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Cryptosporidium species in ducks: parasitological, serological and molecular studies in Egypt.

Kalifa, M.M., Nassar, A.M., Nisreen, E.M., *Abdel-Wahab, A.M.

Parasitology Department, Faculty of Veterinary Medicine, Cairo University, 12211 Giza, Egypt.
*Corresponding author: *marwaehab40@yahoo.com*

Abstract

The present work was initiated to study the incidence, morphology and molecular identification of *Cryptosporidium* spp. infecting ducks. Incidence of *Cryptosporidium* spp. in ducks was 39.9 % (365 out of 915 ducks). Seasonal incidence showed that the highest rate was in winter (74.6%) while the lowest one was in autumn (7.1%). *Cryptosporidium* spp. oocysts were spherical to ovoid in shape with smooth wall. It contains 4 sporozoites and a residium. Comparative study between M.Z.N. and ELISA showed that examination of the bursal and intestinal contents of each of 100 Muscovy ducks by M.Z.N. stain revealed that 33% and 28% were *Cryptosporidium* spp. positive respectively. While ELISA showed that 55% of these ducks had antibodies against *Cryptosporidium* species. Immunoblotting of *Cryptosporidium* spp. antigens of ducks revealed that the bands at the MW 68, 38-42, 20 and that lower than 15 KDa considered to be specific as they react versus *Cryptosporidium* positive duck serum only. Analysis of the PCR products of *Cryptosporidium* spp. from ducks revealed that the positive amplification of the target fragment was 435bp for ducks. Sequencing of the PCR products revealed that the investigated species was *C. meleagridis* in ducks (the accesion number in gene bank was KM581271).

Keywords: *Cryptosporidium* spp., ducks, incidence, ELISA, immunoblotting, PCR.

Introduction

Ducks are hardy animals and good scavengers. About 700 million ducks are kept around the world. More than 500 million are in Asia while the reminder numbers of ducks are in other parts of the world, including Africa and Latin America **(Van der Meulen and Den Dikken, 2004)**. Ducks can be reared for eggs and meat for own use or for sale. Other products from ducks, which can be sold, include down, feathers, and fattened livers**.** The ducks of the world are of two types, the Muscovy ducks (*Cairina moschata*) and the mallard ducks (*Anas platyrhynchus*) **(Harrison and Greensmith, 1993)**. There are several breeds of ducks in Egypt, Native, Sudanese, White Peckin, Mallard Muscovy and Campbell (**AbouLaila et al., 2011**).

Cryptosporidium species are one of the most prevalent protozoan pathogen develop in the bursa of fabricius, cloaca, intestine, trachea and kidney of birds **(Dubey et al., 1990**). It causes diarrhea and unthriftiness, in addition to pathological changes in infected ducklings as epithelial hyperplasia, loss of cilia and dilated mucous glands in trachea. In bursa of fabricius, it causes hyperplasic hypertrophied epithelium, thickened stroma and infiltration of lamina propria with mononuclear cells and heterophils **(Mousa, 2000**). In birds, three main *Cryptosporidium* spp. have been described namely *C. baileyi, C. galli* and *C. meleagridis* **(Slavin, 1955, Current et al., 1986, Ryan et al., 2003)**. Several researches referred to

infection with another species of *Cryptosporidium* in birds like *C. parvum* which was recorded as natural infection in different wild and pet birds **(Ryan, 2010** and **Graczyk et al., 1996)**.

C. meleagridis is the third most common *Cryptosporidium* parasite in human (**Xiao, 2010)** and has the ability to transfer from infected birds to human **(Chappell et al., 2011).**

Cryptosporidium spp. among ducks are well documented all over the world (**Richter et al., 1994, Adejinmi and Oke, 2011, Jasim and Marhoon, 2015)**. In Egypt there were information only on the incidence and description of *Cryptosporidium* spp. by light microscope **(Abu-Siry, 1993, Abdel-Wanis et al., 1996, Mousa, 2000, El- Madawy, 2001, AbouLaila et al., 2011 and Nagwa et al., 2013)** but there is no previous work on the serological and molecular diagnosis. So, the present work was initiated to study the incidence of *Cryptosporidium* spp. infection in ducks using parasitological [modified Ziehl-Neelsen stain (M.Z.N.)] and serological [Enzyme Linked Immuno-Sorbent Assay (ELISA)] methods, characterization of *Cryptosporidium* spp. antigens by using Sodium Dodecyl Sulphate-Polyacrylamide Gel electrophoresis (SDS-PAGE) and immunoblotting , molecular identification of *Cryptosporidium*-positive samples by polymerase chain reaction (PCR) and sequencing of the PCR products.

