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CLINICAL AND PATHOLOGICAL SCORING OF AVIAN COCCIDIOSIS IN BASRA PROVINCE, IRAQ

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ABSTRACT

A current study was conducted to detect poultry coccidiosis in Basrah province. 81 samples of broiler and layer chicken were collected from (Al-Basra Veterinary Hospital in Al-Mashreq, poultry houses in Al-Zubair, veterinary clinics and veterinary laboratories).Period of study from December 2020 to May 2021. The results of the study showed different clinical signs on birds that were abnormal (diarrhea, wasting, lethargy, dehydration and weight loss). The results of the grossly showed petechial hemorrhage of different sizes diffused into the intestinal lining, severe mucosa secretion mixed with blood and congestion along the intestinal wall. The results of microscopy parasitic examination for the detection of *Eimeria* species were *E.praecox*(42.1%) *E.mitis*(15.78%) *E.tenella*(15.78%) *E.necatrix*(10.52%) *E.acervulina*(5.26%) *E.maxima*(5.26%) *E.bruneti*(5.26%). *E.praecox* had the highest incidence (42.1%) and the most common is the *E.mitis* and *E.tenella*.Histopathological results of poultry coccidiosis revealed severe infiltration of inflammatory cells in more than three villi in the intestinal mucosa, the development stages of *Eimeria* in the villus epithelium, and the presence of some red blood cells. The results of the polymerase chain reaction (PCR) test, based on 20 samples, showed conclusively the presence of poultry coccidiosis in the Basrah province. The results showed the presence of *Eimeria* DNA in the chickens intestine, Where seven of the total number of samples showed positive. The current study, which concluded the rates of severity and distributionof villus infection with the various coccidial stages, as follows: mainseverity score (63.15%) and distributionscore was (47.36%). The current study concludedthat the recorded seven type of *Eimeria* species in Iraq/Basrah Provence from broiler and layer chickens also results of present study revealed that some cases of avian coccidiosis threat commercial poultry population in Iraq. It is must be improved to prevent the occurrence and dissemination of avian coccidiosis.

I. INTRODUCTION :

Coccidiosis is the most common parasitic infectioncaused by an internal parasite of the *Eimeria* genus in poultry that causes major economic losses around the worldIn most tropical and subtropical locations of the world, this disease is endemic,coccidiosis is spread through feco-oral transmission. It is most common in young birds and chickens kept in intensive systems. Avian coccidiosis is characterized clinically by ruffled feathers, dehydration, and a pale comb are all symptoms of bloody diarrhea, as well as intestine thickening, haemorrhage, depending on the *Eimeria* species involved, necrotic enteritis in a specific region of the intestine of chickens during necropsy (Tewari & Maharana, 2011).Loss of epithelial tissue, congestion of blood vessels indicating disruption followed by blood leakage, significant mucosal oedema, necrosis of the submucosa, and loss of villi were among the abnormalities seen in the caecal type and marked hemorrhages and lymphoid cells showing hyperplasia. Also, chicken caecum and intestine showing *Eimeria* oocyst Lesions in the form of complete separation of the mucosal layer from the submucosal layer were identified in intestinal types. Sloughing of the villi and Eimeria oocysts can also be seen in the chicken gut (Babaei *et al.*, 2016). The majority of Eimeria species attack young hens aged 3 to 18 weeks, causing considerable mortality (Morris & Gasser, 2006). The parasite has two phase of life cycles: an endogenous phase in which the parasite divides multiple times in intestinal cells, and an exogenous phase in which ingested sporulated oocysts release sporozoites into the intestinal lumen (excystation) (Hammond & Minr, 1965).*Eimeria* was diagnosed using a variety of techniques, including faecal examination, serological testing, and molecular testing (Orlandi & Lampel, 2000).When analyzing faecal samples, the advent of PCR methods gives excellent levels of sensitivity and specificity (Sweeny *et al*., 2011). The PCR method, which is based on DNA

amplification, has been used to diagnose *Eimeria* parasites in animals. A variety of methods for analyzing parasites produced in vitro or present in tissue samples and clinical materials have shown to be both specific and sensitive (Kawahara *et al*., 2010).This study aimed tooccurrence rate and characteristics of coccidiosis in different avian species,also, Determination the most common type of *Eimeria* and Use lesion scoring technique for determination the severity of disease.

