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Original Article

A Preliminary Survey on *Eimeria* species infecting Chicken in Gharbia Governorate, Mid-Delta of Egypt

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Abstract:

The present study conducted to investigate prevalence of *Eimeria* species in chickens in Gharbia governorate, Mid-Delta, Egypt. From a total of 800 samples examined, 614 (76.75%) were positive for *Eimeria* oocysts. According to chicken breeds, white broiler was the most infected 371/614 (46.37%), then Balady chickens 124/614 (15.5%), then Saso chickens 86/614 (10.75%) and lastly layers 33/614 (4.12%). Regarding age, the most affected ages was in-between 31-40 days old 255/614 (31.83%), followed by 21-30 days old 90/614 (11.24%). All districts were found infected with one or more of *Eimeria* species, the most affected districts were El-Mahalla El-Kubra and Kotoor 95/614 (11. 875% for each district), while El-Santa was the lowest 29/614 (3.625%). Seven species of *Eimeria* viz., *E. tenella*, *E. acervulina, E. mitis, E. praecox, E. necatrix, E. burnetti* and *E. maxima* were recorded with prevalence of 14.5, 13.875, 13.375, 11.25, 9.5, 8.5 and 5.75% respectively. *Eimeria tenella* was found the most predominant species in the study area. This study highlight the high incidence of *Eimeria* species infecting chicken in Egypt and tell new and updated results that might help in controlling such infection.

Key words: *Eimeria*, Chicken, Coccidia, Prevalence, Egypt

INTRODUCTION

Chicken coccidiosis is one of the most important protozoan diseases worldwide. It is responsible for much economic losses in the poultry industry. It caused by infection with one or more of the *Eimeria* species infecting chickens **(McDougald and Reid, 1997; McDouglad, 2003)**. The protozoan parasites of the genus *Eimeria* species multiply in the intestinal tract and cause tissue damage, with resulting interruption of feeding and digestive processes or nutrient absorption, dehydration, blood loss, loss of skin pigmentation and increased susceptibility to other disease agents **(Williams, 1996)**. Nine species of *Eimeria* may infect chickens. The species important in broiler production include *Eimeria tenella*, *E. maxima, E. acervulina*, and *E. mivati*; the species important in breeder and egg- layers are *E. brunetti* and *E. necatrix*. *Eimeria tenella* and *E. necatrix* are the most pathogenic species. *E. acervulina, E. maxima* and *E. mivati* are common and slightly or moderately pathogenic; *E. brunetti* is uncommon but pathogenic when it does occur. *E. mitis*, *E. praecox* and *E. hagani* are relatively non-pathogenic species **(Reid and Long, 1979; Conway and McKenzie, 1991)**. Coccidiosis is one of the most important diseases in commercial poultry production in Egypt. But, there is scanty and controversy information on the occurrence of the different *Eimeria* species in spite of many research papers carried on coccidiosis in Egypt, as **Khilfa, (1982), Ahmed et al. (2003), Abu-Akkada and Awad, (2010), Al-Gawad et al. (2012) and Gadelhaq et al. (2015)**, they all study the coccidiosis in different Governorates of Egypt and Coccidia still cause severe economic losses in poultry industry. So, the present study aims are detection and estimation of different *Eimeria* species infection of different breeds and ages of chicken in Gharbiah governorate, to give new and updated information which may help in control of such infection.

MATERIALS AND METHODS

Study area and design: Gharbia governorate is one of the Mid-Delta governorates, north to Cairo (30.881°N 31.06° E). A cross-sectional survey designed including the 8 districts of the governorate (i.e. El-Mahalla El-Kubra, Kafr El-Zyat, Samannoud, Tanta, Zifta, El-Santa, Kotoor and Bassyoun) to find the prevalence of different *Eimeria* species involved in chicken coccidiosis and factors associated with this infection.

Samples: From August 2013 till the end of December 2014 a total of 800 fecal samples (100 samples from each district) were collected from broilers and layers chickens of

different breeds, ages and management practices. Each sample labeled individually; preserved after collection in separate clean polyethylene bags then in plastic containers, kept in refrigerator at 4°C with 2.5% Potassium dichromate and transferred to the lab of Department of Parasitology, Faculty of Veterinary Medicine, Kafrelsheikh University for further examination.