Materials and Methods

1. Collection of samples:

1. a. Intestinal contents and bursal samples:

Intestinal contents and bursal samples were collected from each of 915 ducks (820 Muscovy, 70 Mallard and 25 Native) during the period from January to December 2014 at age varied from one week to 6 months.

1. b. Blood samples:

One hundred random serum samples were collected from the previously examined 820 Muscovy ducks. The intestinal, bursal and serum samples of these 100 ducks were used for comparative study between M.Z.N. stain technique and ELISA for diagnosis of *Cryptosporidium* spp. infection.

2. Diagnosis of *Cryptosporidium* spp.:

2.1. Parasitological examination:

The intestinal contents and bursal samples were stained by M.Z.N. stain (**Henriksen and Pohlenz, 1981).** The morphological parameters for 50 *Cryptosporidium* oocysts were measured under light microscope.

2.2. Serodiagnosis of *Cryptosporidium* spp. in the 100 duck sera by ELISA:

2.2.1. Preparation of antigen:

The *Cryptosporidium* spp. oocysts used for preparation of antigen were collected from naturally infected ducks. The oocysts were purified according to **Khalil, 1993.** The protein content of the prepared antigen was measured according to **Lowry et al., 1951**.

2.2.2. ELISA procedure (Catty and Raykundalia, 1989):

The antigen concentration after checkerboard titration was 3µg protein / well, serum dilution was 1:100 and anti-chicken IgG horse reddish peroxidase (1:1000 dilutions). The optical density was read at 450 nm with a micro-ELISA reader system.

3. Characterization of *Cryptosporidium* spp. antigens by using Sodium Dodecyl Sulphate-Polyacrylamide Gel electrophoresis (SDS-PAGE) and immunoblotting:

3.1. SDS-PAGE:

Three different types of antigens were prepared from purified *Cryptosporidium spp.* oocysts of naturally infected ducks, *Cryptosporidium baileyi* of naturally infected chickens and *Cryptosporidium parvum* of naturally infected calves. These antigens were electrophoresed by SDS-PAGE according to **Laemmli, 1970**.

3.2. Immunoblotting:

The unstained protein bands of the previously prepared antigens (ducks, chickens and calves) were electrophoretically transferred from SDS-PAGE to a nitrocellulose sheet using modified technique according to **Towbin et al., 1979.**

4. Molecular identification of *Cryptosporidium* spp.:

4.1. DNA Extraction:

Cryptosporidium spp. oocysts were isolated from the bursa of naturally infected ducks and chickens. The oocysts were purified and stored -20°C until used for DNA extraction. DNA was extracted by DNA extraction Kits (Qiagen).

4.2. PCR amplification:

PCR amplification for *Cryptosporidium* spp. was processed using 18s rRNA gene and primers (forward and reverse) as shown in Table 1. Cycling protocol used in PCR for identification of *Cryptosporidium* spp. was done according to **Johnson et al., 1995**. PCR products were placed on 1 % agarose gel for electrophoresis and stained with ethidium bromide using 100 bp DNA ladder as size marker (fermentas, Germany): they visualized under UV trans-illuminator and photographed using gel documentation system **(Sambrook et al., 1989)**.

Table1: primers of *Cryptosporidium* spp.

Primer	Sequence
Forward	5'-AAGCTCGTAGTTGGATTTCTG-3'
Reverse	5'-TAAGGTGCTGAAGGAGTAAGG-3'

4.3. Sequencing of the PCR products and submission to Genbank:

Purified PCR products were sequenced in both orientations by the dideoxy chain-termination method using the amplification primers which described above. Gene sequencing was carried out using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) at Bethesda, Maryland USA using 3730XL DNA sequencer. The whole experimental process was monitored through LIMS (Laboratory Information Management System). The results of sequencing were analyzed by BLAST web tool of the Genbank (NCBI). http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome. Analysis runs on all releases of Microsoft Windows operating system (BIOEDIT version 4.0.2 software) **(Tamura et al., 2007).** The results of sequencing were analyzed by BLAST web tool of the GenBank (NCBI). Sequence submission to the GenBank was conducted following the instructions offered by the web tool BankIt of GenBank http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank.