II. MATERIALS AND METHODS

Intestinal samples were taken from 81 hens of various ages, sexes, and species between December 2020 and May 2021. Coccidiosis was diagnosed based on clinical indicators and microscopically evaluated using a direct wet smear to establish the presence of oocysts or used (floutation method). Gross lesions according to ((Davis & Morishita, 2001)the Intestinal samples were obtained straight from the fresh positive case, placed in a clean plastic container, and snugly closed, with protective measures such as donning disposable gloves removed. The age of the bird, the type of bird, and the date of sample were all documented. The samples were transferred in ice bags to a pathology laboratory at the University of Basrah's College of Veterinary Medicine for histopathologic testing (Bancroft *et al.*,2018).The primers were used in Conventional PCR for detection of *Eimeria* spp in tissue of chicken intestine. The gene was then amplified by PCR technique (Patra *et al*., 2010) for confirmation, used a special primers**.**The primers were used in present study are listed in Table (1).

Table (1.1): Primers sequences

Gene	Primer Sequences (5´- 3´)	Product size	Reference
	F*: CTGTGAATCCATCGGA R : ATCGCATTTCGCTGCGTCCT	520bp	(Patra *et al*., 2010)

The ITS1 gene was amplified using the primers listed in Table (1). The total volume of the reaction tubes is 20µl, consist of 5µl Master Mix, 1.5µl of both the forward and reverse of the primers for each gene, 3µl of DNA The volume was filled by adding nuclease-free water to the template. Electrophoresis was used to separate the extracted DNA samples by mixing 5µl from DNA with of loading dye and loaded into the dedicated wells, then exposed to an electric field (70V for 45-60 min). The thermocycling program of ITS1 gene was listed in Tables 2.

Table (1.2): Program of ITS1gene (Patra *et al*., 2010)) .

Step	Temperature, °C	Time	Cycle
Initial denaturation	94	5 min	1
Denaturation	94	50 sec	30
Annealing	62	50 sec	
Extension	72	1 min	
Final extension	72	5min	1

III. RESULTS

Clinical Diagnosis and Incidence:the present study a total of 81 chickens were clinically examined. Two different types of chicken's species which were broiler and layer Some of birds showing significant clinical signs of suspected coccidiosis includ diarrhea and dehydration as in figure(4.1),

Figure (4.1) suspected coccidiosis diarrhea and dehydration

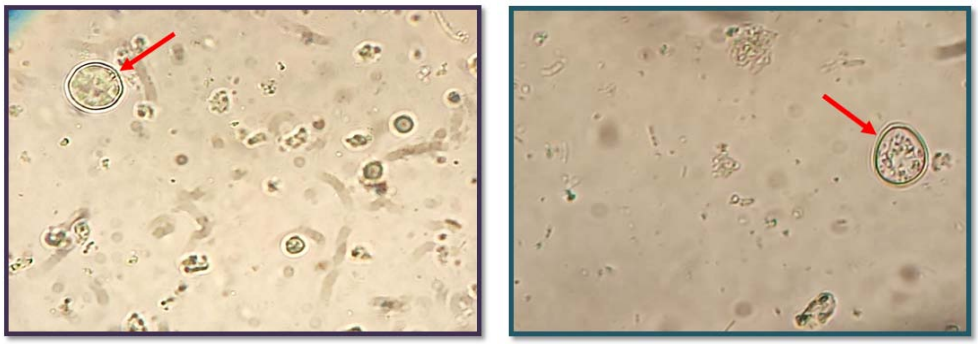
Incidence results: The current result of parasitic study of total 81 birds (broiler 42 (51.86%) cases and 39 (48.14%) layercases showed that the 18 (22.2%)cases of coccidiosis in broiler and 1 (1.23%) case in layer as in table (4.1).

Table (4.1): Percentage incidence of *Emeriosis* incidence in broiler and layer chickens.

