Studies on Coccidia of Egyptian Balady Breed Chickens

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Abstract: A total of 711 Balady breed chickens of different ages and sex were collected from houses and farms of 4 localities: Cairo & Giza, governorates Western delta governorates; El-Gharbiyah, El-Behiera, Kafer El- Sheikh, Eastern governorates; El- Sharqiyah, Ismailia & Upper Egypt governorates; Qina and Aswan, during the period between September 1999 - August 2003 were sacrificed and their intestine were examined for the presence of *Eimeria* species. Microscopical identification of Eimeria oocysts species revealed that 21.24% of these chickens were found infected with five species of Eimeria; which were *E.necatrix* (58.27%), *E.tenella* (25.82%), *E.acervulina* (19.20%), *E.mitis* (10.59%) and *E.maxima* (4.66%), respectively. It was found that chickens of 1-21 day old were found free from infection (0%), while chicken of 64 – 84 day old showed high infection rate (62.37%).The high rate of infection was noticed in winter season (45.13%), while the lowest rate was recorded during summer season (1.86%). The highest incidence of *Eimeria* species (37.16%) was found in (Cairo & Giza).While, the lowest incidence (7.32%) was found in Delta areas. The prepatent period, age resistance beside histological examination of the five previously identified *Eimeria* species, which were experimentally isolated and propagated, was also studied.
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1. Introduction

Avian Coccidiosis is the major problem in poultry worldwide; it causes serious problem and causing huge economic loss to poultry industry **Jadhav et al. (2011).** The occurrence of different *Eimeria* species combinations and the intensity of infection vary considerably, both globally and locally **Oikawa et al. (1979), Williams (1996)** and **Amer et al. (2010)** and with time **Braunius, (1986b)** and **Haug et al. (2008).**

Coccidiosis also, causes weight loss, lower feed conversion rate, delayed sexual maturity and decrease of egg production. **Lobago et al. (2005).** Lesions of the intestinal mucosa and loss of pigmentation may also become apparent during the latter stages of infection **Conway & McKenzie (1997), Mc Dougald & Reid (1997)** and **Amer et al. (2010).**

In Egypt, numerous research papers were carried on coccidiosis of commercial white broilers, but few of them carried on Egyptian Balady breed chicken, which seem to be more resistant to infectious diseases **Abu El-ezz (1994).** Therefore, this study was designed to determine the incidence of coccidiosis in local strain (balady breed chickens) and to identify the prevalent *Eimerian* species in 4 different localities in Egypt. Experiment was planned to study the isolation and identification of the most predominant *Eimeria* species by morphology and detection of microscopic lesions as well as studying the pathogencity of each isolates species.

2. Materials & Methods

A total of 711 sacrificed Egyptian Balady breed chickens of different sex and ages (1-21days), (22– 42 days), (43 – 63 days), (64 - 84 days) and (< 84 days) were collected from 4 localities in Egypt; Cairo & Giza, Western delta governorates; El-Gharbiyah, El-Behiera, Kafer El-Sheikh, Eastern governorates; El- Sharqiyah, Ismailia and Upper Egypt governorates; Qina & Aswan. The study was conducted from September 1999 to August 2003.

Concentration floatation technique was applied for the collection of *Eimeria* oocysts from intestinal content of chickens **Davies et al. (1963).** Isolation of *Eimeria* oocysts was depended on the measurements by using a calibrated ocular micrometer at 400x magnification **Long** and **Reid (1982),** 30 random oocysts from each sample were identified by a combination of the following criteria according to **Conway** and **Mckenzie (1997);** (1) Location & characteristics of intestinal lesions (2) oocyst morphology (3) Sporulation time of *Eimeria* species. *Eimeria* oocysts measured and categorized into three groups (Table 1): a small oocysts group, 17.8-14.1µm; *Eimeria mitis* in the middle part of intestine (ileum) and 18.2-14.1µm *Eimeria acervulina* (duodenum), a medium group sized oocysts, 20.1-16.9µm; *Eimeria necatrix* (ileum) and 21.3-17.9µm *Eimeria tenella* (caecum); a large oocysts group, 29.9-23.8µm; *Eimeria maxima* (ileum).