5. Statistical Analysis:

Data on morphological parameters were analysed on the computer statistical package SSPS using Chi-square test. Differences expressed as significant at $P < 0.05$ (**Verzani, 2004**.).

Results

1. Diagnosis of *Cryptosporidium* spp.:

1.1. Incidence of *Cryptosporidium spp.* infection in the examined 915 ducks by M.Z.N. stain:

The incidence of *Cryptosporidium* spp. in 915 examined ducks was 39.9 % and 36.4% in bursal and intestinal samples respectively (Table 2). It is worth to mention that the positive intestinal samples were included in the positive bursal samples. Seasonal incidence showed that the highest rate was in winter (74.6%) while the lowest one was in autumn (7.1%) as shown in table 3. Concerning the age, it was recorded that 66.5% of the examined young age (1W-2M) was infected with *Cryptosporidium* spp. while the older one (>2-6 M) showed negative results (Table 4).

Table 2: The incidence of *Cryptosporidium* spp. in 915 examined ducks.

Duck breed	No. of examined	Samples			
		Bursal		Intestinal	
		+ve	%	+ve	%
Muscovy	820	340	41.5	310	37.8
Mallard	70	25	35.7	23	32.9
Native	25	0	0	0	0
Total	915	365	39.9	333	36.4

Table 3: Seasonal incidence of *Cryptosporidium* spp. in ducks:

Season	No. of examined	No. of infected	(%)
Winter	283	211	74.6
Spring	194	96	49.5
Summer	269	46	17.1
Autumn	169	12	7.1
Total	915	365	39.9

Table 4: Incidence of *Cryptosporidium* spp. infection in ducks according to age.

Age of ducks	No. of examined	+ve	%
1 week- 2 M.	549	365	66.5
>2-4 M.	301	0	0
>4-6 M.	65	0	0

1.2. Morphology of the detected *Cryptosporidium* spp.:

Light microscopical examination of M.Z.N. stained smears from the bursal and intestinal contents of ducks showed that *Cryptosporidium* spp. oocysts appeared as acid fast (red-pink) on a green background. The sporulated oocysts were spherical to ovoid in shape with smooth wall. It contains 4 sporozoites and a residium. The measurement of 50 oocysts from M.Z.N. stained smears were 4.5-6.0 µm in length (mean 5.55 µm, S.D. 0.56) and 4.5- 5.0 µm in width (mean 4.8 µm S.D. 0.245).

1.3. Results of examination of 100 Muscovy duck samples by M.Z.N. stain and ELISA:

Examination of the bursal and intestinal contents of each of 100 Muscovy ducks (from 820 Muscovy ducks) by M.Z.N. stain showed that 33% and 28% were infected with *Cryptosporidium* spp. oocysts respectively. While examination of the sera of these 100 Muscovy duck by ELISA revealed that 55% had antibodies against *Cryptosporidium* species. All M.Z.N. stain positive samples were out of the 55% ELISA positive samples (Table 5).

Table 5: Results of examination of 100 Muscovy duck samples by M.Z.N. stain and ELISA.

No. of samples / Test	M.Z.N. stain				ELISA	
	Bursal		Intestinal		Serum	
	+ve	%	+ve	%	+ve	%
100	33	33	28	28	55	55

2. Characterization of *Cryptosporidium* spp. antigens by SDS- PAGE and immunoblotting:

2.1. SDS- PAGE:

Characterization of the three purified *Cryptosporidium* spp. antigens by SDS- PAGE revealed that *Cryptosporidium* spp. of ducks were fractionated into 6 bands at molecular weight (MW) 75, 69, 57-64, 48-53, 22-25 and 18-20 KDa. While *C. baileyi* of chickens were fractionated into 6 bands at MW 75, 69, 57-64, 55-57, 22-25 and 18-20 KDa. In addition to *C. parvum* of calves were fractionated into 5 bands at level of 75, 69, 51-53, 22-25 and 18-20 KDa (Fig.1).