Laboratory examination:

Samples processed for morphological examination by concentration floatation technique. The processed samples were examined with calibrated light microscope fitted with scale-measure ocular lens according to **Conway and McKenzie (2007)** to determine the oocysts shape and index (Length/Width). *Eimeria*-positive samples were further processed by McMaster-chamber method according to **Conway and McKenzie (1991)** to determine the *Eimeria* oocyst count. Also, *Eimeria*-positive samples were sporulated in 2.5% potassium dichromate according to **Davies et al. (1963) and Wash et al. (1985)**. After sporulation, oocyst count and checking of the morphology, shape index were determined again to identify the *Eimeria* species. Oocysts of different *Eimeria* spp. images were captured using digital camera, scale bar was determined using method of **Otify (2012)** and software ImageJ 1.49v.

Statistical analysis: The statistical analysis was done by using the statistical package Microsoft Excel software. Data were compiled and represented in tables.

RESULTS

Out of a total 800 examined samples; 614 (76.75%) were positive for *Eimeria* oocysts. According to chicken breeds, white broiler was the most infected 371/614 (46.37%), then Balady chickens 124/614 (15.5%), then Saso chickens 86/614 (10.75%) and lastly layers 33/614 (4.12%). Regarding age (Table 1), the most affected ages was in-between 31-40 days old 255/614 (31.83%), followed by 21-30 days old 90/614 (11.24%). All districts were found to be infected with one or more of *Eimeria* species (Table 2), the most affected districts were El-Mahalla El-Kubra and Kotoor 95/614 (11. 875% for each district), while El-Santa was the lowest 29/614 (3.625%). Seven species of *Eimeria*

Table 1: Prevalence of different *Eimeria* infection detected among different ages of examined chicken

Age range	Positive samples		Negative samples		Total	
(day)	Number	% from total	Number	% from total	Number	% from total
10 – 20	40	5	10	1.25	50	6.25
21 – 30	90	11.24	20	2.50	110	13.75
31 - 40	255	31.83	44	5.50	299	37.38
41 – 50	80	10	11	1.39	91	11.38
51 – 60	28	3.44	20	2.50	48	6
61 – 70	25	3.21	20	2.50	45	5.62
71 – 80	25	3.21	17	2.12	42	5.25
81 – 90	50	6.20	20	2.50	70	8.75
>90	21	2.61	24	3	45	5.62
Total	614	76.74	186	23.26	800	100

$Chi^2 = 33.45$, Significant at $p < 0.01$

Table 2: Prevalence of *Eimeria* detected in different districts in Gharbia Governorate, Egypt

District	Positive	% from total	Negative	% from total
El-Santa	29	3.625	71	8.875
Kafr El-Zyat	93	11.625	7	0.875
Kotoor	95	11.875	5	0.625
Tanta	81	10.125	19	2.375
Bassyoun	96	12	4	0.5
Zifta	49	6.125	51	6.375
El-Mahalla El-Kubra	95	11.875	5	0.625
Samannoud	76	9.5	24	3
Total	614	76.75	186	23.25

Table 3: Prevalence, oocyst length, oocyst width and shape index of different *Eimeria* species detected from examined samples

Eimeria species	Positive	% from positive	% from total	Oocyst Length in µm	Oocyst Width in µm	Shape Index (L/W)
				Mean ± SE	Mean ± SE	Mean ± SE
E. preacox	90	14.65	11.25	22.35±0.38	17.39±0.30	1.29±0.00
E. tenella	116	18.89	14.5	19.93±0.28	17.01±0.19	1.17±0.01
E. maxima	46	7.49	5.75	22.74±0.72	15.59±0.53	1.46±0.01
E. brunetti	68	11.07	8.5	22.73±0.55	16.99±0.43	1.34±0.00
E. necatrix	76	12.37	9.5	20.45±0.57	18.25±0.49	1.12±0.00
E. mitis	107	17.42	13.375	17.46±0.41	17.03±0.34	1.02±0.01
E. acervulina	111	18.07	13.875	22.71±0.49	18.47±0.39	1.23±0.00

