

# Isolation and Identification of Eimeria from Field Coccidiosis in Chickens

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**Abstract:** Oocysts of Eimeria species were collected from 8 Native breed chicken flocks aged 7-8 weeks. These chickens were suffering from bloody dropping, loss of weight, low conversion rate and variable mortalities 3-12% in 6-10 days. Eimeria species' oocysts were sporulated and tested for their infectivity and pathogenicity in male commercial chicks aged 14 days old. The infected chicks showed general signs of ruffled feathers, off food, huddling together with loose dropping and/or bloody dropping with total mortality reached to 90%. The post mortem examination showed hemorrhagic foci in the duodenum, hemorrhagic mucosa in mid intestine and bloody caecal core in two caeci. Eimeria species developmental stages in duodenum, intestine and caecum were histopathologically detected at the 6<sup>th</sup> day post infection. The obtained sporulated oocysts were identified according to morphological features, and the calculated shape index were 1.14, 1.19, 1.25 and 1.23 suggestive to be E. tenella, E. necatrix, E. acervulina and E. praecox; respectively. Chicks kept individually in a wire cage were inoculated with one sporulated oocyst for obtaining pure isolate from morphologically identified 10 isolates and for detection the site of infection and histopathological features. Egyptian four local isolates in a pure form were obtained. These isolate, including E. tenella, E. acervulina, E. necatrix, and E. praecox. This isolates were passed in the chicks 14<sup>th</sup> day old from increasing their number.

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**Keywords:** Oocysts; Eimeria species; sporulated oocyst; morphological features

## 1. Introduction:

Coccidiosis still as one of the most important worldwide diseases of poultry, the disease caused by protozoa of the Phylum Apicomplexa which undergoes a direct life cycle with transmission between hosts by way of resistant oocysts. In the host, the parasite grows and multiplies intracellular in epithelial and subepithelial cells, usually in the gut inducing enteritis (Gordon and Jordan, 1982). Flocks infected as a result of mild to severe exposure usually shows a marked decrease in food and water consumption and birds become depressed and tend to huddle. Decreased weight gains occur as a result of the disruption of the intestinal mucosa where minimal absorption is taking place. Diarrhea may result as the host is trying to flush the organism from the body, which may induce dehydration. Lesions of the intestinal mucosa and loss of pigmentation may also become apparent during the latter stages of infection (Conaway and McKenzie, 1991; Edgar, 1992; Lillehoj and Trout, 1993; McDougald and Reid, 1997).

From the above mentioned our study was planned for isolation and identification of Eimeria from the field infected cases by morphology and detection of microscopic lesions in sight of infection with one oocyst as well as studying the pathogenicity of mixed field isolates.

## 2. Materials and methods

Samples for isolation of Eimeria oocysts:

Field Eimeria species' oocysts were isolated from dropping, litter samples and intestine with the caecum of chickens from chicken flocks from naturally infected native breed chicken flocks aging 7-8 weeks suspected to be infected with coccidiosis.

Isolation of Eimeria oocysts:

Eimeria oocysts were isolated according to the method of Davies et al. (1963) and Ryley et al. (1976), then kept for sporulation in 2.5% for sporulation according to Long et al. (1976). The McMaster chamber method was used for the oocyst count as mentioned by Long et al. (1976).

**Solutions:**

Potassium dichromate solution was used in 2.5% for sporulation and preservation of the parasite. Saturated Sodium chloride solution was used for flotation technique. Formole saline was used for fixation of tissues during histopathological work according to El-behairy, (2005).

**Ration:**

Anti coccidial-free commercial ration, containing not less than 21% crude protein, not less than 2.7% crude fat and not more than 2.6% crude fiber was used.

**Chicks:**

Two hundred and thirty; one day- old chicks (layer cocks) obtained from commercial hatchery were used for inoculation of one oocyst and pathogenicity of both purified and mixed isolates throughout this work. These chicks were fed on autoclaved ration after addition of commercial amino acids, vitamins and minerals.

**Histopathology:**

Intestinal tissues were fixed for 1-2 hour and washed it several changes of 50% alcohol for 4-6 hour and stored in 70% alcohol then washed in 80%, 90%, 95% and absolute, finally the specimens were embedded in paraffin wax, sectioned and stained with H&E. Urara et al. (2005).

**Lesion score:**

The gross lesion score was recorded according to Johnson and Reid (1970) and Conway (1979).

