Plant Archives Vol. 20, Supplement 2, 2020 pp. 1135-1139 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ISOLATION AND IDENTIFICATION OF INTESTINAL PROTOZOA FROM OSTRICHES IN MIDDLE OF IRAQ

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Abstract

Samples were collected from the feces of 50 ostriches, which were examined by direct smears. With and without centrifugation; cysts and oocysts of protozoa and nematode eggs were examined in fecal sample. The results of this work were as follows: Trophozoites and cysts of *Entamoeba* spp., cyst of *Giardia* spp., oocyst of *Cryptosporidium* spp., oocyst of *Eimeria* spp. and *Isospora sp. egg* were recorded, After data analysis, it was concluded that Ostriches were infected enzootically by nematode and protozoan species (Mix parasite infection). There are a few records on infections with intestinal protozoa in ostriches of Iraq. *Eimeria* spp. (protozoa), belong to parasites causing the most serious economic losses in ratites in the world. As farming of these big birds is still in the beginning, many years of research and development are necessary to reach levels of medical and technological progress that is nowadays practiced in poultry industry. The current study aims to record and identify intestinal protozoa on ostriches in the middle of Iraq.

***Keywords*:** Coccidia, Helminth, protozoan, Ostriches, *Eimeria spp.*

Introduction

In birds, protozoa are present in the digestive system (includes oropharynx, intestine and cloaca), which is an important and main cause of disease, as well as in the blood flows and inner parts of the body (Silvanose *et al.,* 1998). A number of intestinal protozoans, including *Eimeria* spp., *Isospora* spp., *Sarcocystis* spp., *Cryptosporidium* spp., *Giardia* spp., *Trichomonas* spp., *Histomonas* spp., *Hexamita* spp. and *Toxoplasma gondii* have been isolated from ostrich chicks. Often, they cause serious diarrhea in ratites (Jurajda, 2002; Cooper, 2005; Martinez-Diaz *et al*., 2013; Gallo *et al*., 2014). The incidence of infective parasitic stages such as eggs, larvae, cyst, oocysts, and if combined with stress or poor husbandry conditions (e.g. poor hygiene) It could cause clinical diseases (Pantchev, 2008). The clinical symptoms of infection by intestinal parasite are as follows: anemia, anorexia and weight loss, (Barton and Seward, 1993), lethargy and general muscle weakness (Mukaratirwa *et al*., 2004). The pathology is represented by an intestine mucosa thickening as parasites were observed at high level concentrations, and nodular areas in the distal third of the infected caeca, also hemorrhagic areas abutting small ulcers surrounded by edema. It can be said from what was mentioned that double (mixed) infections Mostly present in ostriches all over the world, But sometimes it cannot be distinguished due to morphological similarities. There are several parasites acclimatized with some avian species, at the same time birds may be infected with different parasites. Therefore, the parasitic species and their importance to the host may differ among the bird species. Stages of infectious parasite (oocysts and eggs) does not always cause an infection and a parasitism is not always combining with clinical disease (Papini *et al*., 2012). In restrictive birds, nematodes and other endoparasites like coccidia must be keep under observation. The re-infection rate of direct life-cycle parasites is higher in captive birds comparing with free-ranging conspecifics, which may be lead to a big parasite load and high severity of disease (Sasseville *et al*., 1988; Lierz *et al*., 2010). The infection is diagnosed histopathologically on part of the infective organs or by identification of oocysts in faecal samples (Deeming, 1999; Jurajda, 2002).

