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Application of Lesion Scoring Technique for the Assessment of Pathology and Treatment of Coccidiosis in Broiler Chicks

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ABSTRACT

This study was conducted to assess the pathology of coccidiosis and its response to ionophore treatment rusing lesion scoring technique in experimentally infected broiler chicks. For this purpose, day-old broiler chicks were kept in floor pen condition and each inoculated with 50,000 sporulated oocysts of mixed field isolates of *Eimeria* species. The birds of infected groups developed characteristic acute stages of coccidiosis within a period of 3-4 days well supported by the post-mortem observations. The lesions included ballooning of intestine, serosal pinpoint hemorrhages, thickness of intestinal wall, coagulative necrosis, white plaques/streaks, mucosal pinpoint haemorrhages, mucoid and bloody faecal contents. Lasalosid sodium was better in curtailing the lesions as compared to monensin and slainomycin as assessed by lesion scoring technique.

Key Words: Lesion scoring technique, *Eimeria* species, ionophore antibiotics

INTRODUCTION

Coccidiosis is a major disease problem, which attained much concern in the recent years owing to the intensification of world poultry industry. The disease is of varying intensity depending on the species of *Eimeria* involved and climatic conditions. Therefore, it is deemed necessary to gain knowledge about the pathology of local isolates of *Eimeria* in order to devise its effective control.

This paper describes the numerical ranking of gross lesions associated with mixed *Eimeria* infection at various stages of the disease; and the comparative of efficacy of some ionophores in reducing the pathological lesions of the disease.

MATERIALS AND METHODS

Extraction and sporulation of oocysts. Different *Eimeria* species were collected from the infected guts (n = 22) of birds naturally infected with coccidiosis and scanned for location of gross lesion and the presence of oocysts. The contents of infected guts and scrapings were mixed and soaked overnight in 2.5% Potassium dichromate solution. The suspension was filtered through a fine sieve and filtrate was centrifuged (1500 rpm) for 2-3 minutes. The supernatant was discarded and sediment was re-suspended in saturated solution of Sodium chloride and centrifuged. The top layer was piped out, mixed with water, kept overnight and supernatant was discarded. The sediment containing oocysts was re-suspended in 2.5% Potassium dichromate solution (Solusby, 1982). The solution containing oocysts from different field isolates of *Eimeria* was poured into petridishes and kept at 30°C for 24-72 h with forced aeration and humidity. The spourlated oocysts were stored at 4°C in Potassium dichromate solution (Soulsby, 1982; Graat *et al*., 1994) and adjusted to 50,000 oocysts per 2 mL of inoculum (Hodgson, 1970; Long *et al*., 1976) for experimental inoculation to birds.

Experimental birds. Day-old broiler chicks (n = 150) were kept in floor pen condition for 30 days without administering them any coccidiostat. These birds were divided into five different groups (A, B, C, D and E) and each group was further divided in three sub groups of 10 birds each. Birds in groups A, B, C and D were inoculated with 50,000 sporulated oocysts orally into the crop and the birds of group E were administered tap water of equal amount. Birds of group A, B and C were given broiler ration treated with lasalocid sodium, monensin and salinomycin, respectively on day 3rd post-infection. Birds of group D were kept as infected untreated while group E as uninfected untreated controls.

Lesion scoring technique. To assess the progress of the disease, a comprehensive post-mortem examination was performed at five-day intervals on six birds from each group. The entire intestine was pulled out unbroken from the slaughtered birds. The whole gut was divided into four parts, duodenum and jejunum as upper intestine (U), middle intestine (M), ileum and rectum as lower intestine (L) and caecum (C). Beginning with the duodenum the intestine was slit opened and both the mucosal and unopened serosal surfaces were examined for the presence of any lesions due to coccidiosis. The lesions used in lesion scoring technique using method of Johnson and Reid (1970) with modifications included ballooning, pinpoint haemorrhages; thickening of intestine, mucosal petechiation, coagulative necrosis, white streaks; bloody faecal contents, mucoid contents from serosal surface, mucosal surface and intestinal contents. Lesion scoring was conducted under direct sunlight and scoring scale was devised from 0 to +4 as

follows: 0 = no lesion, 1= mild lesion, 2= moderate lesion, 3= severe lesion, 4= extremely severe lesion/death

