International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Role of Coxynil in Pathology of Coccidiosis in Broilers

N. Kalaivanan

Department of Pathology, Bombay Veterinary College, Mumbai, India

1. Introduction

Coccidiosis constitute one of the most economically important diseases chickens, disease causing chicks mortality coccidiosis ranks next to Ranikhet disease and Salmonellosis. The poultry lose hot only through mortality but also due to morbidity resulting from malnutrition lowered performance like reduced weight gains, egg production and deceased feed conversion and also though cost of anticoccidial drugs. The US poultry industry more than $1.5 billion in annual losses due to coccidiosis and in India it estimated 5.6 crores though anticoccidial in annual losses. Currently, chemotherapy is used extensively to control coccidiosis, but drug resistance in field strains, of parasites mandates development of lower drugs emerging to control this disease. The anticoccidial are sued prophylactecally rather than therapentically, this very important that the drugs are free from toxicity, not only to growing fluids who receive them through life, but also to those who may eat fresh or eggs of treated fluids and thus ingest drug residues. To prevent or delay the emergence of resistance, the idea of drug rotation has each introduced, two or three urelated drugs are used sequentally, the change from one drug to another leing made either when the diet us changes during repcausing of crop of broiler. The problem is to discover two or three such new drugs at the same time to mix together. The present study was undertaken in order to understand the "Role of Coxynil in Pathology of Coccidiosis in Broilers" chicks at department of pathology, Bombay Veterinary College, Mumbai.

2. Materials and Methods

Experimental design
One day old broiler chicks were brought Random sample poultry performance testing center, Aarey Milk Colony, Mumbai, were reared in a metal cases on were floors. A total of 120 day old broiler chicks were divided into four equal treatment groups of 30 chicks in each and were maintained under standard managemental practices for 6 weeks period. The fluids were treated as shown below and control fluids [group A and D] were fed broiler ration without anticoccidial at libitum. The [group B and C] treatment fluids were fed ration containing coxynil and libitum from 1st week dose as shown in table. For infections at 30 days of old, age, control fluids were transferred to a second room and maintained as above- mentioned.

Isolation of *Emeria tenella Oocysts*:
The oocysts were isolated by Sedimentation method from the caecal contents collected from slaughterhouse caeca which shown positive lesion for coccidiosis. Later the collected oocysts were identified as *Eimeria tenella* based on location of lesion, microscopically by shape index and also by sporulation time. The numbers of oocysts were calculated in one ml of sediment at per standard procedure.

Treatment Schedule

S. No.	*Group*	*Treatment of Coxynil*	*Oocysts challenge (age at)*	*Dose of oocysts*
1	A. Control	--	--	--
2	B. Medicated Control	@ 250 mg/kg feed	--	--
3	C. Medicated infected	@ 250 mg/kg feed	At 30 days of age	75,000/ fluid
4	D. Infected Control	--	At 30 days of age	75,000/ fluid

Sporuation of oocysts
The collected oocysts were sulyected to sporulation by the sediment was mixed with an excess of 25 percent Potassium Dichromate solution and powered into petridishes, care taken to maintain the depth of fluid at room temperature (27-32^{0}C) for 24-36 hours. The mixture was maintains oxygen requirement. The 95% sporulation was observed at 36 hours.

Oocysts challenge
The excess of Potassium Dichromate were washed with phosphate buffer saline twice or thrice by centrifugation and sedimentation method. The number of sporulated oocysts adjusted 75,000 per ml for oocysts challenge. The experimental fluids were challenged at dose of 75,000 per fluids.

Experimental observation
The following deservation was made in order to reserve "Role of Coxynil on Pathology of coccidiosis at various infection period and these observation were compared among the different groups.

1. Growth and Weight Gain
The fluids from each group weight individually at weekly interval and the mean value different groups analyzed statistically. Also the reductions in body weight gain at end of experiment and at 14th day after infection were calculated as follows.

Gain in body weight (in gms) = weight at 14th day of infection –weight 0th day of infection.

Percentage of reduction in body weight:

$$100X\,1-\frac{\Delta \text{ Body weight gain of infected group}}{\Delta \text{ Body weight gain of uninfected group}}$$

2. Clinical Symptoms, morbidity and mortality:
The experimental fluids were observed for clinical symptoms, morbidity and mortality at the time of feeding and watering at various period of observation.