2.2. Immunoblotting:

Nitrocellulose (NC) strips containing fractionated *Cryptosporidium* spp. of duck antigen were treated versus different sera. The data in (Fig. 2) revealed that 12 Protein fraction bands (PFB) corresponding to Molecular weight (MW) 135 ,100, 68, 58-62, 38-42, 30-35, 25, 18-20, 17, 15, 12 and 11 KDa (Lane 1) were demonstrated after treatment of NC strip versus infected duck serum. In the same time treatment of similar strip by serum of *C. baileyi* of infected chickens revealed 7 reacted bands corresponding to MWS >135, 100, 75, 48-62, 35, 30 and 25 KDa (Lane 2) while treatment versus calf infected serum revealed 5 band at MWS 100-135, 75, 48-62, 35 and 32 KDa (Lane 3). In the same time, only 3 bands (MW 32-35, 18, 17 KDa) were recorded in fractionated duck *Cryptosporidium* spp. versus negative duck serum (Lane 4). From the previous reaction, the bands at MW 68, 38-42, 20 and that lower than 15 KDa were considered to be specific for duck strain as they react versus serum of infected duck only. In the same time some PFB at MW 100, 62, 35and 25 KDa were considered to be common between antibodies in the tested sera. In the same time the fractions at MW 20-38 KDa were considered to be specific for chickens as it react versus chicken *C. baileyi* infected serum only (Lane 5 & 6). Bands at MW 34, 25 and 16 KDa were considered to be specific versus infected calf serum after treatment of fractionated calf *C. parvum* (Lane 7 &8).

3. Molecular characterization and sequencing of *Cryptosporidium* spp. isolated from ducks and chickens:

Analysis of the PCR products on agarose gel electrophoresis revealed the positive amplification of the target fragment of *Cryptosporidium* spp. with correct size 435bp for ducks and chickens (Fig. 3). Duck and chicken specimens revealed similarity nearly 99% to *C. meleagridis* and *C. baileyi* according to accession number in gene bank (KM581271 and KM581270) respectively (Table 6).

Table 6: Genotype of *Cryptosporidium* by using basic local alignment:

sample	Accession No.	Isolation source	Species
1	KM581270 http://www.ncbi.nlm.nih.gov/nuccore/KM581270	Bursal content	Chicken
2	KM581271 http://www.ncbi.nlm.nih.gov/nuccore/KM581271	Bursal content	Duck

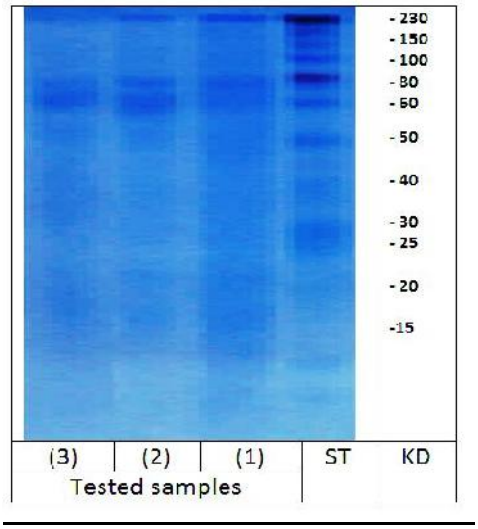

Fig.1: SDS-PAGE demonstrated different protein fractions in *Cryptosporidium* spp. purified oocyst:
ST: standard (1): *Cryptosporidium* spp. of ducks (2): *C. baileyi* of chickens (3): *C. parvum* of calves.

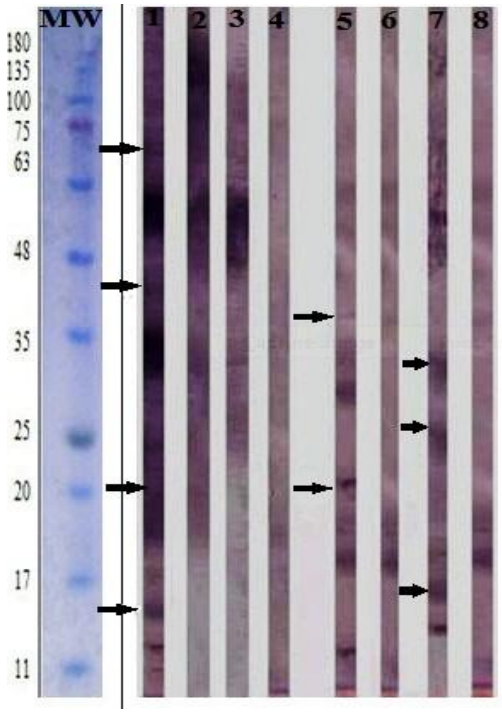

Fig. 2: ***Cryptosporidium*** **spp. fractionated antigens reacted versus positive and negative sera using immunoblotting.**