**Identification of Eimeria:**

Obtained field sporulated oocysts were identified according to:

**1. Morphology:**

Morphological identification by measuring their length and width of 10 oocysts having similar morphological features using the ocular micrometer followed by calculation of oocyst index according to Edgar and Siebold (1964) and Norton and Joyner (1981).

**2. Purification and detection of infection site:**

For each morphologically identified 10 oocysts (table1); 10, 14 day old individually wire caged Eimeria infection free chicks were inoculated with one sporulated oocyst. Other 5 chicks were left as non-inoculated control. From the 3<sup>rd</sup> to the 10<sup>th</sup> dpi dropping of the inoculated birds was individually collected and examined by concentration flotation technique with a collection of the shed oocysts for

sporulation. At the 10<sup>th</sup> day, all birds were sacrificed individually with a collection of intestinal content after examination. The suspected intestinal lesions were subjected to histopathological examination. Results are shown in table (2) figs. (2, 3 and 4).

**Pathogenicity of isolated field oocysts:****1. Mixed oocysts:**

Twenty, one day- old male LSL chicks were used. At the 14<sup>th</sup> day of life, 5 random birds were sacrificed, and their intestinal contents were examined to be free from coccidian infection. Ten chicks were orally inoculated each with 0.5 ml containing approximately  $5 \times 10^4$  sporulated oocysts, while the other 5 chicks were kept as uninfected controls. The inoculated chicken was kept in isolated wire floor cages and fed on autoclaved ration and water.

From the 3<sup>rd</sup> to 10<sup>th</sup> dpi, dropping of the inoculated birds was individually collected and examined by concentration flotation technique. At 10<sup>th</sup> dpi; all inoculated birds were sacrificed and their intestinal contents were collected for oocyst collection. The obtained results are shown in table (2) and fig. (5).

**2. Purified sporulated oocysts:**

The collected purified sporulated oocysts 6 isolates from inoculated chicks with one oocyst were inoculated each in 5, 14 day old chicks, and other 5 chicks were kept as non inoculated control (Table 3). From the 3<sup>rd</sup> to 10<sup>th</sup> dpi dropping of the inoculated birds were individually collected and examined by concentration flotation. Dead birds and live birds to the 10<sup>th</sup> day all birds were sacrificed and examined then their intestinal contents were collected for collection of oocysts. Results are shown in (table 3).

**3. Results:**

Field's cases from eight chicken flocks showing, signs of bloody drooping, loss of weight, low conversion rate and mortalities ranging from 3-12 % in 6-10 days.

Dead birds showed intestinal lesions, including ballooned intestine, petechial hemorrhages and white foci in the middle intestinal mucosa. Caecal lesions ranged from hemorrhages to present of caecal cores. Duodenum of some cases showed thickened mucosa with petechial hemorrhages. All specimens were positive in rate of 100%.

**1. Morphology and measurements:**

Detected oocyst measurements (table 1) indicated that obtained oocysts were from mixed infection. The calculated oocyst index (table1)

indicated that the measured oocysts were to be *E.tenella* (1.14), *E.necatrix* (1.2), *E. acervulina* (1.25), and morphological feature Fig. (1), indicated additional two species suspected to be *E. maxima* and *E. mitis*. There are many species were detected from the same bird or flock.