Gross lesion examination and lesion scores: Investigated sacrificed chickens in the laboratory by cervical dislocation using the technique described by **Zander (1978).** The gastrointestinal tract was grossly examined carefully. The intestinal portions were divided into 4 sections, the upper part (duodenum and

jejunum), the middle part (ileum), lower part (distal ileum and rectum) and cecal pouches. Intestinal gross lesions in any part of the sections were graded from 0 to 4 based on lesion score key **Conway** and **McKenzie (1997).** The lesion score zero represents absence of lesion and lesion score four is for very severe intestinal /cecal mucosa lesion and fatal cases. The location of the lesion was recorded; intestinal contents from the respective sections were taken and duplicate mucosal scrapping smears made from each section of the intestine.

From each part of infected intestine of *Eimeria* oocysts species was collected the content and prepared according to **Conway** and **McKenzie (1997)**. Then identification of each species of *Eimeria* depending on the three criteria previously recorded. Each species of *Eimeria* was spread out in shallow Petri dish 2.5% potassium dichromate solution for sporulation. **Ryley et al. (1976).** The isolated oocysts were counted by Mc Master Technique **Long et al. (1976).**

Selected number (10^3) of identified species of sporulated oocysts; (*E.necatrix, E.acervulina*, *E.maxima & E.mitis*) and (10^4) for *E .tenella,* were inoculated orally to experimentally chickens for propagation and histopathological studies of investigated *Eimeria* species.

Experimental infection: In this experiment 5 isolated oocysts which are; *E.mitis*, *E.acervulina, E.necatric, E.tenella & E.maxima,* were inoculated in 28 old age Balady breed chickens. In this experiment 80 Balady free chicks reared from one day old in disinfected wire cages. The ration used for the chicks was completely free from antibiotics and anticoccidials drugs. The eighty balady chicks were divided into 6 groups; the first 5 groups (10 chicks/ group) were inoculated by 10^3 sporulated oocyst of each pre-isolated species of *Eimeria* through its crop. The last group (30 chicks) was kept as non infected control group (Table2). For examination of intestinal lesions and endogenous stages of the parasites, one of the infected chickens from each group was slaughtered at 3 to 8 day's post-inoculation (dpi) depending on the *Eimeria* species. Faeces were collected once daily and examined for the presence of oocysts and detection prepatent time, Oocysts count and morphology were also determined **Thebo et al. (1998).** The rest of infected chickens were slaughtered on 9^{th} of infection to detect the P.M lesions and histopathological changes for each *Eimeria* species. The intestinal tissues of infected chickens showing gross lesions were fixed by formal saline 10% to apply histopathological staining according to **Carleton et al. (1967).**

3. Results

From table (3): it was found that 151(21.24%) chickens from total 711 were infected by five *Eimeria* species (in four different localities from Egypt). The rates of infection by *Eimeria* species were 58.27%, 25.82%, 19.20%, 10.59% and 4.66% for *E.necatrix E.tenella, E.acervulina E.mitis* and *E.maxima*, respectively. (Fig.1); showed unpopulated oocysts of *Eimeria* spp.; (A).*E. maxima*, (B) *E.acervulina*, (C) *E. mitis* (D) *E.tenella and* (E). *E.necatrix*. Mixed infection was recorded in 33% of positive samples.

Table (4); revealed that the Cairo & Giza regions showed highest incidence of infection (37.16%) followed by Eastern delta regions (36.30%).While, the lowest incidence was recorded in Western delta regions (7.32%).

Concerning the age susceptibility, it was found that chickens of 1-21 days age showed low rate of infection (0%), while chicken of 64 – 84 day old showed high infection rate (62.37%).

Regarding the season's variation, table (5) showed that a high rate of infection has been noticed during the winter season (45.13%), while lower rate of infection was recorded during summer season (1.86%). Autumn and spring seasons showed 18.30% & 18.21 % rate of infection.

From experimental results in table (6), determined the prepatent period from each infected groups they recorded 100 hrs. for *E. mitis* & *E.acervulina*, 120 hrs. for *E. maxima* & *E.tenella* and 168 hrs. for *E. necatrix*. Also, described the predilection sites and P.M lesions for different *Eimeria* species which beginning from 4^{th} dpi. for *E. mitis, E.maxima, E. necatrix and E. tenella,* While, started on the 5^{th} dpi. for *E.acervulina.* The clinical signs concentrated mainly in loss of weight, severe anemia and bloody diarrhea in *E.tenella.*

Tissue specificity and gross lesions were preliminary diagnostic of samples especially for *E.acervulina* (Fig.2), *E.necatrix* (Fig.3) and *E.tenella* (Fig.4), during the experimental infection gross lesions.