Duck Ag	**Chicken Ag**	**Calf Ag**
Lane 1: duck Ag versus + Ve duck serum Lane 2: duck Ag versus + Ve Chicken serum Lane 3: duck Ag versus + Ve Calf serum Lane 4: duck Ag versus – Ve duck serum	Lane 5: chicken Ag versus + Ve Chicken serum. Lane 6: Chicken Ag versus – Ve Chicken serum.	Lane 7: Calf Ag versus + Ve Calf serum. Lane 8: Calf Ag versus – Ve Calf serum.

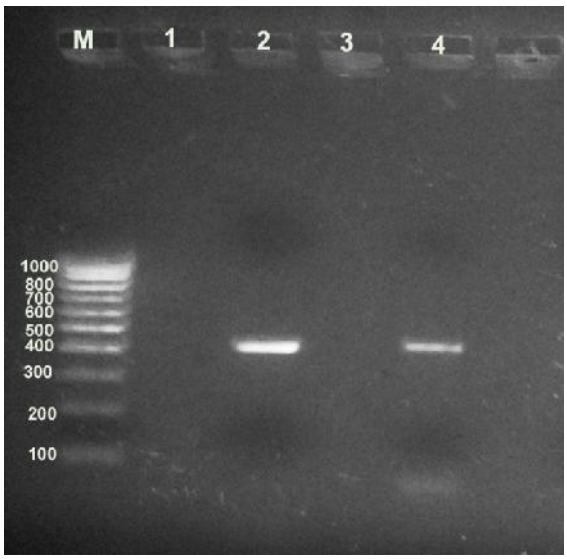

Fig.3: Agarose gel stained with Ethidium bromide showing the PCR amplification products of ***Cryptosporidium*** **spp.**

Lane M: 100bp DNA size marker; Lane 2: Positive PCR signal for *Cryptosporidium* spp. from chickens (435bp). Lane 4: Positive PCR signal for *Cryptosporidium* spp. from ducks (435bp).

Discussion

Cryptosporidiosis was reported in a variety of mammalian species including human as well as avian species as turkeys, chickens, ducks, geese and quails **(Fayer** and **Ungar, 1986)**. The present study showed that examination of each of 915 duck samples by using M.Z.N. stain technique revealed that 39.9% was *Cryptosporidium* spp. positive. This result was similar to that reported by **Mousa, 2000** (40%) in Egypt. On the other hand, number of authors showed dissimilar results where 26.8%, 24.2%, 16%, 0% and 13.8% was recorded by **Abu-siry (1993), El- Madawy (2001), Abdel-Wanis et al. (1996), Abo-Laila et al. (2011)** and **Nagwa et al. (2013)** at different localities in Egypt respectively. Also, our results disagreed with that reported on other countries by **Wang and Leiw, 1990** (66%) in Taiwan, **Richter et al., 1994** (57%) in Frankfurter, **Kuhn et al. (2002)** in Southern New Mexico (49%), **Majewska et al., 2009** in Malaysia (33.3%) and **Wang et al., 2010** (16.3%) in China. The difference between our results and those authors might be attributed to the variation of the number and breeds of examined ducks. Also, might be due to difference in the investigated localities, type of samples, methods of diagnosis, hygienic measures, bird management and environmental conditions. Seasonal incidence showed the highest incidence was in winter (74.6%) while the lowest one was in autumn (7.1%). Our results agreed with **Nagwa et al. (2013)** in Egypt where the incidence was 44.2% and 2.4% in winter and autumn respectively. This result disagreed with **Mousa, 2000** in Egypt and **Mohammed (2009)** in Iraq whom reported high incidence in summer and spring (15.3% and 84%respectively). While the lowest one was 6.1% and 68% in winter and summer respectively. In relation to age, the rate of *Cryptosporidium* infection in the present work was 66.5% in young ages (1W-2M). Other authors as **Mousa (2000)** in Egypt (1-6 weeks), **Richter et al. (1994)** in Germany (1-5 weeks) and **Wang et al. (2010)** in China (11-30 day) recorded 30.7%, 57.03% and 40.3% respectively.