## 2. Site of infection:

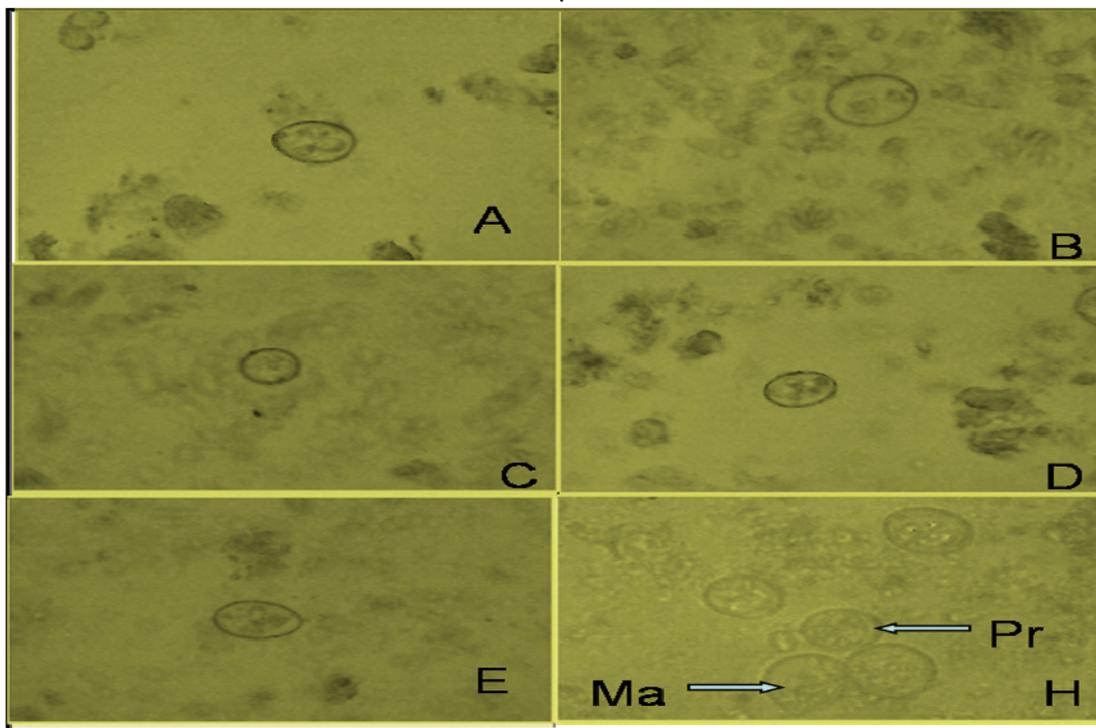
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obvious signs could be detected; oocyst could be detected in dropping of 27% chicks at 5<sup>th</sup> dpi. Variable intestinal lesions could be detected in oocyst shedders in the form of hemorrhagic foci in duodenum, hemorrhagic mucosa in the middle part and hemorrhagic caecal mucosa were detected in 27 % of inoculated chickens; depending on suspected inoculums. The collected intestinal and caecal content at the 10<sup>th</sup> dpi proved to contain oocysts. Ten isolates were obtained including 1 *E. tenella*; 2 *E. maxima*; 2 *E. necatrix*; 3 *E. acervulina*; and 2 *E. praecox*.

## 3. Pathogenicity:

Birds infected with mixed oocyst showed signs of ruffled feathers, off food and huddling together at the 3rd dpi with loose and/or bloody droppings. Dead birds were 2, 6 and 1 at 6th, 7th and 9th dpi; respectively; with total 9/10 mortality (90%). The examined intestine from died and sacrificed birds at 10 dpi showed lesion scores of 2, 1, 1 and 3 in upper, middle, lower and caeci. Histopathological examination of tissue sections at the 6th dpi (fig., 5) revealed the detection of developmental Eimerial stages in duodenum, mid intestine and cecum.

Date, number and percentage of positive mortalities recorded in chicks inoculated with identified semi pure *Eimeria* strains (table 3). One chick was died at the 5th dpi in *E. tenella* inoculated (gr 1). Two chicks were died, 1 at 6th and 1 at 7th (40%) in *E. necatrix* infected (gr 3). *E. acervulina* infected groups (4) and (5) showed each 1 dead bird at 7 and 6 day (20%); respectively. There were no mortalities in birds of groups 2, 4 and 7-10. Signs and lesions were representative of the inoculated species with variation in severity between the same species, especially in *E. necatrix* and *E. acervulina* inoculums.



**Fig (1): Obtained measured Eimerial oocysts from chickens**

A: *E. tenella*; B: *E. maxima*; C: *E. mitis*; D: *E. necatrix*; E: *E. acervulina*; H: *E. maxima* (Ma) and *E. praecox* (Pr).