Material and Methods

50 fresh feces samples were collected directly from ostriches for both sexes from middle of Iraq. Samples were taken four times from each animal with a three-day break between each collection with numbering of animals when taking the sample. placed in a clean container and stored in the freezer until examined in the laboratory. A fecal sample centrifuge to separate it and determine the parasitic stages present in it, centrifugation fecal flotation in Sheather's sugar solution (Sheather, 1923), samples were performed, and then two techniques were used: staining with 2% Lugol's Iodine Solution and the positive one for oocysts mixed with a 2.5% potassium dichromate solution ($K_2Cr_2O_7$), samples passed through two layers of gauze and aerated to facilitate sporulation, then examined microscopically. A digital camera Strapped to a binocular microscope was used to photograph the parasites, and Sample Images Software was used for cyst, oocyst and egg measurements in micrometers (μm), and also using ocular micrometer to find size of infected phases of protozoa.

Results and Discussion

During this study, a total of 50 fecal sample in Ostriches were examined, of which 18/ 50 (36%) were found to be infected with one or more species of intestinal protozoa.

A total of 5 species of intestinal protozoa parasites (cyst/oocyst) were identified, namely, *Cryptospordium* sp. (4%), *Eimeria* sp,(24%), *Entamoeba* sp (2%), *Giardia* sp (2%) and *Isospora sp* (4%). (Table 1 and Table 2). (Fig. 1, 2, 3, 4 and 5)

Table 1 : Total infection with of intestinal protozoa in Ostriches

Animal	No. of Samples Examined	No. of positive	Percentage (%)
Ostriches	**50**	**18**	**36**

Table 2 : Prevalence of intestinal protozoa species in Ostriches

Intestinal protozoa	No. of Samples Examined	No. of positive	Percentage (%)
Cryptospordium sp	50	2	4
Eimeria sp	50	12	24
Entamoeba sp	50	1	2
Giardia sp	50	1	2
Isospora sp	50	2	4

Results indicated mixed infection intestinal protozoa with nematode and cestode in Ostriches. In this study the prevalence *(Eimeria sp*+ Ascaridia s*p*), (*Cryptospordium* + Ascaridia s*p*+ *Raillietina* egg), *(Entamoeba sp*+ Ascaridia sp), (*Giardia* sp+ Ascaridia s*p*+ *Raillietina* egg), (*Eimeria sp* + Trichostrongylidae) infection rate recorded (20%) , (14%) , (4%),(8%) and (16%). (Table. 3) (Figure 6, 7, 8 and 9)

Table 3: Prevalence of mixed infection intestinal protozoa with other parasite in Ostriches.

Mix infection	No. of Samples examined	No. of case	Percentage (%)
Eimeria sp+ Ascaridia s*p*	50	10	20
Cryptospordium + Ascaridia s*p*+ *Raillietina* egg	50	7	14
Entamoeba sp+ Ascaridia s*p*	50	2	4
Giardia sp+ Ascaridia s*p*+ *Raillietina* egg	50	4	8
Eimeria sp +Trichostrongylidae	50	8	16

Table 4 shows the prevalence of intestinal parasites according to the family, group and stage to which the parasite belongs

Table 4: Prevalence of intestinal parasite according to Family parasite in this research

Family parasite	Parasite	Parasite group	Stage
Cryptospordiidae	*Cryptospordium*	Protozoa	Oocyst
Hexamitidae	*Giardia*	Protozoa	Cyst
Eimeriidae	*Eimeria*	Protozoa	Oocyst
Entamoebidae	*Entamoeba*	Protozoa	Cyst
Eimeriidae	*Isospora sp*	Protozoa	Oocysts
Ascarididae	Ascaridia	Nematode	Egg
Trichostrongylidae	Trichostrongylus.	Nematode	Egg
Davaineidae	*Raillietina*	Cestode	*Egg*