The morphological parameters of 50 *Cryptosporidium* spp. oocysts by M.Z.N. stain were spherical to ovoid in shape with smooth wall. It contains 4 sporozoites and a residium. Its measures varied from 4.5-6.0 x 4.5-5.0 µm. Previous authors not give definite identification morphometrically to the species infecting ducks **Mousa (2000)**, **El- Madawy (2001)** and **Mohammed (2009)** where they mentioned that ducks were infected by *Cryptosporidium* species. The measurements by light microscope not give accurate identification for *Cryptosporidium* spp. infecting ducks but the identification of the detected species will be confirmed by molecular identification.

Examination of the bursal and intestinal contents of each of 100 Muscovy ducks (from 820 Muscovy ducks) by M.Z.N. stain showed that 33% and 28% were infected with *Cryptosporidium* spp. oocysts respectively. While examination of the sera of these 100 Muscovy ducks by ELISA revealed that 55% had antibodies against *Cryptosporidium* species. All M.Z.N. stain positive samples were out of the 55% ELISA positive samples. The incidence of *Cryptosporidium* by ELISA was higher than that recorded by M.Z.N. stain. This might be due to the ducks were chronically infected so the oocysts not detected due to their low numbers in the bursal and intestinal contents but the immune system was stimulated to release *Cryptosporidium* antibodies.

Identification of the specific protein fractions using SDS and immunoblotting revealed that the bands at the MW 68, 38-42, 20 and that lower than 15 KDa considered to be specific for duck strain as they react versus serum of infected duck only. In the same time, the fraction at MW between 20-38 KDa in the fractionated chicken antigen considered specific versus *C. baileyi* infected chicken serum only. While that at MW 34, 25 and 16 KDa in the fractionated calf antigen considered specific versus *C. parvum* infected calf serum. In the same time protein fractions corresponding to MW 100, 62, 35and 25 KDa appear as common bands of antibodies in the different tested sera. The specific fractions of *C. baileyi* of chickens recorded in the present study (20-38 KDa) were occurred in the same range previously recorded by **Ditrich et al., 1993** (15.5 to 33 KDa). Immunoblotting of *C. parvum* in the present study showed fractions at MW 34, 25 and 16 KDa. On the other hand, fractions at MW 17-69 KDa and 105-300 KDa were detected by **Lorenzo et al. (1995)** and **Kramer et al. (2007)** respectively. The difference in the number of bands with this author may be related to type of used sera. The common bands 100, 62, 35and 25 KDa were considered as cross-reacted band between duck, chicken and calf sera. The difference in bands might be due to difference in genotypes, and level of anti-bodies in the tested sera.

In the present work analysis of the PCR products on agarose gel electrophoresis revealed the positive amplification of the target fragment of *Cryptosporidium* with correct size 435bp for ducks and chickens. Also, Duck and chicken specimens resulted nearly 99% homologus to *C. meleagridis* and

C. baileyi according to accession number in gene bank (KM581271 and KM581270) respectively. **Champliaud et al. (1998)** identified the same base pair (435) of *Cryptosporidium spp.* in quail and chickens. While **Wang et al. (2010)** identified *C. baileyi* in chicken and ducks by PCR and **Bomfim et al. (2013)** reported that the *Cryptosporidium* sequence from duck shared high similarity (99%) with GenBank sequences from *C. baileyi* obtained from birds (GU816042, GU816039, GU816040, GU816041, and GU816043). *C. parvum* species isolated from chicks showed 99% sequence similarity with *C. parvum* sequences from bovine origin (HQ009805 and EF175936). **Wang et al. (2014)** in China identified 3 genotypes from broiler chickens (*C. baileyi*, *C. meleagridis*, and avian genotype II) by PCR and sequencing. In addition, **Jasim and Marhoon (2015)** recorded *C. baileyi* in broiler chickens and ducks with 18SrRNA by PCR.

From the current results, it was concluded that the *Cryptosporidium* spp. infecting ducks according to morphometric and molecular identification might be *C. meleagridis*.

References

Abdel-Wanis, N.A., Amin, A.H., Hassan, N.M. and Ahmed, N.E. (1996): Studies on the effect of *Cryptosporidium* on the immune response to duck virus hepatitis vaccine. Assiut. Vet. Med. J. 34 (68): 138-146.