RESULTS AND DISCUSSION

All the birds of infected groups (A, B, C, & D) developed characteristic acute stages of coccidiosis within a period of 3-4 days which were also supported by the post-mortem observations in the latter stages. These lesions included ballooning of intestine, serosal pinpoint haemorrhages, thickness of intestinal wall, coagulative necrosis, white plaques/streaks, mucosal pinpoint haemorrhages, mucoid and bloody faecal contents. The results of post-mortem lesions scoring of birds disclosed that the average development of intestinal ballooning was better differentiated in the upper and middle portion of intestine than of the lower intestine and caecum of all infected groups as compared to the control (uninfected untreated). Significantly higher differences ($P<0.001$) in scores of ballooning were observed among all the infected groups and also within each infected group at different time spaces (Table I).

Table I. Effect of ionophores medication on lesion score (ballooning) in broilers infected with *Eimeria* species

Days (interval)	Group A	Group B	Group C	Group D
30-35				
U	0.48±0.22	0.56±0.17	0.88±0.22	0.44±0.24
M	1.92±0.26	2.08±0.15	2.44±0.45	2.25±0.29
L	1.56±0.17	1.24±0.33	1.04±0.12	1.00±0.44
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
35-40				
U	0.16±0.07	0.20±0.13	0.25±0.12	0.56±0.24
M	1.80±0.14	0.60±0.17	2.61±0.14	3.02±0.20
L	0.72±0.32	0.00±0.00	1.04±0.15	1.24±0.21
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
40-45				
U	0.00±0.00	0.00±0.00	0.24±0.12	0.64±0.27
M	1.76±0.33	0.92±0.19	1.64±0.25	2.14±0.59
L	0.72±0.27	0.52±0.19	0.56±0.21	1.85±0.50
C	0.00±0.00	0.00±0.00	0.00±0.00	0.32±0.32
45-50				
U	0.20±0.06	0.00±0.00	0.00±0.00	0.00±0.00
M	0.27±0.10	0.48±0.19	1.80±0.42	1.98±0.55
L	0.28±0.23	0.60±0.17	1.64±0.28	1.20±0.24
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

U= Duodenum and jejunum as upper intestine, M= Middle intestine, L= Ileum and rectum as lower intestine, C= Caecum

Post-coccidial development of serosal pinpoint haemorrhages (Table II) were demonstrated at all parts of intestine in all birds of infected groups. These haemorrhages were well marked at the middle and lower parts of intestine. Highly significant difference was evident in scores of pinpoint haemorrhages when matched among the infected groups. Differences among infected groups in curtailing the lesions lasted till the termination of experiment. Lasalosid sodium was better in curtailing the lesions as compared to other ionophores antibiotics. Post coccidial elevation in scores of thickness of intestinal mucosa was recorded (Table III).

Table II. Effect of ionophores medication on lesion score (serosal pin point haemorrhages) in broilers infected with *Eimeria* species

Days (interval)	Group A	Group B	Group C	Group D
30-35				
U	0.24±0.14	0.20±0.12	0.88±0.10	0.64±0.11
M	1.00±0.14	2.04±0.39	2.48±0.18	1.00±0.14
L	1.36±0.29	1.48±0.33	1.08±0.32	1.64±0.44
C	1.00±0.20	0.48±0.25	0.00±0.00	1.44±0.27
35-40				
U	0.60±0.32	0.80±0.80	0.20±0.00	0.36±0.15
M	1.76±0.24	1.76±0.46	2.56±0.33	0.24±0.22
L	0.96±0.25	2.40±0.29	1.04±0.29	2.44±0.13
C	0.00±0.00	1.96±0.17	0.00±0.00	3.04±0.09
40-45				
U	0.00±0.00	0.00±0.00	0.20±0.11	0.24±0.09
M	0.36±0.24	1.28±0.22	1.56±0.14	2.64±0.16
L	0.20±0.06	1.40±0.14	0.60±0.14	1.44±0.24
C	0.00±0.00	0.76±0.14	0.00±0.00	0.80±0.20
45-50				
U	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
M	0.00±0.00	0.64±0.17	1.80±0.11	1.64±0.14
L	0.00±0.00	0.40±0.21	1.64±0.07	2.08±0.54
C	0.00±0.00	0.80±0.41	0.00±0.00	1.00±0.11