Histological finding of duodenum of experimentally infected chicken with *E. acervulina* showed presence of hyperplastic changes in the epithelial mucosa with activation of goblet cells, sometimes there was epithelial desquamation. The lamina propria was infiltrated with inflammatory cells Plate (1A-B), accompanied with hemorrhagic areas (Plate 2A). Gametocyte was observed (Plate2B) and the muscular layers suffer from edema. In addition, plate (2 A-H) explains the histological finding of the middle part of small intestine of naturally infected balady chicks with *E. necatrix* which showed its characteristic coagulative necrosis and focal hemorrhagic areas and deeply embedded gametocyte in tunica musculosa and serosa.

Moreover, the caecum of naturally infected balady chicks with *E.tenella* Plate (3A-C): showed considerable numbers of oocysts in lamina propria beside sever hemorrhage and complete desquamation of epithelium and edema of muscular tissue.

Table (1): Identification of five *Eimeria* species in Balady chickens.

Site of lesion	Postmortem lesions	Shape	Size of oocyst (µm)	Shape index	Sporulation time	Species of *Eimeria* identified
Ileum	Mucoid, enteritis	Ovoid	17.8-14.1	1.26	18 hrs.	*E.mitis*
Duodenum	Transverse Whitish band on duodenal loop.	Ovoid	18.2-14.1	1.29	17 hrs.	*E.acervulina*
Ileum	Balloning of intestine Mucoid blood filled exudates	Oblong Ovoid	20.1-16.9	1.19	18 hrs.	*E.necatrix*
Caecum	Haemorrhages & clotted blood in caecal pouches	Ovoid	21.3 -17.9	1.19	18 hrs.	*E.tenella*
Ileum	Thickened intestine wall. Patechiae.	Ovoid	29.9-23.8	1.25	30 hrs.	*E.maxima*

Table (2): Experimental infection of 50 Balady free chicks (28 days) old with five *Eimeria* species.

Main Group	1	2	3	4	5	Control
No. of chicks	10	10	10	10	10	30
Eimeria spp.	*E.necatrix*	*E.tenella*	*E.acervulina*	*E mitis.*	*E. maxima*	0
Inoculation dose	10^3	10^4	10^3	10^3	10^3	0

Table (3): Incidence of *Eimeria* species in examined chickens.

Species	Number of examined chickens (711)	Number of infected chickens (151) 21.24%	No. inf. chickens	%	Mixed infection
E.necatrix			88	58.27	9
E.tenella			39	25.82	28
E.acervulina			29	19.20	18
E.mitis			16	10.59	16
E.maxima			7	4.66	7

Table (4): Incidence of *Eimeria* spp. in four different localities in Egypt.

localities group	No. of ex.	No. of inf.	Incidence (%)	Isolated species
Cairo & Giza group	148	55	37.16	*E.acervulina, E.maxima, E.mitis, E.necatrix and E.tenella*
Western Delta area (El-Gharbiyah, El-Bihiera, Kafer El-Sheikh)	287	21	7.32	*E.acervulina, E.maxima, E.mitis and E.tenella*
Eastern Delta governorate (El Shrquiyah &Ismailia)	157	57	36.30	*E.acervulina, E.maxima, E.necatrix and E.tenella*
Upper Egypt (Qina & Aswan)	119	18	15.13	*E.acervulina, E.maxima, E.mitis, E.necatrix and E.tenella*
Total	711	151	21.24	*E.acervulina, E.maxima, E.mitis, E.necatrix & E.tenella*

Table (5): Seasonal incidences of *Eimeria* spp. in Balady breed chickens:

Season	Examined	Infected	%	Isolated species
Summer	107	2	1.86	*E.necatrix*
Autumn	213	39	18.30	*E.acervulina, E.necatrix and E.tenella*
Winter	144	65	45.13	*E.acervulina, E.mitis, E.necatrix & E.tenella*
Spring	247	45	18.21	*E.acervulina, E.maxima, E.mitis &E.tenella*
Total	711	151	21.24	*E.acervulina, E.maxima, E.mitis, E.necatrix and E.tenella*