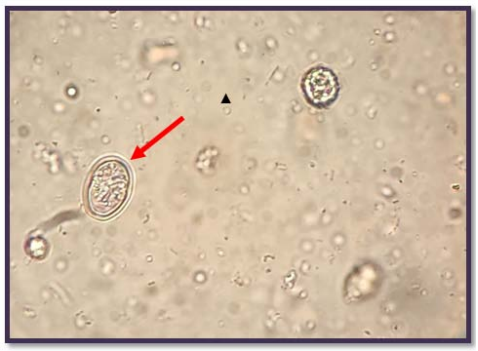
Types of parasites	Broiler	Layer	Total
E.praecox	8 (42.1%)	0 (0%)	8 (42.1%)
E.mitis	3 (15.78%)	0 (0%)	3 (15.78%)
E.tenella	3 (15.78%)	0 (0%)	3 (15.78%)
E.necatrix	2 (10.52%)	0 (0%)	2 (10.52%)
E.acervulina	1 (5.26%)	0 (0%)	1 (5.26%)
E.maxima	1 (5.26%)	0 (0%)	1 (5.26%)
E.bruneti	0 (0%)	1 (5.26%)	1 (5.26%)
	18 (94.74%)	1 (5.26%)	19 (100%)

The results showed that the infected broiler were higher than layer with a percentages of infection of 18 (94.74%) and 1 (5.26%) respectively. Furthermore, infection by *E.praecox* was 8(42.1%) in broiler while it was (0%) in layer. Similarly results showed that infection by *E.mitis* was 3(15.78%) in broiler while the layer it was (0%) as in table (4.1).On the other hand the results showed that infection by *E.tenella* was 3(15.78%) in broiler while the layer was (0%). The infection by *E.necatrix* was 2(10.52%) in broiler while the layer was (0%) as in table (4.1). In addition results demonstrated that infection by *E.acervulina* was 1(5.26%) in broiler while the layer was (0%), as well table (4.1) reveal that infection by *E.maxima* was 1(5.26%)in broiler while the layer was (0%). Finally the infection by *E. brunetti* was (0%) in broiler while the layer was 1(5.26%) as in table (4.1).

Microscopical detected Eimeria species: The parasitic study also demonstrated the *E. praecox* appeared in 8(42.1%) characterized by ellipsoidal to supspherical oocyst , colorless with tow smooth layer , polar cap present, micropyle absent as in figure (4.1).the strains of *E. praecox* supspherical oocysts**.**The *E.tenella* showed 3(15.78%) and characterized by broad ellipsoidal shape, colourless covered with tow smooth layers, micropyleabsent ,polar cap present as in figure (4.2). The *E. necatrex* showed 2(10.52%) and characterized byellipsoidal shape his colourless with smooth layer , micropyle absent and polar cap present as in figure (4.3).



A



B

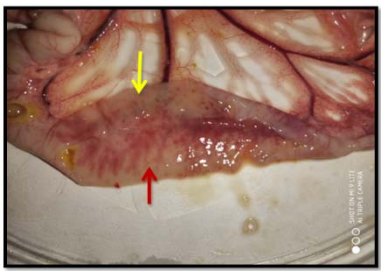
C

Figure (4.2):*E. praecox*oocyst isolated from floatation method (red arrow) 40X.

Figure (4.3): *E. tenella*oocyst isolated from floatation method (red arrow) 40X.

Figure (4.4) *E. necatrex*oocyst isolated from floatation method (red arrow) 40X.

Macroscopical results: infected birds showed there are a severe petechial hemorrhages and ecchymotic hemorrhages that diffused in the jejunum epithelium as in figure (4.5). in addition, An excessive amount of blood is retained in the tissue (hyperemia) as well as to ballooning like appearance of the intestine as in figure (4.6). There was mucoid to blood-tinged exudates (cecum) as in figure (4.7).



D

E

F

Figure (4.5): Severe petechial haemorrhages (yellow arrow) and ecchymosis haemorrhages (red arrow) that diffused in the jejunum epithelium.