Table III. Effect of ionophores medication on lesions score (intestinal thickness) in broilers infected with *Eimeria* species

Days (interval)	Group A	Group B	Group C	Group D
30-35				
U	2.20±0.14	3.04±0.14	3.20±0.14	3.16±0.23
M	2.08±0.21	2.44±0.17	3.20±0.11	3.24±0.16
L	1.40±0.25	1.40±0.22	2.12±0.10	1.16±0.13
C	0.68±0.18	0.48±0.15	1.00±0.14	0.16±0.13
35-40				
U	1.40±0.30	2.20±0.26	1.56±0.31	1.64±0.25
M	1.00±0.24	1.40±0.22	0.76±0.30	0.56±0.15
L	1.56±0.22	1.40±0.22	0.80±0.21	0.88±0.16
C	0.60±0.14	0.24±0.16	1.28±0.26	1.16±0.17
40-45				
U	1.8±0.14	1.68±0.18	1.4±0.35	1.76±0.26
M	0.80±0.14	1.00±0.19	0.52±0.22	0.76±0.16
L	1.2±0.34	1.48±0.13	0.68±0.18	1.84±0.15
C	1.00±0.24	0.00±0.00	0.24±0.16	0.24±0.10
45-50				
U	2.28±0.20	2.04±0.19	1.16±0.25	1.16±0.18
M	0.80±0.17	1.00±0.26	0.92±0.15	0.88±0.26
L	1.24±0.22	1.80±0.15	0.60±0.14	1.48±0.30
C	3.04±0.16	0.20±0.15	0.24±0.16	0.24±0.18

The mucosal thickness was at its climax on day 35 post-experiment in all infected groups. The mucosal thickness and their scores gradually decreased towards the end of experiment following the use of ionophores treatment. No significant difference in mucosal thickness scores was observed among *Eimeria* infected groups. There was development of mucosal petechiation (Table IV) recorded in different treatment groups, which indicated the presence of coccidiosis caused by *E. necatrix*, *E. tenella*, *E. maxima* and *E. brunetti*.

Table IV. Effect of ionophores medication on lesions score (mucosal petechiation) in broilers infected with *Eimeria* species

Days (interval)	Group A	Group B	Group C	Group D
30-35				
U	0.20±0.11	0.20±0.15	0.28±0.10	0.72±0.10
M	0.76±0.07	1.56±0.14	0.64±0.15	1.21±0.14
L	1.24±0.13	1.00±0.24	0.64±0.19	0.88±0.24
C	0.64±0.19	0.20±0.02	0.00±0.00	0.20±0.00
35-40				
U	0.44±0.10	0.44±0.12	0.00±0.00	0.80±0.17
M	0.80±0.06	0.84±0.15	0.80±0.13	0.64±0.12
L	0.32±0.05	1.80±0.21	0.68±0.10	1.40±0.20
C	0.20±0.15	1.24±0.13	0.20±0.02	1.20±0.19
40-45				
U	0.00±0.00	0.00±0.00	0.60±0.06	1.20±0.06
M	0.40±0.09	0.68±0.15	0.64±0.15	0.80±0.24
L	0.00±0.00	1.00±0.24	0.64±0.12	1.00±0.06
C	0.00±0.00	0.88±0.16	0.00±0.00	0.56±0.16
45-50				
U	0.00±0.00	0.20±0.06	0.40±0.04	1.08±0.15
M	0.00±0.00	0.44±0.07	0.20±0.06	1.00±0.29
L	0.00±0.00	0.24±0.02	0.00±0.00	0.40±0.03
C	0.00±0.00	0.68±0.06	0.72±0.05	1.08±0.10

The development of petechiation was well noted at the middle and lower potions of intestine, as well as in caeca. Significant differences in petechiation scores were displayed between the birds of infected treated and infected untreated groups. Treatment with salinomycin and lasalocid gave better results in reducing scores of mucosal petechiation than monensin.