**Table (1): Dimensions ( $\mu\text{m}$ ) and shape index of Eimeria species oocyst isolates.**

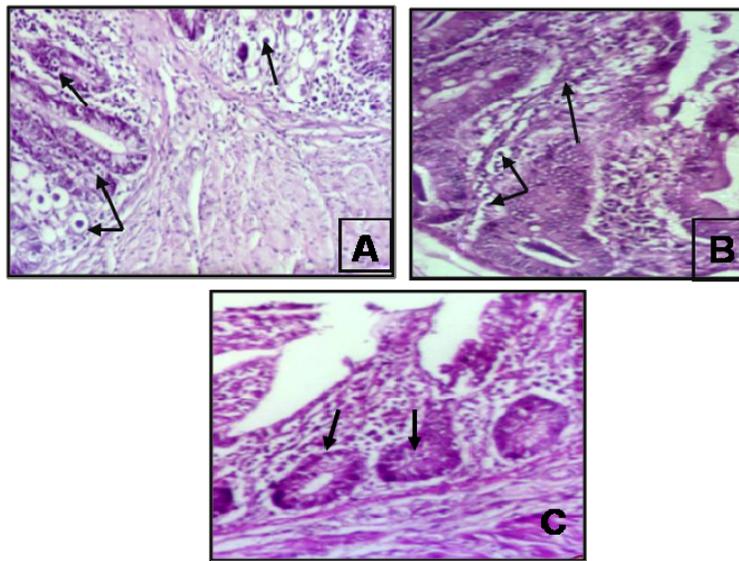
Isolates	Dimensions		Shape index	Suspected species
	Length	Width		
1	21.39	18.745	1.14	<i>E. tenella</i>
2	21.275	17.71	1.20	<i>E. necatrix</i>
3	20.815	17.48	1.19	
4	22.770	17.94	1.27	<i>E. acervulina</i>
5	21.965	17.48	1.25	
6	20.240	16.675	1.21	<i>E. necatrix</i> ,
7	22.310	17.48	1.27	<i>E. acervulina</i>
8	22.425	17.825	1.26	
9	23.690	19.205	1.23	<i>E. praecox</i>
10	19.205	15.755	1.22	<i>E. necatrix</i>

**Table (2): Mortality of chicks infected with mixed sporulated oocysts at 14<sup>th</sup> day old chicks.**

Group	Infection	No. of chicks	Days post infection						Mortality	
			5	6	7	8	9	10	Total	%
1	+	10	0	2	6	0	1		9	90
2	-	5	0						0	0

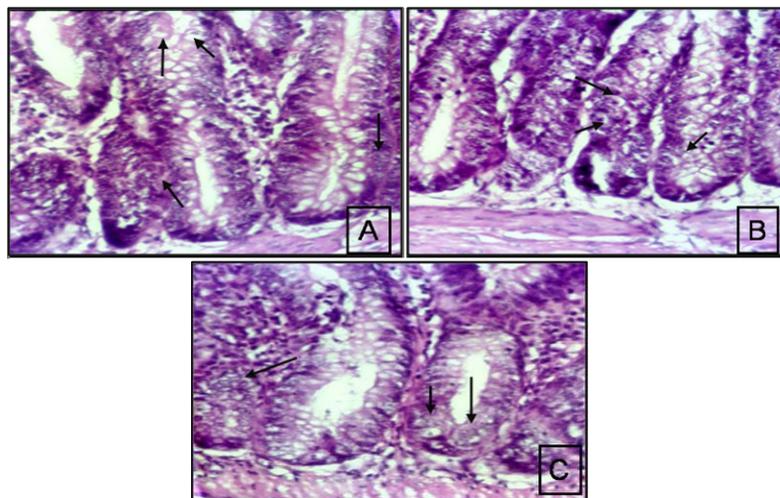
**Table (3): Mortality of chicks infected with the purified sporulated oocyst at 14<sup>th</sup> day old chicks (n=5 chicks).**

Group No.	Suspected species	Days post infection				Mortality	
		5	6	7	8 - 10	Total	%
1	<i>E. tenella</i>	1	-	-	-	1	02
2	<i>E. necatrix</i>	-	-	-	-	0	0
3		-	1	1	-	2	40
4	<i>E. acervulina</i>	-	-	-	-	0	0
5		-	-	1	-	1	20
6		-	1	-	-	1	20
7-8	<i>E. E. necatrix</i>	-	-	-	-	0	0
9-10	<i>E. maxima</i>	-	-	-	-	0	
11	control	-	-	-	-	0	0