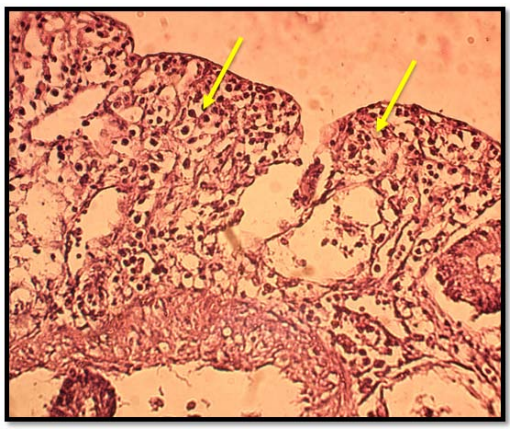
Figure (4.6): Excessive blood retention in the duodenum congestion as well as to ballooning like appearance of the duodenum (black arrow).

Figure (4.7) : Mucoid to blood-tinged exudates (cecum) (Yellow arrow)

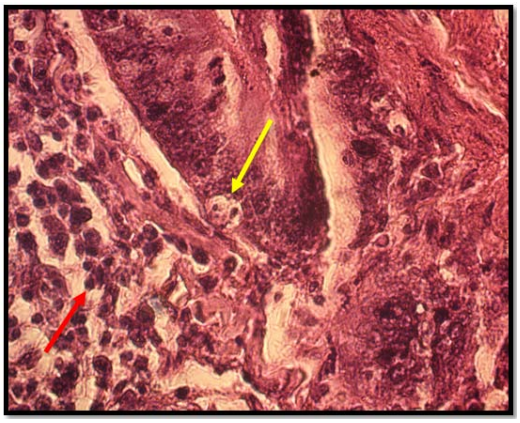
Microscopical and pathological scoring: The distribution of pathological lesions of avian coccidiosis showed that the majority of scoring appeared in score 2, then score 3 and score 1 which showed 9 (47.36%), 6 (31.57%) and 4 (21.05%) respectively, while the score didn't show any scoring degree in score 0 and score 4 as in table (4.2).While the severity score of pathological lesions showed that the majority of scoring appeared in score 1, then score 2 which showed 12 (63.15%) and 7 (36.85%) respectively, while the score didn't show any severity scoring degree in score 0, and score 4 as in table (4.2).

Table (4.2): Pathological distribution and severity scoring of infected birds.

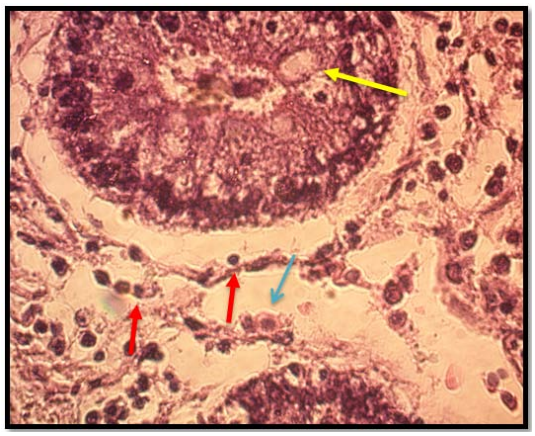
	Score 0	Score 1	Score 2	Score 3	Score 4
Distribution score (D.S.)	0 (0%)	4 (21.05%)	9 (47.36%)	6 (31.57%)	0 (0%)
Severity score (S.S)	0 (0%)	12 (63.15%)	7 (36.85%)	-	0 (0%)



Microscopical results include the intestinal mucosa a severe infiltration of inflammatory cells mainly mononuclear inflammatory cells in the more than three atrophied villi referred to distribution score (DS)= 3 and severity score (SS)=2 as in figure (4.8), in addition there are a severe infiltration of inflammatory cells mainly mononuclear inflammatory cells as well as a visible developmental stages of *Eimeria* in the villus epithelium referred to DS= 1 and SS=1 as in figure (4.9).The intestinal mucosa showed a severe infiltration of inflammatory cells and eosinophils, also there are a visible developmental stages of *Eimeria* oocysts referring to DS= 2 and SS=2 as in figure (4.10).