SE= Standard Error, significantly different at P < 0.01

Table 4: Prevalence of different *Eimeria* species detected in examined chickens of each district of Gharbia Governorate, Egypt

	E. preacox	*E. tenella*	*E. maxima*	*E. brunetti*	*E. necatrix*	*E. mitis*	*E. acervulina*	Total
El-Santa	2	7	0	3	5	6	6	29
Kafr El-Zyat	13	7	10	9	16	22	16	93
Kotoor	18	24	5	16	12	11	9	95
Tanta	12	6	5	8	15	24	11	81
Bassyoun	18	19	10	7	9	17	16	96
Zifta	5	13	3	8	4	4	12	49
El-Mahalla El-Kubra	8	20	11	5	10	16	25	95
Samannoud	14	20	2	12	5	7	16	76
Total	90	116	46	68	76	107	111	614

viz., *E. tenella, E. acervulina, E. mitis, E. praecox, E. necatrix, E. burnetti* and *E. maxima* recorded with prevalence of 14.5, 13.875, 13.375, 11.25, 9.5, 8.5 and 5.75% respectively (Figure 1 and Table 3). *Eimeria tenella* was the most predominant species in the study area. The length, width and shape index (length/ width) for each *Eimeria* species oocysts was also recorded (Table 3). Each district has more the one *Eimeria* species (Table 4).

DISCUSSION

Chicken coccidiosis is one the most important economic diseases in Egypt and worldwide. In the current study, the incidence of *Eimeria*-infection was 72.75% which go in line with results of **Khilfa (1982)** who mentioned that the incidence of coccidiosis among chicken in Egypt with different ages were 84.24% and **Abu-Akkada and Awad (2010)** result that detected the infection rate as 20- 100% and mortality is 20-60% with severe reduction in body gain and feed efficiency. But in the other hand, the results of the current work disagree with those of **Ahmed et al., (2003)** who reported that the prevalence was 43.9% in Egypt, **Al-Gawad et al. (2012)** who examined balady breed chicken in (Cairo, Giza, El-Gharbia, El-Behiera, Kafr El-Sheikh, El-Sharqiyah, Ismailia, Qina and Aswan) the incidence was 21.24% and recorded Cairo and Giza regions showed highest incidence of infection (37.16%) followed by Eastern delta regions (36.30%). While, the lowest incidence was recorded Western delta regions (7.32%), also our results was higher than that obtained by **El-Gaos (2014)** who stated that the incidence of coccidiosis in broiler chickens in Dakahlia governorate was 45%. In comparing our results with those in other areas of the world, our results on coccidiosis incidence agrees with **Gari et al. (2008)**, But, our results are higher than reported before by **Lundén et al. (2000)** recorded that 19.3%incidence in layer farms in Sweden at the age ranged between 19-32 weeks and 25.8% . **Ashenafi et al. (2004)** indicated that 25.8% of chicken were infected with *Eimeria* spp. in Ethiopia and **Naphade (2013)** in India the incidence of coccidiosis was 9.18%, in broiler chicken. The variation in-between the incidence of *Eimeria* in the current study and other researcher's studies is understandable. Generally speaking, the incidence and