Table (6): Illustrations of Prepatent period and gross lesions of experimentally infected Balady chickens.

gp. no.	Species	Prepatent period (hrs)	Clinical signs Day post Infection (dpi)	PM lesions	Gross lesions
1	*E.mitis*	100	Decrease in weight gain from 5th dpi.	4th day	Slight enteritis in middle part of intestine (+1)
2	*E.acervulina*	100	Decrease in weight gain from 5th dpi.	5th day	Duodenum had lesions from pinpoint white necrotic focci to sever ladder like white batches (+1 to +3)
3	*E.maxima*	120	Decrease in weight gain from 5th dpi.	4th day	Slight enteritis in middle part of intestine (+1)
4	*E.tenella*	120	From 3rd dpi. Severe depression, wing drops, strains, white diarrhea to bloody, no mortality.	4th day	Lesions began from 4th day as typhlitis ranging from slight to bloody. Lesion score from +2 to +4 in 100% of chicks
5	*E.necatrix*	168	From 4th dpi. Sever anemia, wing drops, strains, bloody diarrhea.	4th day	Bloody enteritis in middle part of intestine, lesion score (+2 to +3)

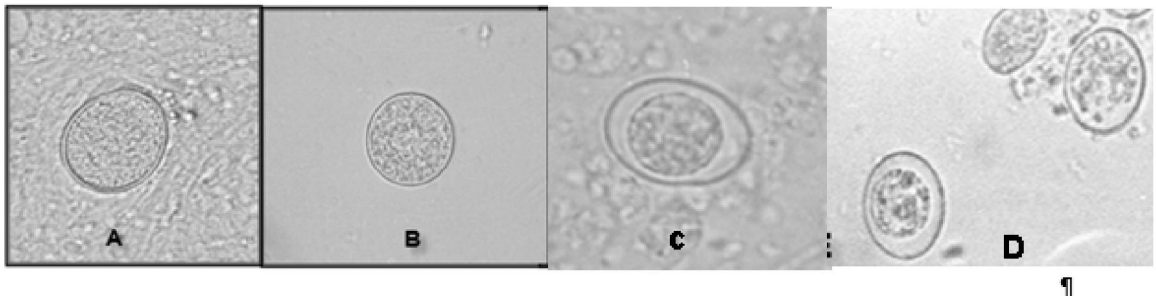

Fig.1; unpopulated oocyst of *Eimeria* spp.; (A). *E.acervulina*,, (B) *E. mitis* (C) *E.tenella* (D). *E.necatrix* (X400).

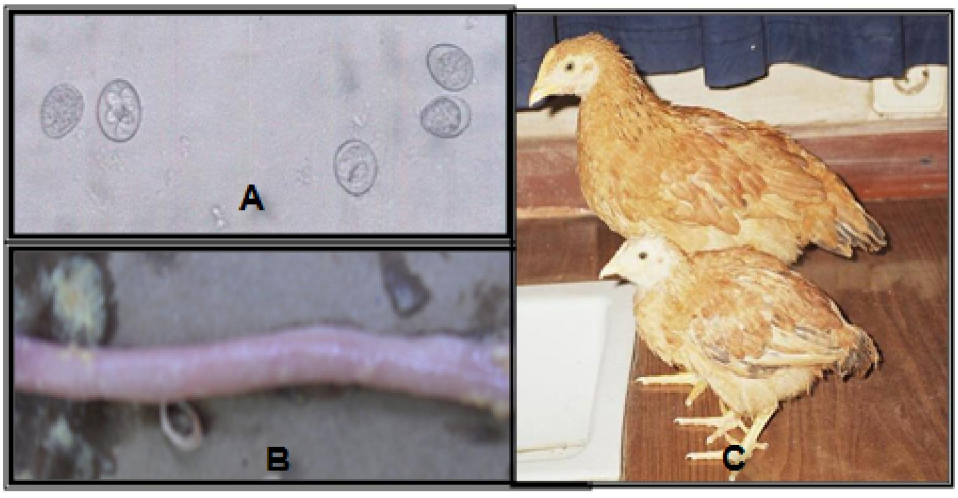

Fig.2: *Eimeria acervulina* (A) sporuolated oocysts direct smear X100 (B) White necrotic focci appear from the serosal surface of duodenum 6th dpi. (C) Infected chicken against control chicken showing loss of weight