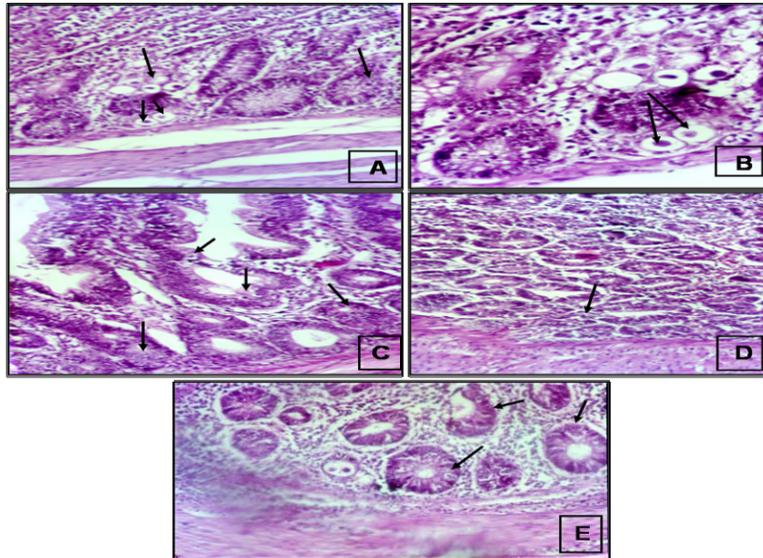
**Fig (2): Chicken duodenal sections stained with H&E of birds infected with oocysts suspected to be *E. acervulina* showing:**

- A: Submucosal parasitic stages (oocysts) (arrows) [X 200].  
 B: Parasitic stages in the glandular epithelium (arrows) [X 100].  
 C: Few parasitic stages (arrows) [X 200].



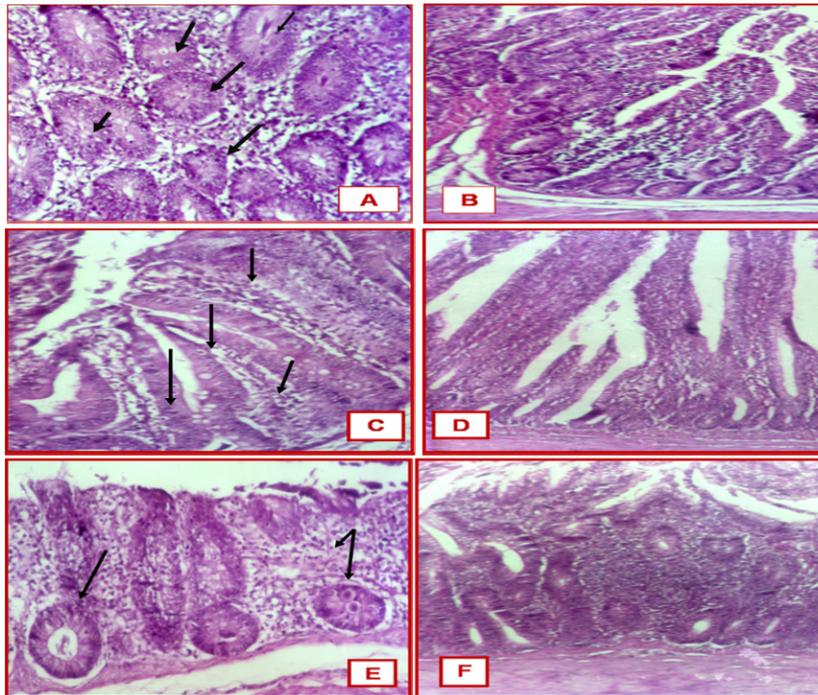
**Fig (3): Sections in middle intestine of chickens infected with oocysts suspected to be *E. necatrix* showing:**

- A: Mucosal invasion with parasitic stages (arrows) [X 200].  
 B: Mucosal invasion with parasitic stages (arrows) [X 100].  
 C: Parasitic stages in the mucosal epithelium (arrows) [X200].



**Fig (4): Caecal sections of chicken infected with oocysts suspected to be *E. tenella* showing:**

- A: Mucosal parasitic stages (arrows) [X 100].  
 B: high power of the previous lesions (arrows) [X 400].  
 C: Parasitic stages in the caecal glands. (arrows) [X100].  
 D: Glandular parasitic stages (arrows) [X 100].  
 E: Glandular parasitic stages (arrows) [X 200].



**Fig (5): Tissue sections from different intestinal parts of *Eimeria* infected and noninfected control hickens at 6 days post infection stained with H&E.**

- A: Duodenum of infected : different parasitic stages in duodenal glands (arrows) . B Deudenum.of non infected: normal histology.  
 C: Intestine of infected : different parasitic stages (arrows). D: Intestine of non infected: normal histological.  
 E: Cecum of Infected: Eimerial stages (oocysts) (arrows). F: Cecum of non infected: normal tissues.