The clinical symptoms were noted includes:
a) Appearance of dullness.
b) Appearance of loose droppings.
c) Appearance of mucus mixed faces and
d) Appearance of blood in droppings.

3. Test for occult blood
The droppings from 15 fluids in each treatment group were collected regularly in an 24 hours intervals and selected bendizine lost to differentiate. The degree symptoms among the treatment groups through out the infection period.

4. Packed cells volume and Haemoglobin levels:
The unclotted blood were collected by using 2% EDTA as anticoagulant on 0,4,5,6 and13th day of post infection from four fluids in each group to find out the PCV and Hb level.

5. Oocysts per gram of faces:
The space under the floor of age divided in to four equal part of each treatment groups and three grams of droppings were collected from pooled sample of respective partition separately on 0,4,6,7,8,9,10th day of post infection. The oocyst were counted by using stolls method.

6. Lesion Score:
Four fluids from each group sacrificed on 4th, 5th and 6th day of post infection. In post mortem examination caecal lesions were scored by 0 to +4 scoring systems as described earlier by Johnson and Reid (1970) as follows.

Score t1:
For a few scattered patechiae, which were reddish purple in colour on unopened caecum, which lesion may get extended into the lower small intestine between the caeca. There was no thickening of caeca wall.

Score t2:
For numerous petechiae, which apparent on the secosal surface. Bleeding, which appeared on the 5h to 6th day of infection was more marked on the mucosal surface that is typical t1 score, slight thickening of intestinal wall.

Score t3:
For more bleeding with clotting appearing in the distal end of pouch. The clot balance hyadened at the sloughed mucosal surface joined the bloody material to form core. Absence of normal caecal contents, caecal became practically non-functional. The serosa of unopened caecum showed the petechial as coalesced and eroding the entire surface.

Score t4:
For severe bleeding, much thickened caecal wall and eroding mucosal surface. The unopened caecum was distended with blood at the distal end but left contracted and shortened. Larger number of deaths occurred on 6th day infection.

The fluids, which did during observation period, were autopsied and location and severity of the lesion wee noted.

7. Histopathological study
Caeca from sacrificed fluids were fixed and preserved in 10% formalin for histopathological studies by H78E staining.

8. Haemagglutination inhibition titre against NDV:
Clotted clot collected in sterile vials from 4 fluids in each treatment group. Serum separated and 1+2 titre performed against NOV by using vaccinational strains as antigen.

Table 1: Average Body Weight of Fluids From Different Groups.

Group	A	B	C	D
1	50.4	50.4	50.4	50.4
2	124.00	124.00	124.00	124.00
3	213.83± 4.972	221.3± 6.38	223.53± 7.14	209.13± 7.64
4	436.17± 15.54	350.38± 12.57	382.52± 15.50	332.035± 15.1998
5	592.68± 23.02	634.23± 30.69	636.1± 26.04	596.035± 28.37
6	949.29± 33.99	984.07± 44.78	852.5± 40.56	683± 46.50
7	1206.78± 40.79 bd	1367± 55.59 bc	1127.44± 59.99 ab	883.18± 66.41 acf

P<0.05 Figures having common superscript did not differ significantly.

Table 2: Percentage of reduction in body weight gain at 14th day of infection (Compared with group)

A	C	D
16.19%	32.9%	60.8%

3. Result and Conclusion

1. Body weight gain:
Average weekly body weight recorded during the experimental period is presented table No.1

The result showed that numerically lower body weight at end of experiment was observed in-group D, which was infected control followed by medicated infected group.

Birds receiving coxynil at the rate of 250mg/kg of feed in the experimental period showed maximum body weight 1367±55 gm at end of sixth week in groups as against body weight of 1127.44±5999 gm in parallel groups, forever was challenged on 30th day of age with coccidial oocyst.

Along the unchallenged fluids of group A and group B, numerically highest weight noted in group B than group A, which not received neither coxynil nor oocyst. Among challenged fluids with coccidial, birds from group C shows numerically highest body weight than group D, which was untreated.