2



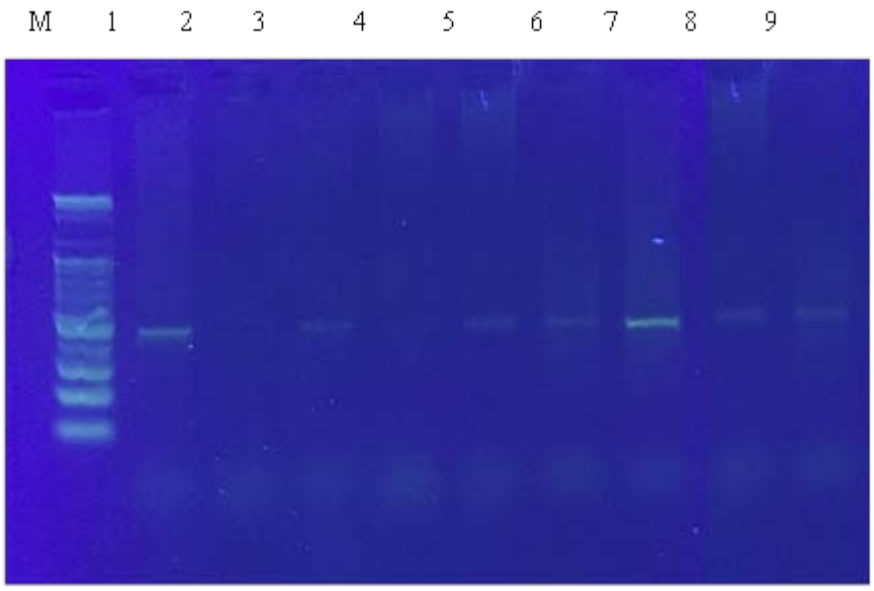
3

Figure (4.8): Histopathological section of intestinal mucosa showed a severe infiltration of inflammatory cells mainly mononuclear inflammatory cells (yellow arrows) in the more than three atrophied villi referring to DS= 3 and SS=2. H&E stain. 40X.

Figure (4.9): A significant infiltration of inflammatory cells was seen in histopathological sections of the intestinal villus. mainly mononuclear inflammatory cells (red arrows); also there are a visible developmental stages of *Eimeria* (yellow arrow) in the villus epithelium referring to DS= 1 and SS=1. H&E stain. 100X.

Figure (4.10): Histopathological section of intestinal sub mucosa showed a severe infiltration of inflammatory cells and eosinophils (red arrows);also visible there are red blood cell (blue arrows) also there are a visible developmental stages of *Eimeria* oocysts (yellow arrow) referring to DS= 2 and SS=2. H&E stain. 100X.

Molecular results (PCR detection of Eimeria genes:PCR purified for *ITS1* gene of *Eimeria* by *Eimeria* DNA extraction from sample by the forward primer and reverse primer were



performed to verify the specific present of an (approximately 520bp long) DNA product of *TSI1* gene . twenty tow total samples that were examined by PCR technic (figure 4.24).

Figure (4.11) Design of Eimeria partial ITS1 gene electrophoresis on agarose gel PCR produces (approximately 520 bp long) 1&9: Field samples were positive. Agarose gel electrophoresis of ITS1 genus-specific gene amplification,M: ladder, 1,3, and 5-7,9: positive results; 2: negative results AND 4:negative control.

IV. DISCUSSION

Clinical Diagnosis and Incidence: The clinical signs reported in this study was as following mild signs such as diarrhea, dehydration and depression in line with previous studies such as Abbas *et al*., (2013); Pérez-Fonseca *et al*., (2016) andForeyt, (2013) . The parasitic study showed higher *Eimeria* infestation in broiler more than in layer case, that may be due to crowding behaviour, oocyst accumulation, immunity was probably low and wetting of litter with watery droppings; This results agreement with Nematollahi, *et al*., (2009) and Rashid *et al*.,(2019)w' tioned that the avian coccidiosis It affects the epithelial cells of birds between the ages of 3 and 18 we(500 vding behavior, limited environment, oocyte buildup, and wetting of litter with watery droppings may all contribute to the greater incidence rate in adult broilers. This study recorded 7 species of *Eimeria*para 100 have been identified in Basrah provincethese were*E.prae(* %)*E.mitis*(15.78%)*E.tenella*(15.78%)*E.necatrix*(**10**.52%)*E.acervulina*(5.26%)*E.maxima*(5.26 %)*E.bruneti*(5.26%). The current study recorded 7 species of *Eimeria*E.praecox, *E. acervulina, E. brunetti, E. maxima, E. mitis, E.necatrix,* , and *E. tenella*; this result in lined with Gadelhaq, *et al*, (2015) who showed that the *Eimeria* species naturally infecting chickens. The present results partially disagree with the results mentioned by Al Se, Mohenned *et al* (2013) who reported that the *E.praecox* was nill infection and *E.tenella* (7.1%), *E.brunetti* (7.1%) while less than ratios *E.acervulina*(28.5%), *E.maxima* (14.2%), *E. mitis* (21.4%) and *E.necatrix* (21.4%); This result is attributed to the difference in the type of chickens reared in the farms and the local chickens and thus the difference in immunity of bird against types *Eimeria*. The infection with *Eimeria praecox* in broiler appeared higher than other *Eimeria* species, that because the *E. praecox* produced large number from schizonts in it is life cycle; this result as similar to McDougald, *et al*, (1997)*Eimeria praecox* was conclusively identified in 56% of the samples by producing typical oocysts in the faeces.