#### 4. Discussion:

Coccidiosis is one of the oldest important described poultry worldwide poultry parasites as Raillet and Lucet (1891) measured oocysts from ceca of chickens and described a new species named by Raillet (1913) to *E.tenella* and Fantham (1910) described details of the life cycle. There are 8 *Eimeria* species affecting including *E.tenella* (Raillet and Lucet, 1891 and Fantham, 1909), *E. acervulina*, *E.maxima*, *E.mitis* (Tyzzer, 1929), *E. necatrix*, *E.praecox* (Johnson, 1930) *E.brunetti* (Levine, 1942) and *E.mivati* (Edgar and Seibold, 1964). Tyzzer (1929) and Johnson (1930 and 1933) detailed described of *Eimeria* species characteristic lesions, importance of sanitation and hygiene in reducing environmental contamination with the infective stage of the parasites and advocated exposure of the host to low numbers of oocysts to allow acquire protective immunity.

Coccidia are almost universally found wherever chickens are raised. It was exceedingly rare to find any commercial chicken flock not affected by *Eimeria* (Williams, 1999). Their strict host specificity eliminates wild birds as sources of infection. The most common means of spread of coccidia is mechanical, by personnel that move between pens, houses, or farms. Coccidial infections are self-limiting and depend largely on the number of oocysts ingested and on the immune status of the bird (McDougald, 2003).

Examined 8 naturally Field cases showing signs, intestinal lesions and mortalities ranging from 3-12 % in 6-10 days was resulted in the collection of 8 mixed *Eimeria* isolates. Our results subjected the wide distribution of *Eimeria* in poultry flocks. Williams, (1999) stated that commercial chicken flocks free from coccidian are extremely rare. The detected oocyst measurements (table, 1) and morphological features (fig., 1) indicated, that the obtained field oocysts are due to mixed infection and there are many species of *Eimeria* isolated from the same bird or flock. These finding were similar to those of Williams (1996) who found that multi species' infections were predominated in 95% farms; up to 6 species occurring together.

Results of passage of one oocyst (purified) isolates to indicate that we obtained four purified local isolates; *E. tenella*, *E. necatrix*, *E. acervulina* and *E. praecox*. The shape index of these species were 1.14, 1.19, 1.25 and 1.23 respectively according to Edgar and Seibold (1964) and Norton and Joyner (1981). These results clearly showed that 4 *Eimeria* species are prevalent in tested chicken flocks. Khilfa (1982) stated that on the morphological basis of *Eimeria* oocysts from litter samples from different age chickens flocks the *E. tenella*, *E. necatrix*, *E.*

*acervulina*, *E. mitis*, *E.praecox*, *E.maxima* and *E. hagani* were prevalent, especially the first 4 species (10-70%).

Using the site of infection as a criterion for supportive identification of *Eimeria* species 14 days old chicks 4 species were only conferred. This criterion was used previously by Karim and Trees (1990) who identified 5 *Eimeria* species; *E.acervulina*, *E.tenella*, *E. maxima*, *E. brunette* and *E. necatrix*; based on a lesion seen at post mortem examinations of naturally infected birds, dimensions of oocyst and lesion seen in experimentally infected chicks with single oocyst.

In the present study 27% inoculated in chicks, each by one sporulated oocyst with was positive. This result agreed with Chapman and Rose (1986) where the success rate was 25% using direct injection of a single sporozoite into the caecum of chicks. Disagree with Stephan et al. (1997) who obtained 52.6% success rate when packing the gelatin block carrying an oocyst into the crop of 10-day-old chick. Our result was higher than those of Shirley and Harvey (1996) who had success rate of 12.5 % for single sporocyst of *E. tenella* given orally.

Regarding pathogenicity of field mixed to isolate to 14 days old chicks, the obtained signs of ruffled feathers, off food and huddling together at the 3rd dpi with loose and/or bloody droppings. The recorded of 90% mortality in the period of 6-9 dpi indicates that the obtained mixed inoculums was pathogenic. The examined intestine from died and sacrificed birds at 10 dpi showed lesion scores of 2, 1, 1 and 3 in upper, middle, lower and caeci. Histopathological examination of tissue sections at the 6th dpi (fig 5) revealed the detection of developmental *Eimeria* stages in duodenum, mid intestine and cecum.

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