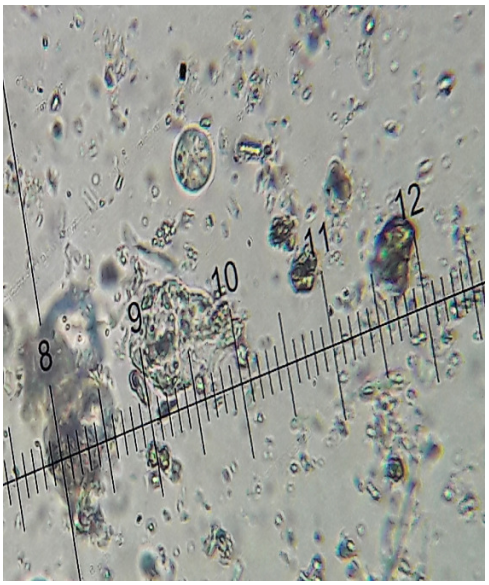

Fig. 1: ***Oocyst of Cryptospordium*** **sp**

Fig. 2 : Oocyst of Eimeria sp

Fig. 3 : Cyst of *Entamoeba* sp

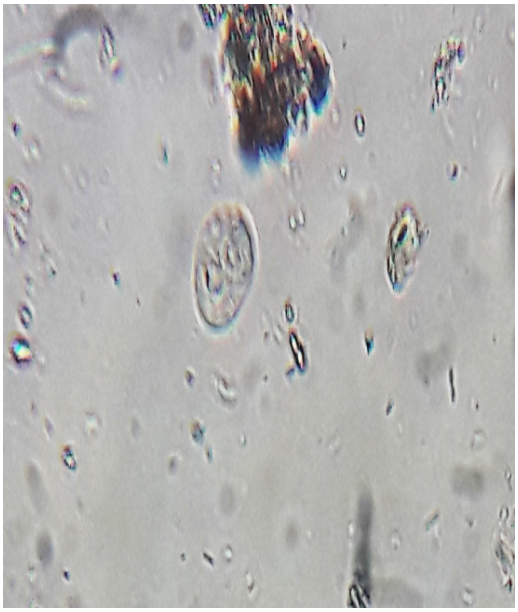

Fig. 4 : Cyst of Giardia

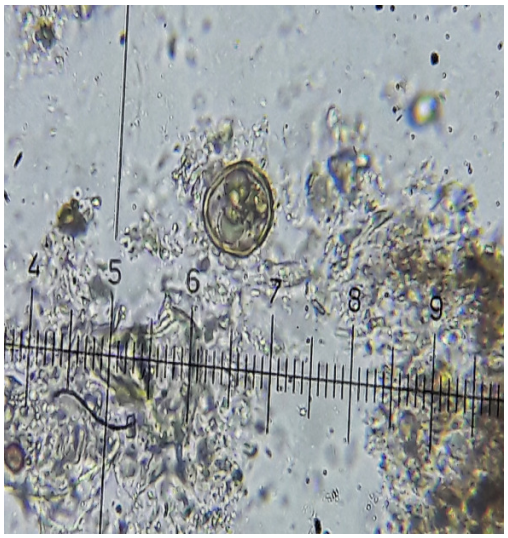

Fig. 5 : *Isospora sp* oocyst

Fig. 6 : Ascaridia sp

Fig. 7 : Trichostrongylidae egg

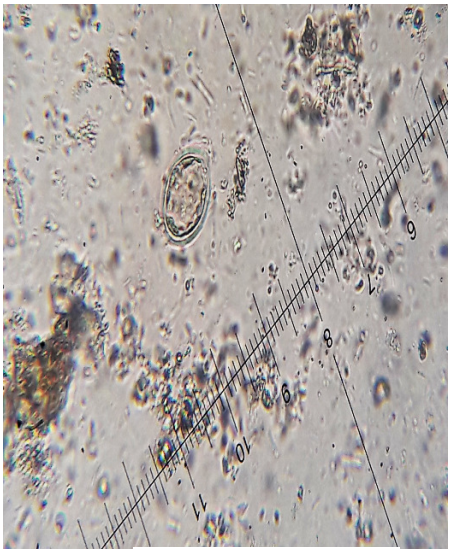

Fig. 8 : Trichostrongylus sp egg

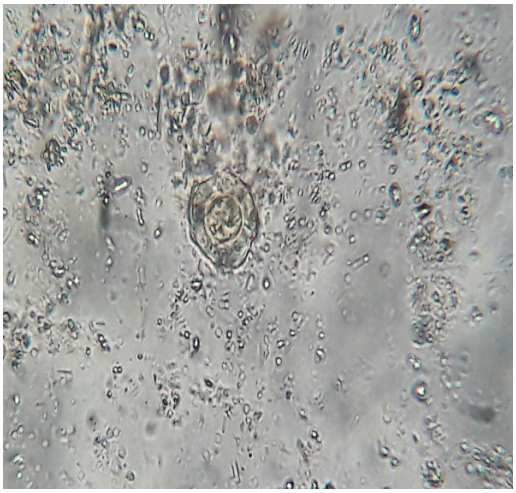

Fig. 9 : ***Raillietina sp egg***

The overall prevalence of gastro-intestinal parasites (protozoa) of Ostriches was (36%) from 18/50 sample. The spread of gastrointestinal parasites can be significant in places where living conditions and basic sanitation are minimal or nonexistent. Many parasites: protozoa of the *Entamoeba* and *Giardia*, helminthes of *Ascaris* and many species of cestodes are transmitted by contaminated water or food, while other helminthes are transmitted by larvae that are available in the soil (Neves, 2005). This work recorded infection rate with many species of intestinal protozoa *(Cryptospordium sp, Eimeria sp, Entamoeba sp*, *Giardia* sp and *Isospora dromaii*) with rate (4%,24%, 2%, 2 % and 4%) respectively and this agreed with (Gallo *et al*., 2019) who recorded Trophozoites and cysts of *Entamoeba* spp. and *Giardia* spp., oocysts of *Eimeria* spp. and *Isospora dromaii* .also agreed with (Sotiraki *et al.,* 2001), small diameter (10-15 µm) uninucleate cysts of *Entamoeba* spp. were identified in the feces of ostriches; these cysts were much smaller than the uninucleate and multinucleate cysts. While (Radhy *et al*., 2013) reported rate of 40% infected with oocysts of *Giardia spp* in Al-Zawraa zoo in Baghdad city . Ostrich as an ecosystem could be infested with its own specific parasites as well as the external and internal parasites of other bird, some parasites of ruminants and raccoons. So, more comprehensive studies are needed to illustrate different aspects of parasitic infestations of this animal and their influence on the infested birds (Eslami *et al*., 2007).

