined animals because it allows them to interact with wild animals more easily. The enclosures and treatment areas must be properly disinfected, and intermediate-host populations must be reduced through targeted treatments and effective sanitary administration. (Melo et al., 2022).

This increased both chemical and natural dewormers being used during this period. Even though no helminths parasites were detected during this study, it is highly likely that birds can suffer from helminths infestation and more research should be conducted on this topic.

CONCLUSION
In conclusion, the present study demonstrated for the first time the prevalence of endoparasites in ostriches raised in Punjab. Ostriches showed a very low prevalence of endoparasites which is a good aspect of ostrich farming in Pakistan. Maintenance of good sanitary conditions, feeding management, and avoidance of contact with domestic animals at farms might be found as helpful factors for ostrich farming. However further studies on problems of ostrich production such as feeding management which can negatively affect the production need to be investigated in Pakistan. This study will essentially be helpful to the researchers and local veterinarians to develop strategies for the treatment and control of diseases.

ACKNOWLEDGMENT
The authors are highly indebted to Punjab University New Campus, Lahore Research Grant Project for the
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provision of funds to complete this task. The help of Zoo/Zoo Safari management and the cooperation of various private ostrich farms for the collection of samples is commendable.

ETHICAL APPROVAL
The study was approved by the Institutional ethical review committee.

CONFLICT OF INTEREST
No conflicts of interest were reported by the authors.

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