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-Shiekh, 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.


Keywords: Five Eimeria species - Egyptian Balady Breed Chickens – Incidence – Histopathology.

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 coccidioses 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 pathogenicity of each isolates species.

2. Materials & Methods
A total of 711 sacrificed Egyptian Balady breed chickens of different sex and ages (1-21 days), (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, 17.8-14.1 μm; Eimeria mitis in the middle part of intestine (ileum) and 18.2-14.1 μm Eimeria acervulina (duodenum), a medium group sized oocysts, 20.1-16.9 μm; Eimeria necatrix (ileum) and 21.3-17.9 μm Eimeria tenella (caecum); a large oocysts group, 29.9-23.8 μm; Eimeria maxima (ileum).

Lesions of the lower part (caecum); a large oocysts group, 29.9-23.8 μm; 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 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 (10^3) 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.necatrix, 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 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.necatrix, 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 orally to experimentally chickens for propagation and histopathological studies of investigated Eimeria species.

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 4th dpi. for E. mitis, E.maxima, E. necatrix and E. tenella, While, started on the 5th dpi. for E.acervulina. The clinical signs concentrated mainly in loss of weight, severe anemia and bloody diarrhea in E.tenella.

**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 muscosa 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.

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

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

<table>
<thead>
<tr>
<th>Main Group</th>
<th>No. of chicks</th>
<th>Eimeria spp.</th>
<th>Inoculation dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>No. of chicks</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Eimeria spp.</td>
<td><em>E. necatrix</em></td>
<td><em>E. tenella</em></td>
<td><em>E. acervulina</em></td>
</tr>
<tr>
<td>Inoculation dose</td>
<td>$10^3$</td>
<td>$10^4$</td>
<td>$10^5$</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of examined chickens (711)</th>
<th>No. of infected chickens (151)</th>
<th>%</th>
<th>Mixed infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. necatrix</em></td>
<td></td>
<td></td>
<td>88</td>
<td>58.27</td>
</tr>
<tr>
<td><em>E. tenella</em></td>
<td></td>
<td></td>
<td>39</td>
<td>25.82</td>
</tr>
<tr>
<td><em>E. acervulina</em></td>
<td></td>
<td></td>
<td>29</td>
<td>19.20</td>
</tr>
<tr>
<td><em>E. mitis</em></td>
<td></td>
<td></td>
<td>16</td>
<td>10.59</td>
</tr>
<tr>
<td><em>E. maxima</em></td>
<td></td>
<td></td>
<td>7</td>
<td>4.66</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>localities group</th>
<th>No. of ex.</th>
<th>No. of inf.</th>
<th>Incidence (%)</th>
<th>Isolated species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cairo &amp; Giza group</td>
<td>148</td>
<td>55</td>
<td>37.16</td>
<td><em>E. acervulina, E. maxima, E. mitis, E. necatrix and E. tenella</em></td>
</tr>
<tr>
<td>Western Delta area (El-Gharbiyah, El-Bihiera, Kafer El-Sheikh)</td>
<td>287</td>
<td>21</td>
<td>7.32</td>
<td><em>E. acervulina, E. maxima, E. mitis and E. tenella</em></td>
</tr>
<tr>
<td>Eastern Delta governorate (El Shreqiyah &amp;Ismailia)</td>
<td>157</td>
<td>57</td>
<td>36.30</td>
<td><em>E. acervulina, E. maxima, E. necatrix and E. tenella</em></td>
</tr>
<tr>
<td>Upper Egypt (Qina &amp; Aswan)</td>
<td>119</td>
<td>18</td>
<td>15.13</td>
<td><em>E. acervulina, E. maxima, E. mitis, E. necatrix and E. tenella</em></td>
</tr>
<tr>
<td>Total</td>
<td>711</td>
<td>151</td>
<td>21.24</td>
<td><em>E. acervulina, E. maxima, E. mitis, E. necatrix &amp; E. tenella</em></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Season</th>
<th>Examined</th>
<th>Infected</th>
<th>%</th>
<th>Isolated species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>107</td>
<td>2</td>
<td>1.86</td>
<td><em>E. acervulina</em></td>
</tr>
<tr>
<td>Autumn</td>
<td>213</td>
<td>39</td>
<td>18.30</td>
<td><em>E. acervulina, E. necatrix and E. tenella</em></td>
</tr>
<tr>
<td>Winter</td>
<td>144</td>
<td>65</td>
<td>45.13</td>
<td><em>E. acervulina, E. mitis, E. necatrix &amp; E. tenella</em></td>
</tr>
<tr>
<td>Spring</td>
<td>247</td>
<td>45</td>
<td>18.21</td>
<td><em>E. acervulina, E. maxima, E. mitis and E. tenella</em></td>
</tr>
<tr>
<td>Total</td>
<td>711</td>
<td>151</td>
<td>21.24</td>
<td><em>E. acervulina, E. maxima, E. mitis, E. necatrix and E. tenella</em></td>
</tr>
</tbody>
</table>
Table (6): Illustrations of Prepatent period and gross lesions of experimentally infected Balady chickens.

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

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) sporulated oocysts direct smear X100 (B) White necrotic focii 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 7<sup>th</sup> dpi.

Fig. 4: *E. tenella* (A) sporulated oocysts X100. (B) Infected chicken showing diarrhea (C&D) Two coeci of infected chicken showing bloody content 6<sup>th</sup> 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).
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 respectively 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 against coccidiosis which showed no mortality, less severe lesion than the other lines, the white Leghorn lines were the most suscetible.

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 cecal 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. Moreover, 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. praecox 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 was varied in related to different selected climatic zones; there were 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 factors.

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, Lunnen &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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References


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