The current study recorded mixed infections of intestinal protozoa with Nematode and cestode like *(Eimeria* sp. with Ascaridia *sp*), *(Cryptospordium,* Ascaridia *sp and Raillietina* egg) (*Eimeria sp* with Trichostrongylidae), this results agree with (Eslami *et al*., 2007) who findings revealed the presence of *Libostrongylus douglasii* and Oocyst of *Eimeria* sp. was found in ostrich on farm of Garmsar and agree with (Gallo *et al*., 2019), who observed infection intestinal protozoa with eggs belonging to the Ascaridida order were found in the feces. While (Hoberg *et al*., 1995) recorded *Libyastrongylus entatusn* sp. (Nematoda : Trichostrongylidae) from Ostriches in the North of America. It can be concluded that Ostriches infected enzootically by nematode and protozoan species.

Last but not least, it can be said that the results of this work indicate that captive animals, on the one hand, can serve as reservoirs for many types of protozoa, and on the other hand, can be a potential source of infection for both domestic animals and human caregivers, although some adverse stress conditions are crowded, such as those which obtain in captivity, have often compromised the existing reservoir status, (John *et al.,*1982).

Acknowledgement

The authors would like to extend their sincere thanks and appreciation to all those who contributed and assisted the employees of this work especially the employees of the laboratories of (Iraq Natural History Research Center and Museum)- University of Baghdad, in carrying out this study, greetings to all.

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