Macroscopical study: The microscopical results of infected birds showed there are a severe petechial hemorrhages and ecchymotic haemorrhages: that because of congestion of blood vessels lead to disruption it and occur hemorrhages.This result agree with Sharma *et al.*, (2015)who mentioned that blood vessel congestion indicates a disturbance, which is followed by bleeding. An excessive amount of blood is retained in the tissue (hyperaemia) as well as to ballooning like appearance of the intestine; that is due to mechanisms of inflammation it is began from reserve excessive amount of blood to site inflammation called (hyperaemia) also ballooning like appearance due to heamotaxis of white blood cell combined with odema this result agreed with El-Naggar, (2017) who mentioned that inflammation such as hyperaemia, odema, also agreed with Sharma *et al*., (2015) how The post-mortem examination revealed a severely inflated gut with haemorrhages. The intestine was frequently discovered oedematous. The blood-tinged exudate occurs as a result the distraction of blood vessels due to heavy

congestion these findings similarly mentioned by Sharma *et al*., (2015)The caecum was swollen with clotted blood and haemorrhagic patches on the caecal wall due to significant congestion of submucosal blood vessels.

The microscopical study: The microscopical study showed that the most majority of Distribution Scoring (DS) appeared in score 2 and score 3 which characterized by severe infiltration of inflammatory cells and atrophy of intestinal villi this result agreed with Sharma *et al.*, (2015)how mentioned that the invasion of heterophils and mononuclear cells, as well as villous atrophy, and the severity of the lesion may vary and distribuion with Williams *et al.*, (2009)who mentionLesions with a score of 2 or 3 are found in a small number of birds. Because of the large number of *E. praecox* results with a standard deviation (SD) of zero and the scarcity of *E.acervulina* lesions, statistical analysis was not appropriate. that is because deferent virulence species of *Eimeria*. Also agree with Chanie *et al*., (2009); Tewari & Maharana, (2011)They discovered a large number of oocysts, schizonts, and extensive tissue destruction in the caeca, which showed the severity of the E.tenella infection. The second generation schizont, which produced significant tissue damage, hemorrhage, disruption of the caecal glands, and destruction of the mucosa and muscularis layer, was reported as the most harmful stage induced by E. tenella.

The microscopical study showedalso that the most majority of Severity Scoring (SS) were score 1 and score 2 which showed visible development stages of *Eimeria* in the villus epithelium also development stages of *Eimeria* oocysts; this agreed with Amer *et al*., (2010) how revealed that the detection of developmental *Eimerial* stages in duodenum, mid intestine and cecum. also agreed with Sharma *et al*., (2015)how mentionedIn the epithelial cells of the gut, merozoites, schizonts, and microgametes were identified. Coccidial oocysts were found in the lamina propria of the intestine and the epithelial cells of the submucosal glands of the caecum.

V. CONCLUSION

The study recorded seven type of *Eimeria* species in Iraq/Basrah Provence from broiler and layer chickens also results of present study revealed that some cases of avian coccidiosis threat commercial poultry population in Iraq. It is must be improved to prevent the occurrence and dissemination of avian coccidiosis . Coccidiosis in chicken is associated with major economic losses. Finally, the significance of this research focuses the finding of coccidiosis in the region and provides molecular clues for future parasite research.

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