2. Reduction in body weight gain:
At end of sixth week, as well as end of 14th day of infection reduction in body weight noted in-group A among unchallenged fluids than group B, which was received

coxynil of the rate of @250mg/kg of feed. Among the challenged fluids from group C and group D, lightest percentage of reduction in weight gain noted in the group D (60.8%) than group C (32.9%) when compared with fluids from group B, which was received only coxynil throughout the experiment.

The above result indicated that 16.19% weights gain more in- group B than group A, among the unchallenged fluids. Among the unchallenged fluids 41.6% weight gain higher in-group C than group D, which was untreated. Comparatively 60.8% more weight gain noted in-group B than infected unchallenged fluids.

It was noted that at 5th day of post infection all dead fluids had more than 1 kg. Body weight individually in- group both C as well as D and also all live fluids had lesser than kg in group D. Due to growth prompting effect of coxynil more numbers of fluids in group C had body weight of more than 1.2kg than in group D. This pattern of dead suggestive for growth stress and also variation in strains of fluids. This leads to reduction in body weight noted higher in both challenged group.

At end of sixth week average body weight around 1300gm, this value lesser than normal average body weight of broilers at end of sixth week and big variation in weight gain in a group due to mixed strains of broiler chicks in this trial.

Table 3: Clinical Symptoms in different treatment groups

Sr. No.	*Description*	*A*	*B*	*C*	*D*
1	Appearance of dullness	--	--	3DPI	3DPI
2	Appearance of loose dropping	--	--	4 DPI	4 DPI
3	Loose droppings (degree)	+	--	++	++++
4	Appearance of blood in droppings (Occult)	--	--	3DPI	3DPI
5	Appearance of blood in droppings on 3DPI (Occult)	--	--	33%	33%
6	Blood in droppings (Degree) on 6 DPI (gross)	--	--	+++	++++
7	Appearance of mucus	+	--	++	+++
8	Disappearance of blood in droppings (Gross)	--	--	8DPI	9DPI
9	Disappearance of blood in droppings on 7 DPI (Occult)	--	--	60%	100%
10	Disappearance of blood in droppings on 8 DPI (Occult)	--	--	6.66%	45.45%
11	Disappearance of blood in droppings on 9 DPI (Occult)	--	--	1%	10%

Table 3.1 Mortality Percentage of different treatment group

Sr. No.	DPI	A	B	C	D
1	5	0	0	05	15
2	6	0	0	--	06
Total		0	0	7	21
Percentage		0	0	16%	66.67%

2. Clinical Symptoms

Clinical symptoms of Different treatment groups were recorded and presented in table No.3.

Birds were apparently normal until 3DPI, a mild degree of dullness noted in birds from both challenged groups from 3rd day of post infection. Birds from groups which were untreated and challenged with oocyst (group A and D) showed mild diarrhoea on 4th day of post infection. The degree of diarrhoea and mucus slightly higher in fluids, which challenged untreated (group D) than group C and A.

Test for occult blood became positive on 3rd day of post infection. But percentage among the groups noted were 33% in-group C and 73% in-group D. Grossly, blood tinched droppings noted on 5 day of post infection and its degree slightly higher in group D than group C. the test became negative for occult blood at 9th day post infection in group C while 10% positive in group D.

3. Mortality:

Morality were recorded and presented in table 3%. The highest 66.67%mortality were recorded group D, which were challenged, untreated, this reduced to 16% in group C which were medicated challenged. Highest percentage of mortality were recorded on 5th day of post infection in groups both C and D.

Table 4: Oocyst per gram of faeces (in thousands) of fluids from different treatment groups

Group/DPI	A	B	C	D
0	O (+)^{c}	O^{c}	O^{c}	O^{c}
4	O (+)^{c}	O^{c}	O^{c}	O^{c}
6	O (+)^{c}	O^{c}	2.245±0.254^{A}	3.76±0.3074^{B}
7	O (+)^{c}	O^{c}	29.1275±0.64^{A}	52.6±4.08^{A}
8	O (+)^{c}	O^{c}	8.898±0.53^{A}	19.275±4.12^{A}
9	O (+)^{c}	O^{c}	1.19±0.36^{A}	5.52±0.943^{B}
10	O (+)^{c}	O^{c}	0.43±0.17^{A}	1.088±0.34^{B}
R/mean	O (+)^{c}	O^{c}	8.38	16.448

P<0.05 Figures with common superscript did not differ significantly with in row.