A well-marked coagulative necrosis and its high scores were recorded in the lower intestine comes after the middle intestine in all inoculated groups. Presence of this type of lesions, which is a common post-mortem finding of *E. brunetti* infection. Increased score of coagulative necrosis was significantly distinct in birds of group D compared to groups A, B and C (Table V). White plaques/streaks were more pronounced in the upper and middle portion of intestine (Table VI), indicating the presence of *E. acervulina* infection along with other species of *Eimeria*. In sever infection these streaks were coalescent in the form of a ladder on the intestinal mucosa. Increase in the lesion intensity was significant in the infected group D **Table V. Effect of ionophores medication on lesions score (coagulative necrosis) in broilers infected with *Eimeria* species**

Days (interval)	Group A	Group B	Group C	Group D
30-35				
U	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
M	1.44±0.23	1.84±0.12	2.00±0.14	2.04±0.00
L	1.80±0.74	2.00±0.83	2.24±0.16	3.16±0.00
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
35-40				
U	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
M	0.84±0.37	0.80±0.22	1.40±0.37	1.00±0.00
L	1.80±0.14	1.88±0.10	1.40±0.15	2.00±0.11
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
40-45				
U	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
M	0.20±0.15	1.24±0.07	2.00±0.14	1.04±0.28
L	0.56±0.25	2.16±0.56	0.20±0.00	1.84±0.07
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
45-50				
U	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
M	0.88±0.24	0.80±0.20	0.40±0.13	1.00±0.28
L	1.24±0.17	1.00±0.28	0.60±0.14	1.64±0.13
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table VI. Effect of ionophores medication on lesions score (white plaques/streaks) in broilers infected with *Eimeria* species

Days (interval)	Group A	Group B	Group C	Group D
30-35				
U	0.00±0.00	0.44±0.16	1.60±0.12	2.4±0.14
M	0.80±0.10	0.00±0.00	2.00±0.14	2.20±0.33
L	0.00±0.00	0.00±0.00	0.20±0.15	0.00±0.00
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
35-40				
U	1.00±0.14	2.24±0.12	1.20±0.15	2.00±0.09
M	0.60±0.32	1.00±0.15	0.40±0.07	1.00±0.14
L	0.00±0.00	0.02±0.07	0.00±0.00	0.00±0.00
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
40-45				
U	0.40±0.00	2.20±0.32	1.00±0.28	2.00±0.36
M	0.00±0.00	1.00±0.20	0.20±0.15	1.00±0.60
L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
45-50				
U	0.00±0.00	0.88±0.16	0.00±0.00	1.64±0.44
M	0.00±0.00	0.00±0.00	0.00±0.00	0.22±0.04
L	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

as compared to treated groups. Mucoid type faecal contents were recorded and supported by their scores of severity in birds of all groups at different times of the experiment (Table VII). Bloody faecal contents were noted in the form of clotted and frank blood in middle, lower and caecal portions of intestine only and scored separately on the basis of their severity. This type of post- **Table VII. Effect**

of ionophores medication on lesions score (mucoid faecal contents) in broilers infected with *Eimeria* species

Days (interval)	Group A	Group B	Group C	Group D
30-35				
U	2.80±0.06	3.00±0.06	2.00±0.09	2.44±0.13
M	0.80±0.14	2.40±0.14	1.40±0.13	2.40±0.16
L	1.00±0.09	1.04±0.33	1.24±0.15	2.00±0.14
C	2.40±0.14	1.44±0.17	0.60±0.09	0.36±0.07
35-40				
U	1.60±0.06	1.04±0.47	1.64±0.12	1.44±0.28
M	1.56±0.12	0.80±0.11	0.80±0.06	0.80±0.17
L	1.36±0.13	0.84±0.29	0.66±0.14	1.20±0.30
C	0.20±0.06	0.32±0.12	0.20±0.15	1.60±0.14
40-45				
U	0.64±0.13	2.24±0.22	1.60±0.43	1.12±0.08
M	0.00±0.00	0.20±0.00	0.80±0.25	0.64±0.07
L	0.99±0.12	0.40±0.06	0.80±0.13	0.48±0.10
C	0.00±0.00	0.44±0.15	0.60±0.11	1.16±0.15
45-50				
U	0.00±0.00	0.40±0.06	0.00±0.00	0.88±0.10
M	0.00±0.00	0.44±0.05	0.40±0.10	0.80±0.06
L	1.00±0.09	0.60±0.08	0.00±0.00	1.20±0.14
C	0.00±0.00	0.00±0.00	0.44±0.10	1.64±0.12