AbouLaila, M., El-Bahy, N., Hilali, M., Yokoyama, N. and Igarashi, I. (2011): Prevalence of the enteric parasites of ducks from Behera governorate, Egypt. J. Protozool. Res. 21: 36-44.

Abu-Siry, S.M.G. (1993): Some studies on protozoal diseases in waterfowls. M.D. Thesis, Poultry Diseases. Fac. Vet. Med., Zagazig Univ.

Adejinmi, J.O. and Oke, M. (2011): Gastro-intestinal parasites of domestic duck (*Anas platyrhynchos*) in Ibadan southwestern Nigeria. Asian J. Poult. Sci. 38: 125-134.

Bomfim, T.C., Gomes, R.S., Huber, F. and Couto, M.C. (2013): The importance of poultry in environmental dissemination of *Cryptosporidium* spp. J. Vet. Sci. 7: 12-17.

Catty, D. and Raykundalia, C. (1989): ELISA and related enzyme immuno assays. In D. Catty (Ed). Antibodies, a practical approach, Vol.II. IRL Press, Oxford. PP. 97-152.

Champliaud, D, Gobet, P., Naciri, M., Vanger, O., Lopez, J., Buisson, J.C., Varga, I., Harly, G., Mancassola, R. and Bonnini, A. (1998): Failure to differentiate *C.parvum* from *C. meleagridis* based on PCR amplification of eight DNA sequences. App. Enviro. Microbiol. Apr. 64(4):1454–1458.

Chappell, C.L., Okhuysen, P.C., Langer, R.C., Akiyoshi, D.E., Widmer, G. and Tzipori, S. (2011): *C.meleagridis* infectivity in healthy adults' volunteers. Am. J. Trop. Med. Hyg. 85: 238-242.

Current, W.L., Upton, S.J. and Haynes, T.B. (1986): The life cycle of *C. baileyi* n. spp. (Apicomplexa, Cryptosporidiidae) infecting chickens. J. Protozool. 33: 289-296.

Ditrich, O., Kopacek, P. and Kucerova, Z. (1993): Antigenic characterization of human isolates of Cryptosporidia. J. Folia Parasitol. 40 (4): 301-305.

Dubey, J.P., Speer, C.A. and Fayer, R. (1990): Cryptosporidiosis of man and animals. pp. 31-49. 2nd ed. CRC Press Boca Raton, Florida.

El-Madawy, R.S. (2001): Studies on some protozoan parasites in birds. M.V.Sc. Thesis (Parasitology), Fac. Vet. Med., Zagazig Univ**.**

Fayer, R. and Ungar, B.L.P. (1986): *Cryptosporidium* species and Cryptosporidiosis. Microbiol. Review. 50: 458-583.

Graczyk, T.K., Cranfield, M.R., Fayer, R. and Anderson, M.S. (1996): Viability and infectivity of *C. parvum* oocysts are retained upon intestinal passage through a refractory avian host. J. Appl. Environ. Microbiol. 62(9): 3234-3237.

Harrison, C. and Greensmith, A. (1993): Birds of the World. Dorling Kindersly, London, UK.

Henriksen, S. A. and Pohlenz, J. F. (1981): Staining of *Cryptosporidium* by a modified Ziehl-Neelsen technique. Act. Vet. Scand. 22: 594-596.

Jasim, A.G. and Marhoon, A.I. (2015): Prevalence and molecular analysis of *Cryptosporidium* spp. isolated from wild and domestic birds. Acta Parasitologica Globalis 6 (2): 65-70.

Johnson, D. W., Pieniazek, N. J., Griffin, D. W., Misener, I. and Rose, J. B. (1995): Development of a PCR protocol for sensitive detection of *Cryptosporidium* oocysts in water samples. Appl. Environ. Microbiol. 61**:**3849-3855.

Khalil, F. A. M. (1993): Studies on *Cryptosporidium* in calves. M.V.Sc. thesis (Parasitology), Fac. Vet. Med., Cairo Univ.

Kramer, F. M., Vesey, G., Natasha Look, L. N., Herbert, R. B., Joyce, M. S. and Lim, V. D. (2007): Development of a *Cryptosporidium* oocyst assay using an automated fiber optic-based biosensor. J. Bio. Engin. 1(3): 21.