4. Coccidial oocyst gram of faeces:

The oocyst per gram of faeces recorded and result were presented in Table 4.

In control birds oocyst discharge noted in non-significant amount. However, no oocyst were deleted by stolls method in experimental birds not challenged with oocyst and maintained did not containing coxynil over all highest oocyst count were Recorded n infected control group (D) 16.448. Result of present study indicate efficacy of coxynil medication on challenged birds as indicated significant reduction in oocyst count (8.38) of group C as against concentrated group D. Also, 6th, 9th and 10th day of post infection in- group C oocyst count significantly declined in comparison to group D, which were received only oocyst.

Table 6: PCV levels of birds from different group

Group/DPI	A	B	C	D
0	30.5 ±0.65	30±0.71	32.38±0.24	30.75±0.75
4	30.25±0.63	30.5±0.65	25±0.41	23.25±0.48
5	33±1.47	29.5±0.29	18.5±0.96	14.25±0.75
6	30.5±1.04	31±0.58	15.25±0.85	13.75±0.75
13	30.75±0.48	31±0.91	24.5±0.65	28.25±0.63
Treatment Mean	31^{A}	30.4^{A}	23^{B}	22.05^{B}

P<0.05, Figures with common superscript did not differ significantly with in row.

Table 7: Hb levels of birds from different treatment groups

Group DPI	A	B	C	D
0	6.95±0.05	7.125±0.125	7.38±0.24	7±0.41
4	7.75±0.323	7.875±0.427	5.625±0.24	4.375±0.24
5	7.88±0.43	0.575±0.33	3.75±0.32	2.75±0.32
6	7.25±0.25	7.87±0.43	3.375±0.38	2.375±0.24
13	7.75±0.32	7.725±0.28	6.25±0.14	6±0.20
R/Mean	7.52	7.64	5.277	4.50

P<0.05 Figures with common superscript did not differ significantly with in row.

5. Packed cell volume and Haemoglobin level:
PCV and Hb level of birds at different periods of observation mentioned in Table No 6 and 7 respectively.

The mean value of birds from unchallenged groups themselves did not show significant different, like wise value from birds, which are challenged also did not show significant difference.

When comparing the PCV and Hb level birds at a specific period observation correlate with lesion scores.

Table 8: Caecal lesion score of birds from different groups

Group DPI	A	B	C	D
4	0	0	1±0	1.75±0.25
5	0	0	1.75±0.25	2.75±0.25
6	0	0	2.25±0.476	3.75±0.25
13	0	0	0.5±0.289	0.75±0.479
Treatment Mean	0^{A}	0^{A}	1.375^{C}	2.25^{D}

P<0.05 Figures with common superscript did not differ significantly with in row.

6. Lesion Score/Post Mortem Observation:
Result of lesion scores recorded and presented in table 8.

The above result indicates that unchallenged birds in group A and B did not show any lesion for scoring at various period of observation.

There is significant reduction in lesion (treatment mean) recorded in- group C against group D and also at various period at observation group C received Lesser than birds from group D.

As shown in photograph of caeca (gross) at 4^{th} day off post infection, caeca larger in group D an group A than caeca from group C and B. The caeca from group B noted shortened and slightly thickened caecal wall as shown in photograph at various period of observation.

At later period of observation (13^{th} day nad6th day post infection) in opened caeca from group C revealed their membrane like core formation around the clotted blood and faecal matters to protect the caecal mucosa from further infection and secondary infection to promote quicker healing of lesions.

7. Histopathological observations
Histopathological observation of caecal section of birds from group A revealed normal caecal glands with mucosal integrity in an orderly arrangement with lesser degree of proliferation into that lamina propria. Some rare section revealed 2^{nd} generation Schizonts with in-orderly arrangement of epithelial cells in mucosa and minimum loss of its mucosal integrity. Few rare sections showed microgranulocyte with its 2^{nd} generation Schizonts in later period of observation. This changes observed due to accidental contamination.

Caecal sections of birds from group B at various period of observation revealed hyperplastic changes into the caecal glands with increased activity and increased secretary vesicles in epithelial cells of the glands. The focal aggrevation of lymphoid cells, proliferative changes into the lamina propria and infiltration mononuclear cells with moderate increased in size of lamina propria.