score (bloody faecal contents) in broilers infected with *Eimeria* species

Days (interval)	Group A	Group B	Group C	Group D
30-35				
U	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
M	1.84±0.12	0.64±0.12	0.44±0.16	1.64±0.23
L	0.40±0.06	0.00±0.00	0.00±0.00	0.80±0.33
C	0.00±0.00	3.44±0.15	2.08±0.10	2.76±0.17
35-40				
U	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
M	1.80±0.21	1.64±0.17	1.76±0.07	2.24±0.12
L	0.84±0.07	0.68±0.08	1.04±0.12	0.64±0.04
C	1.24±0.13	2.80±0.14	2.44±0.32	2.84±0.12
40-45				
U	0.00±0.00	0.00±0.00	0.00±0.10	0.24±0.15
M	0.64±0.12	0.64±0.07	0.88±0.10	2.04±0.15
L	0.00±0.00	0.44±0.07	0.40±0.13	0.84±0.13
C	1.24±0.15	2.24±0.12	1.60±0.13	2.64±0.19
45-50				
U	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
M	0.00±0.00	0.64±0.07	0.20±0.15	1.24±0.26
L	0.20±0.06	0.44±0.07	0.00±0.00	0.84±0.04
C	0.00±0.00	2.26±0.12	0.40±0.22	1.04±0.50

mortem lesions represents the presence of *E. brunetti*, *E. necatrix* and *E. tenella.* Treatment of birds with lasalocid sodium presented a better reduction in scores of bloody faecal contents than birds in other groups treated with either salinomycin or monensin. A significant difference in scores of bloody faecal contents was also noted between the ionophores treated groups (A, B and C) and infected untreated group D (Table VIII).

These findings indicated that the disease is mostly induced with mixed *Eimeria* spp. and produced substantial post-mortem lesions at different parts of intestine of approximately all birds groups, either infected treated or infected untreated. Further lesions once formed persisted till the termination of the experiment. However, their scores were at decline as observed at different times spaces. The ionophore antibiotics used in this experiment assisted positively in reduction of the post-mortem lesions but could not completely abolished them during the experimentation period. The statistical analysis of lesion scoring revealed noticeable differences between the infected treated and infected untreated bird groups. A marked decrease in lesion scores was observed within each ionophores antibiotic treated group with the passage of time. This decrease in lesion scoring was much pronounced in birds in lasalocid treated group compared to infected treated group B and C and infected untreated group D in which the higher lesion score lingered till the cessation of experiment. Comparing to monensin and salinomycin treated groups, the latter appeared better in over powering the coccidial lesions (Bains, 1980; Rotibi *et al*., 1989; Mounz *et al*., 1993). In contrary, Migaki *et al*. (1979), Weber *et al*. (1985) and Salisch and Shakshouk (1989) reported reliable effects of both salinomycin and monensin in controlling post-mortem lesions as well as the disease compared with other ionophores. This could be due to the variable effects of different ionophores on different *Eimeria* species. In Pakistan, ionophores particularly monensin and salinomycin are extensively used as feed added coccidiostat for the control of coccidiosis (Nabi, 1996). The different isolates of *Eimeria* spp. used in this experiment were collected from the natural field outbreaks. The lower efficacy of these two ionophores in the treatment of coccidiosis as observed in the present investigation could be ascribed to the previous resistance conceivably might had developed by *Eimeria* spp. (Raether & Dost, 1985; Chapman & Hacker, 1994). The findings of the present experiment symbolized that the lesions scoring technique could be correlated to the severity of disease and identification of species of *Eimeria* infection.

Based on these results, it could also be recapitulated that the isolates used in the experiment contained *E. acervulina*, *E. necatrix*, *E. maxima*, *E. brunetti* and *E. tenella* (Johnson & Reid, 1970; Brewer & Kowalski, 1970).

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