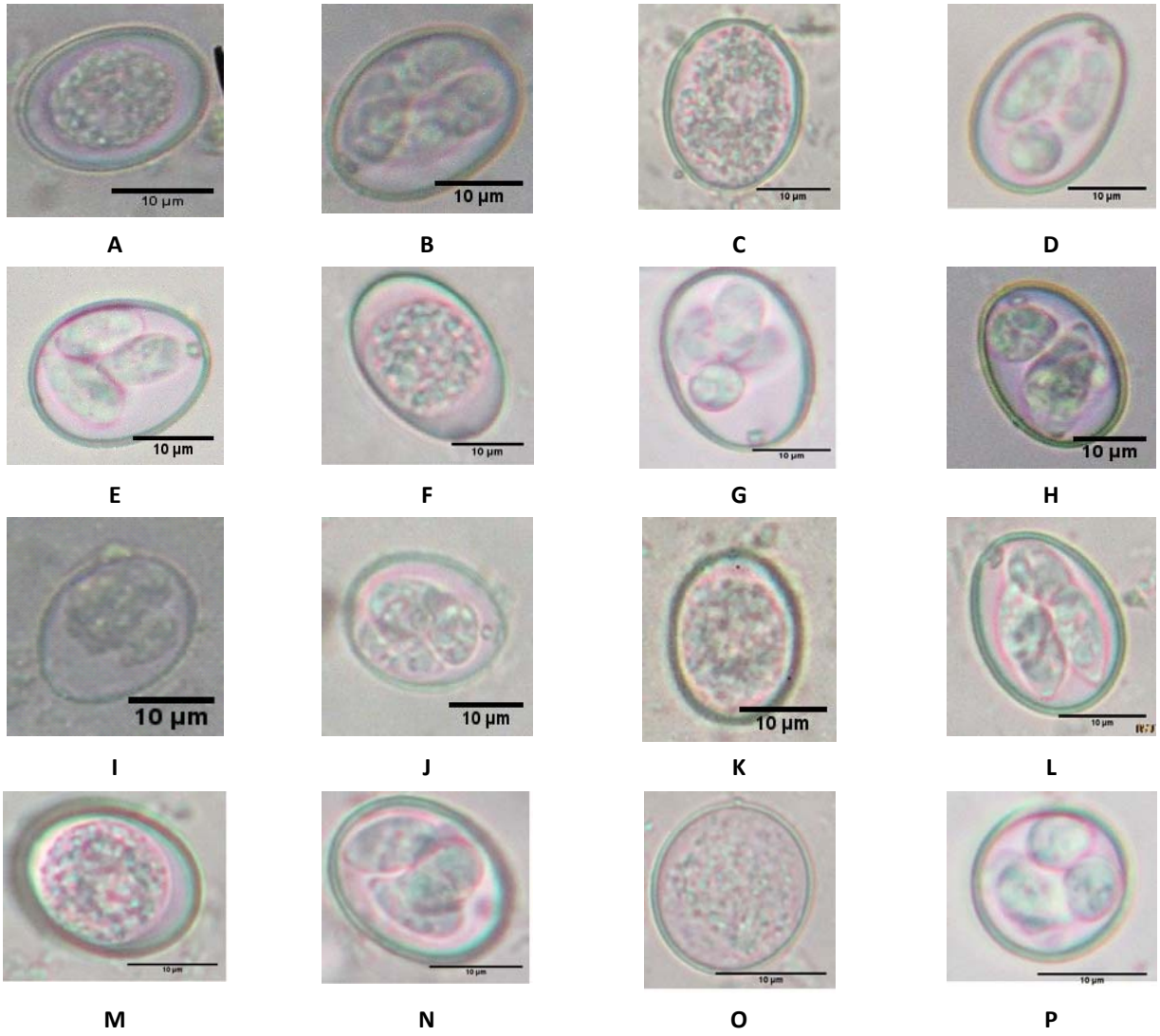

Figure 1: Oocysts of different *Eimeria* spp. isolated from chickens in the current study: *E. necatrix* (A: Non-sporulated, B: Sporulated); *E. maxima* (C: Non-sporulated, D& E: Sporulated); *E. acervulina* (F: Non-sporulated, G& H: Sporulated); *E. brunette* (I: Non-Sporulated, J: Sporulated); *E. tenella* (K: Non-sporulated, L: Sporulated); *E. praecox* (M: Non-sporulated, N: Sporulated) and *E. mitis* (O: Non-sporulated, P: Sporulated).