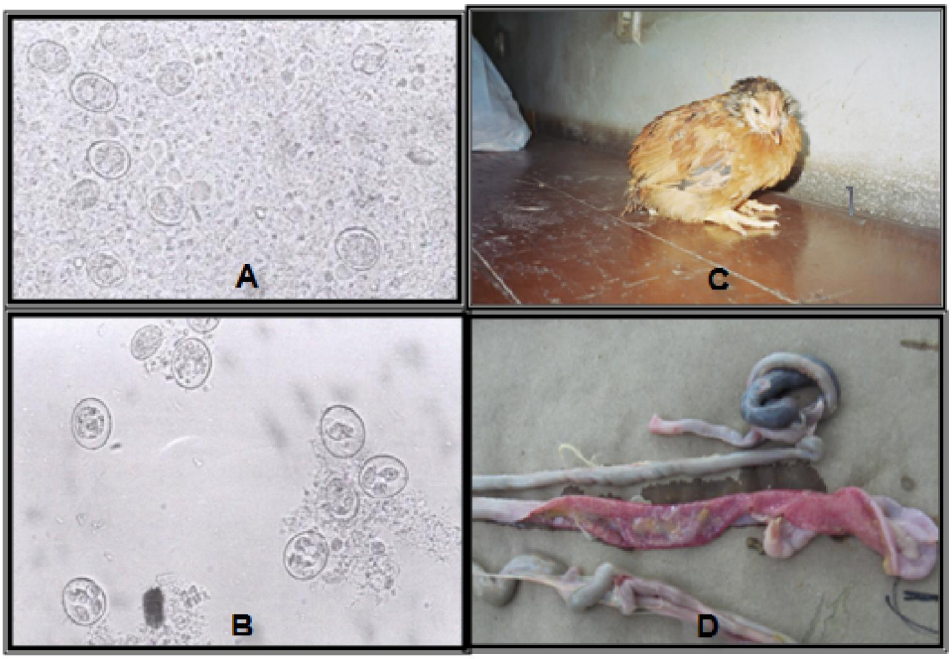

Fig.3: *E.necatrix* (A) unsporulated oocyst (B) sporulated oocysts from direct smear X100. (C) Infected chicken showing depression, ruffling and off food. (D) Ileum showing hemorrhagic enteritis 7thdpi.