Kuhn, R.C., Rock, C.M. and Oshima, K.H. (2002): Occurrence of *Cryptosporidium* and Giardia in wild ducks along the Rio Grande River valley in

southern New Mexico. App. Envirro. Microbiol. 68: 161-165.

Laemmli, U. K. (1970): Cleavage of structural protein during the assembly of the head bacteriophage T_4. Nature, 227: 680-685.

Lorenzo, M. J., Ben, B., Mendez, F., Villacorta, I. and Ares-Mazas, M. E. (1995): *Cryptosporidium parvum* oocyst antigens recognized by sera from infected asymptomatic adult cattle. J. Parasitol. Nov. 60 (1-2): 17-25.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and RandalL, R. J. (1951): Protein measurements with the folin phenol reagent. J. Bio. Chem. 193: 265-275.

Majewska, A.C., Graczyk,T.K., Slodkowicz-Kowalska, A., Tamang, L., Jedrzejewski, S., Zduniak, P., Solarczyk, P., Nowosad, A. and Nowosad, P. (2009): The role of free-ranging, captive and domestic birds of Western Poland in environmental contamination with *C. parvum* oocysts and *Giardia lamblia* cysts. J. Parasitol. Res. 104: 1093-1099.

Mohammed, N.H. (2009): Detection of *Cryptosporidium spp*. in feces of ducks in Nineveh governorate. J. of Iraq Vet. Sci. 23 (1): 1-5.

Mousa, A. A. S. (2000): Studies on cryptosporidiosis in ducks in Kafer El-Sheikh province. M.D. Thesis, Poultry Diseases. Fac. Vet. Med., Cairo Univ.

Nagwa, E.A., El-Akabawy, L.M., El-Madawy, R.S. and Toulan, E.I. (2013): Studies on intestinal protozoa of poultry in Gharbia governorate. Benha Vet. Med. J. 25(2): 78-83.

Richter, B., Wiegand-Tripp, G., Burkhardt, E. and Kaltea, E.F. (1994): Natural infection by *Cryptosporidium* in farm raised ducks and geese. Avian Path. 23: 277-286.

Ryan, U.M. (2010): *Cryptosporidium* in birds, fish and amphibians. J. Exper. Parasitol. 124: 113-120.

Ryan, U.M., Xiao, L., Read, C., Sulaiman, I.M., Monis, P. and Lal, A.A. (2003): A redescription of *C. galli (*Pavlasek, 1999) (Apicomplexa: Cryptosporidiidae) from birds. J. Parasitol. 89: 809-813.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989): Molecular cloning: A laboratory Manual, 2nd ed. Cold Spring Harbor laboratory, Cold Spring Harbor, NY.

Slavin, D. (1955):*C. meleagridis* (sp. nov.). J. Comp. Pathol. 65: 262-266.

Towbin, H., Stachelin, T. and Gordon, J. (1979): Electrophoresis transfer of proteins from polyacrylamide gels to nitrocellulose sheets, procedures and some applications. U.S.A. Proc. Nati. Acd. Sci. J. 76: 4350-4354.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. J. Mol. Biol. and E. 24: 1596-1599.

Van der Meulen, S.J. and Den Dikken, G. (2004): Duck keeping in the tropics. In Agrodok 33, Overgaag A. (Ed.). Agromisa Foundation, Wageningen, the Netherlands.

Verzani, J. (2004): Introductory Statistics. Chapman and Hall/CRC Publishing, pp: 114.

Wang, J.S., and Leiw, C.T. (1990): Prevalence of *Cryptosporidium spp.* in birds in Taiwan. Taiwan J. Vet. Med. and An. Husbandry 56: 45- 57.

Wang, Y., Feng, Y., Cui, B., Jian, F., Ning, C. and Wang, R. (2010): Cervine genotype is the major *Cryptosporidium* genotype in sheep in China. Parasitol. Res. 106: 341-347.

Wang, L., Xue, X., Li, J., Zhou, Q., Yu, Y. and Du, A. (2014): Cryptosporidiosis in broiler chickens in Zhejiang Province, China: Molecular characterization of oocysts detected in fecal samples. Parasite. 21: 36.

Xiao, L. (2010). Molecular epidemiology of cryptosporidiosis: an update. Exp. Parasitol. 124: 80-89.

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