Rare section from group B revealed, the epithelial cells loaded with number of arrested 2^{nd} generation Schizonts as a giant cells, also its mucosal integrity maintained. The number such arrested 2^{nd} generation Schizonts observed more per high power field when compared with section from infected control. This more number due to subsequent evidential oocyst contamination and smaller the size of Schizonts as it was arrested.

Section from group D and C revealed loss of integrity of number, arrangement of epithelial layers with hemorrhage and sloughed epithelial cells with endogenous stages into the lumen. At 4 day of infection the epithelial cells loaded with development and developed 2^{nd} generation Schizonts, mature 2^{nd} generation Schizonts with merozoites at 5thday of infection and micro gramatocyte, epithelial cells loaded with oocyst and also into the lumen at 6^{th} day of post infection were observed on sections from group C an D both.

Sections from group C revealed lesser damage with undamaged caecal glands with hyperplastic, secretary vesicular activity and some arrested endogenous 2^{nd} generation Schizonts than group D. In an average number of endogenous stage at various period of observation observed lesser in group C than group D.

From the Histopathological slides studied, it is concluded that number arrested 2^{nd} generation Schizonts noted in group B of accidental contaminate infection and in group C which were medicated and challenged. In group B in an contaminate infection revealed only arrested 2^{nd} generation Schizonts and also in it further life cycle failed to developed into oocyst. This arrested life cycle cleared when no oocyst discharge in- group B and significant reduction in oocyst discharge in group C taking into the account.

This knowledge on arrested endogenous stages and lesser oocyst discharge assumed the made of action of drug either on IInd generation Schizonts (or) on sexual stage. The arrested 2^{nd} generation Schizonts may be suggested the main target of the drug and it may be suggestive for coccidiostatic action on 2^{nd} generation Schizonts.

Table 9: Mean HI titre (log) against New Castle disease virus in the birds from different treatment groups

Days	A	B	C	D
21	^{A}2.83±0.30	^{C}4.83±0.40	^{B}4.16±0.30	^{A}2.83±0.30
28	^{AB}3.16±0.47	^{B}3.50±0.42	^{A}3.33±0.16	^{AB}3.33±0.42
35	^{B}6.33±0.21	^{D}8.33±0.33	^{B}6.33±0.21	^{A}5.50±0.22
42	^{C}5.66±0.33	^{E}7.16±0.30	^{C}5.66±0.33	^{A}4.16±0.30
Treatment Mean	^{ab}4.495±0.88	^{e}5.955±1.095	^{bc}4.85±0.69	^{a}3.96±0.58

P<0.01 Figures with common superscript did not differ significantly with in row.

8. Antibody medicated immune response –HI titre

In the present experiment overall highest mean HI titre against New Castle disease virus was observed in group B followed by C and A. Where as least titre was recorded in-group D.

In chicks, maintained on feed supplementation with coxynil at the rate of 250 mg/kg shows higher HI titre in comparison to control chicks of group A at various period of observation. Among the experimental birds challenged with oocyst significantly lower HI titre was observed in coxynil concentrated group D compared to group C.

4. Summary and Conclusion

On the basis of observation recorded during the experimental study, it is concluded that coxynil is interfering with developmental stages of coccidial life cycle as evident by arrested endogenous stages in rare section of group C and group B (accidental infection, lowered oocyst in-group C oocyst discharge were not recorded in group B)

It also concluded that coxynil have effect in improving performance of broiler in weight gain as noted 16.19% more weight gain in- group B against group A, 41.6% higher weight gain of group C against group D and 60.8% higher weight gain of group B against group D which were infected.

Immune modulation effect indicated as by significant high HI titre under recorded in- group B against the rest of the group and in-group C against group D.
It also noted that significant reduction in mortality percentage to 16.2(%) in- group C against group D (66.67%).

However, coxynil demonstrated growth promoting effect, immune modulation effect and coccidiostatic effect. Even though coxynil pursuing above mentioned effect, it failed to control the mortality and damage to caecal mucosa in- group C, which may be due to highest dose of oocyst challenge and for that very high level of infection - insufficient dose of coxynil medication.