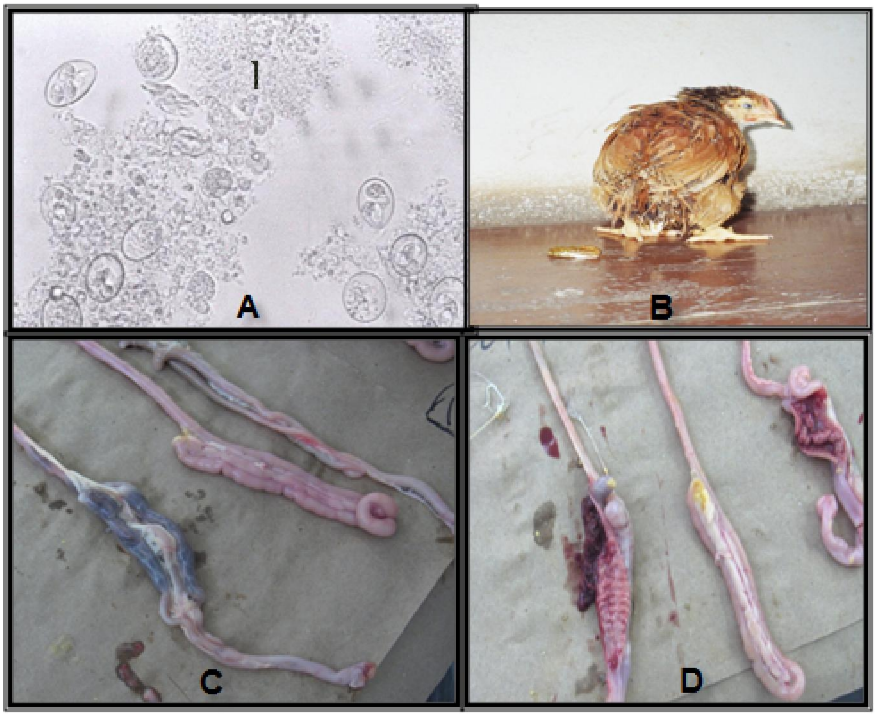

Fig.4*: E. tenella* (A) sporulated oocysts X100. (B) Infected chicken showing diarrhea (C&D) Two coeci of infected chicken showing bloody content 6th dpi.

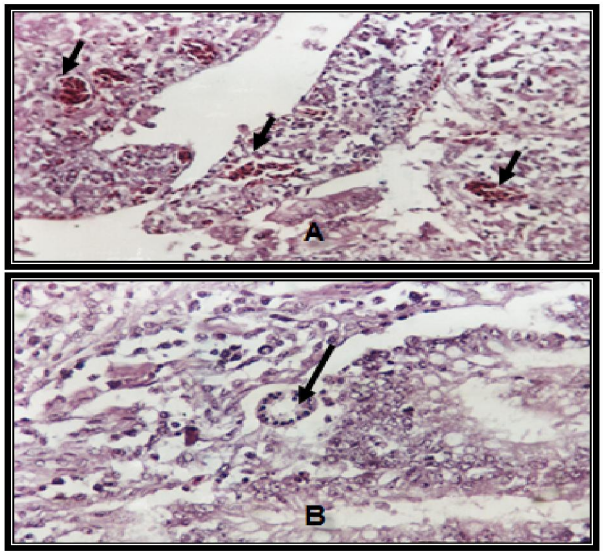

Plate 1 (A&B): showing histological finding of duodenum experimentally infected balady chicks with *E. acervulina*. A: showing focal hemorrhagic area (H. & E. X250).B: showing gametocyte (H. & E. X400).

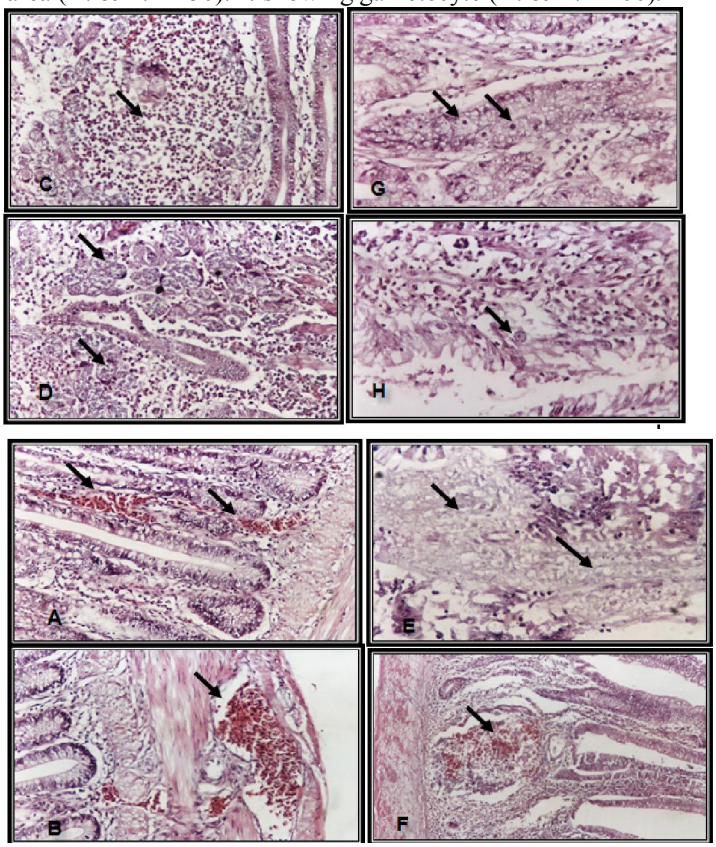

Plate 2 (A-H): showing the middle part of intestine of experimentally infected balady chicks with *E. necatrix*