prevalence of coccidiosis or *Eimeria*-infection is much variant from geographical locality to another and within the same country due to different factors such as, chicken breed, age at sampling, mangemental ways, season, other disease conditions..etc; all these factors and more may affect directly or indirectly to the detected incidence of *Eimeria.* In the present study seven *Eimeria* species were identified and their incidence was recorded as follows: *E. tenella* (18.90%), *E. acervulina* (18.07%), *E. necatrix* (12.38%), *E. maxima* (7.49%), *E. mitis* (17.42%), *E. brunette* (11.07%) and *E. praecox* (14.66%). So, the highest incidence was recorded in infection with *E.tenella* (18.9%), while the lowest was *E.maxima* (7.49%). These results go in line with the results of other researchers from Egypt as **Abu Elezz (1994)** who stated that *E. tenella* was the most prevalent species in balady chickens; **Abu-Akkada and Awad (2010)** they recorded *E. tenella* as the most detected species in Egypt with incidence ranged in-between 20-100% and more recent work of **Gadelhaq et al. (2015)** mentioned that the same 7 *Eimeria* recorded in baldi chickens in Beni Suef province, Upper Egypt. But our results on number and incidence of *Eimeria* species infecting chickens disagree with the results of researchers in Egypt like **Khilfa (1982)** who stated the incidence of different species was as follow: *E. necatrix* 30-90%, *E. tenella* 20-100%, *E. acervulina* 10-80%, *E. mitis* 10-40%, *E. preacox* 5-10%, *E. hagani* 5%, and *E. maxima* 4-10%; **Ahmed et al. (2003)** and **El Behairy (2005)** as they reported absence of *E. brunetti* and *E. praecox* among the examined balady chicks; **Kutkat et al. (2009)** detected