A. showing mild hemorrhagic lamina propia (H&E X250). B. showing congestion in the muscularis (H&E X250)
C. showing inflammatory cells aggregation (H&E X250) D. showing great number of schizonts (H&E X250)
E. showing coagulative necrosis (H&E X 40) F. showing focal hemorrhagic area (H&E X100).
G. showing intracellular oocysts (H& E X400) H. showing gametocyte (H&E X250)

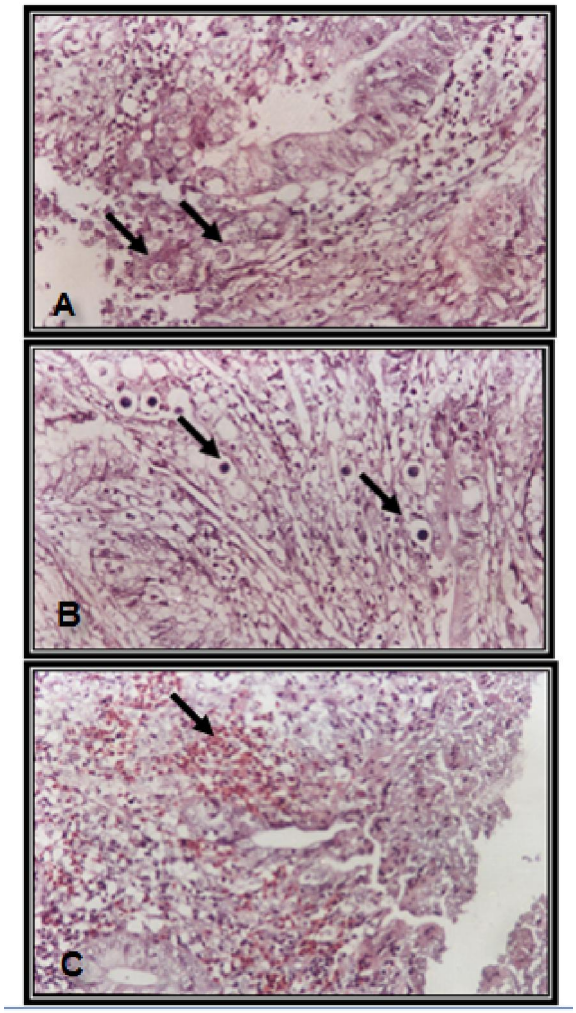

Plate 3(A-C) showing caceum of experimentally infected chickens with *E.tenella*
A. Showing different stages of coccidian (H&E X 250)
B. Showing numbers of intracellular oocysts (H&E X250)
C. Showing sever hemorrhage (H&E X250)

4. Discussion

In the present study, a total of 711 sacrificed Balady chickens of different ages and sex were collected from 4 different geographical localites in Egypt. The incidence of coccidiosis in native breeds was 21.24 %. This result nearly agreed with **Lunden & Thebo (2000) and Ashenafi et al. (2004)** who recorded 19.3 % incidence in layer farms in Sweden at the age ranged between 19-32 weeks and 25.8 % incidence in 190 chicken samples examined in Ethiopia. This result disagree with **Ahmed et al. (2003), Khelfa (1982) and Amer et. al. (2010),** who recorded 43.9 %, 82.24% & 90% rate of infection respectivly in chickens in Egypt. **Norcross &Washko (1970)** and **Allen & Fetterer (2002)** who mentioned that differences in incidence according to age are due to different age susceptibility to different *Eimeria* species**.**

Pinard-van Der Laan (1997) found that Fayomi Line breed was the most resistant agansit coccidiosis which showed no mortality, less sever lesion than the other lines, the white Leghorn lines were the most susceptabile.

In the present study, it was found that the most prevalent *Eimeria* species among the examined chickens were *E. necatrix* & *E. tenella* (58.27% & 25.82%). This result agrees with that reported by **Shakshouk (1984)** who stated that incidence with the same Eimeria species were 32.2% & 67.8% in broilers in Beheira and Alexandria governorates. In addition, this result agree partially with that result recorded by **Abu Elezz (1994)** who stated that, the ceccal coccidiosis *E.tenella* is the most prevalent species in Balady chicks in Egypt. However, **Haug et al. (2008)** who recoded *E. tenella* and *E. maxima* were the most

prevalent species associated with medium-sized and large oocysts, respectively in broiler chickens in Norway.

The present study, revealed that the incidence of *E. acervulina*, *E. mitis* and *E. maxima* incidences were 19.20%, 10.59% and 4.66% respectively. These results agree with **Khelfa (1982)** who stated that the mentioned species incidences were 10-80%, 10-40% and 4-10%, respectively, in the Upper Egypt. **Ahmed et al. (2003)** reported that the presence of *E.acervulina, E.maxima and E.mitis* species was 43.9% in Egypt. This previous result disagrees with the finding of **Kucera (1990), Mc Dougald et al. (1997)** and **Lobago et al., (2005)** who stated that the prevalence differences were normal due to the differences in the epidemiological situation among different countries. Morover, **Haug et al. (2008)** who found the incidence of *E. acervulina and E. maxima* was 100% and 27.5% in broiler chickens in Norway.

The absence of *E. brunetti* & *E. praecox* among the examined balady chicks agree with the finding recorded by **Shakshouk (1984), Ahmed et al. (2003)** and **El Behairy (2005)** and disagree with the finding recorded by **Khelfa (1982)** and **Haug et al. (2008)** who recorded the presence of *E. preacox* in 5-10% and 9.8 % broiler chickens.

Moreover, mixed infection was found in the rate of 33% among native breed Balady chicks under investigation. . This result agree with the finding by **Oikawa et al. (1974), Kucera (1990), William (1996)** and **Lobago et al. (2005),** who noticed the mixed infection with different species of Eimeria in the chickens. The present finding showed a difference in incidence of coccidial infection between different geographical localities, this result agreed with **Shirley (1992)** who stated that the effect of the environment (temperature & moisture) on the course & severity of coccidial infection has a great impact **Ashenafi et al. (2004) and Haug et al. (2008)** who confirmed the incidence of coccidiosis is varied in related to different selected climatic zones; there were a significant difference in coccidiosis prevalence from 42.2% to 13.1% chickens in central Ethiopia and 36.25% to 70.9% in broiler chickens in Norway.

The present study showed different age susceptibility among Egyptian native breed of different *Eimeria* species, *E. acervulina* and *E.tenella* which occur in 4th week and in older ages. In the contrary, *E.necatrix*, *E.maxima* and *E.mitis* infections weren't begin before 42 days of age. All examined samples of age less than 21 days was completely free. This result disagree with normal broiler age susceptibility, finding by **William (1996)** and **Mc Dougald et al. (1997)** who found several species of *Eimeria* oocysts from 15th and before 21th days old in the flocks. The differences of age susceptibility between native breed (Balady) and normal broiler might be explained in relation to genetic factores.

The present study showed clear difference in incidence of coccidiosis, among different seasons of the year, they were 45.13% in winter; 18.30% in autumn & spring and 1.86% in summer. These results agree with **Shirley (1992)** and **Ashenafi et al. (2004)** who explained the effect of humidity percent which increase in winter on the coccidiosis incidence. Moreover, **Lunnden &Thebo (2000)** and **Badawy et al. (2000)** also explained that the stocking density which increase in winter by 30% has a direct effect on the increasing incidence in winter. On the other, hand, **Haug et al. (2008)** found that high incidence (90.7%) of *Eimeria* species was recorded during summer of the year 2003-2004 in Norway.

The experimental infection of Balady breed chickens by Eimeria species isolates is aimed to study the biological characters of each isolate ,and confirming the diagnosis of each species of Eimeria .The protocol which used also by **Kucera (1990), William (1996)** and **Mc Dougald et al. (1997).** The study is aimed also for further immunological investigations of Eimeria species in Balady breed chickens.

The histological finding in this study confirmed the diagnosis of each species as *E. acervulina* showed presence of gametocyte with the characteristic inflammatory cells in duodenal part of intestine. The fact which agreed with **Hein (1971)** *E. necatrix* showed its characteristic coagulative necrosis and focal hemorrhagic areas and deeply embedded gametocyte in tunica musculosa and serosa as had been shown by **Hein (1971).** *E.tenella* showed considerable numbers of oocyst in lamina propria of coecum beside sever hemorrhage and complete desquamation of epithelium and edema of muscular tissue which agreed with the finding by **levine (1985).**

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