only six *Eimeria* species from four Egyptian Governorates (Qalubeia, Sharkeia, Fayoum and Giza) which were *E. necatrix, E. acervulina, E. praecox, E. maxima, E. mitis* and *E. tenella* and **Al-Gawad et al. (2012)** examined balady breed chicken in (Cairo, Giza, El-Gharbia, El-Behiera, Kafr El- Sheikh, El- Sharqiyah, Ismailia, Qina and Aswan) and recorded *E. necatrix* 58.27% *E. tenella* 25.82*%, E. acervulina* 19.20*% E. mitis* 10.59% and *E. maxima* 4.66%; Also, in comparison of our results on number and incidence *Eimeria* species infecting chickens disagree with other researchers in other parts of the world like **Edgar and Seibold (1964)** in Alabama State, USA, found that all flocks had been infected with *E. acervulina* 100%, *E. tenella* 94%, *E. necatrix* 65% and *E. brunetti* 12*%*, **Williams (1996)** stated six species occurring together. *E. acervulina* 100*%, E. mitis* 82*%, E. tenella* 77*%, E. maxima* 73*%, E. preacox* 45% and *E. brunetti* 27% in commercially-reared chickens in France*;* **Ashenafi et al. (2004)** the recorded *Eimeria* species were E*. necatrix, E. acervulina, E. maxima* and *E. tenella* in scavenging indigenous chickens in central Ethiopia; **Lobago et al. (2005)** found prevalent species were *E. brunetti* 45.3%, *E. tenella* 40%, *E. acervulina* 9.7%, *E. necatrix* 4.1% in Kombolcha poultry farms, Ethiopia; **Haug et al (2008)** agreed with our results in recording the presence of *E. preacox* in 9.8% of broiler chicken in Norway; **Lee et al. (2010)** who determined that 7 *Eimeria* species were detected in all the positive farms by PCR. *E. acervulina* 87% and *E. tenella* 62.5% were the most prevalent, followed by *E. brunette* 59.3% and *E. praecox* 37.5 on Korean chicken farms; **Jatau et al. (2012)** found 7 *Eimeria* species were identified as *E. maxima* 58.6%, *E. acervulina* 47.1%, *E. mitis* 30.0%, *E. brunetti* 28.6% E. *tenella* 22.9% *E. preacox* 8.6% in Zaria, Nigeria. While **Thenmozhi et al. (2014)** used COCCIMORPH identification software and revealed the presence of *E. acervulina* (79.49%)*, E. tenella* (72.88%), *E. mitis* (50.62%), *E. maxima* (35.52%) *and E. necatrix* (10.83%) in the farms screened. *E. brunette* was not recorded in any of the farms screened in Chennai, Tamil Nadu, India. Again, the variation in-between the number and incidence of *Eimeria* species detected in the current study and other researchers studies is understandable. It can be attributed to several factors such as the overlapping in-between the dimensions and shape of different *Eimeria* species oocysts; in fact the previously mentioned cause is the main reason that may explain the differences in-between the researchers. Other factors may include the skills of the examiner, using different techniques in identification (e.g. COCCIMORPH and PCR), age at sampling, sampling procedures, chicken breed, poor poultry management where there is overcrowding, leaking water troughs and accumulation of feces are factors that contributed to the high prevalence rate. About morphology and dimension of different *Eimeria* spp. oocysts, our present study indicated that length, width and shape index of *Eimeria* oocysts as follow: *E. preacox* was 22.35-17.39 µm - 1.29, *E. tenella* 19.93-17.01 µm -1.17, *E. maxima* 22.47-15.95 µm -1.46, *E. brunetti* 22.73-16.99 µm -1.34, *E. necatrix* 20.45-18.25 µm -1.12, *E. mitis* 17.46-17.03 µm -1.02 and *E. acervulina* 22.71-18.47 µm -1.23.These results agree with that of in Egypt **Nowar (2007)** recorded *E. acervulina* 20-16 µm, *E. necatrix* 20-18 µm , and *E. mitis* 16-13 µm. Also, these results nearly go in line with **Amer et al. (2010)** who calculated the index of 4 isolate species as *E. tenella* 1.14, *E. necatrix* 1.19, *E. acervulina* 1.25 and *E. preacox* 1.23. Our results also detect the *E. necatrix* length as 20.45 µm and width as 18.25 µm and index as 1.12 which go in line with the results of **Jadhav (2011)** Who detect *E. necatrix* oocysts in broiler chicken in India length as 13.2-22.5 µm (16.2) width 11-18.7 µm (15.7) and index 102-1.4(1.3); **Al-Gawad et al. (2012)** from Egypt detect 5 Eimeria species in balady chicken which agree with our oocysts identification as *E. mitis* 17.8-14.1-1.26, *E. acervulina* 18.2-14.1-1.29, *E. necatrix* 20.1-16.9-1.19, *E. tenella* 21.3-17.9-1.19 and *E. maxima* 29.9-23.8-1.25. The incidence between different breed in Gharbia governorate, was in White broiler 46.37%, Saso broiler 10.75%, Balady broiler 15.5% and Layer 4.12%. The incidence of *Eimeria* species infection detected in the current study in-between different chicken ages in Gharbia governorate was the highest in 30-40 days (31.83%), 21-30 days (11.24%), 41-50 days (10%) and the lowest incidence in age over 90 days (2.61%). These results agree with that of **Nawar (2007)** who examined broiler chicken only in Dakahlia governorate found the highest infection was in age of 25 days 40%, followed by 35 days 29.21% and 15 day 20.8%; **Al-Gawad et al. (2012)** found balady breed chicken in Egypt and find all examined samples of age less than 21 days was completely free. Also these results nearly go in line with that of **El-Gaos (2014)** who studied that broiler chickens in Dakahlia governorate and find the age incidence were 25.6% for 1-14 days, 65.6% for 15-28 days and 43.7% for 29-42 days .In other parts of the world, our

results are in line with the results of **Lunden et al. (2000)** found the incidence of coccidiosis in layer farms in Sweden at the age of 19-32 weeks was 19.3%. But these results disagree with that of **Jatau et al. (2012)** in Nigeria; they found *Eimeria* infection as 44.3% in layer, 37.1% in broiler and 18.6%in indigenous chicken; **Olanrewaju and Agbor (2014)** detected the infection rate among Broiler birds less than 3 months was 60%, layers 66.67%, cockerel 70% and local birds 67%. In birds of 3 months and above, cockerel has the highest prevalence rate of 100%, followed by broiler and layer which have the prevalent rate of 80%. However, local birds have the lowest prevalent rate (50%) in Nigeria. In conclusion, the present study demonstrate high incidence of *Eimeria* spp. infection in mid-Delta of Egypt and provide updated information on such infection which might help in control of it. Further investigations are encouraged.

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Conflict of interest

The authors declare that they have no conflict of interest. This research is a part of N. Amaar study to get M.V.Sc. "Parasitology".

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