

## Control of Coccidiosis in Calves by Vaccination

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### Abstract

The immunizing effect of inactivated sporulated oocyst and inactivated sonicated vaccines against bovine coccidiosis was observed in calves. Indirect haemagglutination (IHA) test was developed for detecting antibodies to coccidian. Serum antibody levels in calves were measured against soluble oocyst (sporulated) antigen. IHA antibody titer was significantly higher ( $P < 0.05$ ) in calves vaccinated with inactivated sonicated vaccines as compared to the calves vaccinated with inactivated sporulated vaccines. Results of the challenge experiments indicated that the inactivated sonicated vaccine gave protection to the challenge calves as immune calves contained high level of antibodies that resisted heavy dose of challenge. Disease was observed in control group (non-vaccinated) calves post challenge whereas vaccinated remained healthy. Then the oocyst count per gram of faeces was significantly higher ( $P < 0.05$ ) in control group (unvaccinated) as compared with the vaccinated groups.

**Keywords:** Calves; Coccidiosis; Vaccination; Sporulated oocyst

### Introduction

Coccidiosis, a protozoal disease of many mammalian, and all domestic livestock species, is caused by infection with species of the genera *Eimeria* or *Isospora*. Clinically it is characterized by enteritis although subclinical infections are frequent. The disease is relatively common in sheep, goats and cattle, rare in horses [1]. The overall prevalence of coccidia was 47.1%, with the highest prevalence in <4 months old calves (51.8%) and the lowest in >12 months old cattle (27.0%). The number of oocysts per gram of feces was significantly higher in young calves than in weaners and adults [2]. Eleven species of *Eimeria* infecting the host, i.e., *Eimeria alabamensis*, *Eimeria auburnensis*, *Eimeria bovis*, *Eimeria brasiliensis*, *Eimeria bukidnonensis*, *Eimeria canadensis*, *Eimeria cylindrica*, *Eimeria ellipsoidal*, *Eimeria pellita*, *Eimeria subspherica*, and *Eimeria zuernii*. The typical host for all isolated species is cattle. The most prevalent species was *E. bovis* (29.7%), while *E. brasiliensis* was the rarest (0.5%). Age-related analysis revealed a higher percentage of *Eimeria* spp. positive samples and higher OPG values in bison under 1 year old as compared to older individuals (93.3% and 50-4050; 37.3% and 50-550, respectively). Additionally, greater eimerian species diversity was present among calves in comparison with older bison [3]. Coccidiosis produces bloody scours, bloody diarrhea, loss of weight and death. Most cases of Coccidiosis occur during winter but outbreaks may occur sporadically throughout the year. Bovine Coccidiosis occurs most frequently in calves between six-to-twelve months of age [4]. The most serious losses are seen in dairy herds where large numbers of calves are kept along with older cattle carriers [5]. Calves become infected by ingesting sporulated oocysts along with their food and water. The severity of disease in calves depends upon the number of sporulated oocysts they ingest. No symptoms are evident if few oocysts are ingested. Disease is severe if large numbers of oocysts are ingested. In some cases death may occur. Sub clinical infection may cause

retarded growth. Overcrowding, poor sanitation and poor nutrition are contributing factors for coccidiosis [6]. Out breaks of coccidiosis in calves and feeder cattle may be handled by mass medication using sulfonamides, amprolium or monensin added in feed or water [4]. Although it is possible to immunize cattle artificially however, the development of commercial vaccines appears difficult and vaccination is not available as an alternative for treatment currently and in the foreseeable future [7]. At present coccidiosis in cattle has neither given importance in most of developing countries, Keeping in view the importance of disease in calves, this research project was design to evaluate an experimental vaccine as a potential candidate of control program.

### Materials and Methods

#### Vaccine Preparation

**Culturing of coccidial oocyst:** Oocysts of *Eimeria bovis*, recovered from the naturally infected calves were sporulated [8]. The fecal samples containing oocysts were placed in Petri-dishes containing 2.5 percent potassium dichromate ( $K_2Cr_2O_7$ ) solution. These Petri dishes were then incubated at 30°C and sporulation time was recorded. Potassium dichromate solution was used as preservative. The examination for recording the sporulation time was initiated at least 12 hours post incubation of the Petri dishes and then continued every day until all the oocysts sporulated.

**Counting of sporulated oocysts:** Oocysts in 1 ml of faecal suspension were counted following the McMaster's counting technique [9].

**Harvesting of oocysts:** The faecal material incubated in Petri-dish was centrifuged at 2000 rpm for 5 minutes and supernatant was discarded. The pelleted oocysts were re-suspended in PBS and re-centrifuged. The procedure was repeated to give four washings. After

last washing the pelleted oocysts were re-suspended at 50,000 oocysts per ml [6].

### Preparation of sonicated antigen

The final concentration of 50,000 oocysts per ml was subjected to ultra-sonication [8] at 60 htz for 5 shots of 1 minute each with an interval of 30 seconds by placing the material on ice. Sonicated oocysts suspension was centrifuged at 2000 rpm for five minutes, supernatants and sediments were collected separately for preparation of vaccines.

### Inactivation of oocysts

Following vaccines were prepared by inactivating oocyst suspension by incubating with 0.3% formalin for 48 hours [10].

Supernatant from sonicated sporulated oocyst (50000 sporulated oocysts/ml)

Sediment from sonicated sporulated oocyst (50000 sporulated oocysts/ml)

Un-sonicated sporulated oocyst (50000 sporulated oocysts/ml)

### Safety Test

All the prepared vaccines were cultured to check the presence of any live oocyst Method of cultivation [8]. These vaccines were also checked for the presence of any bacterial contamination by inoculating the material of vaccines on blood agar and vaccines found unreliable were discarded.

### Immune response of experimental vaccine

The immune response of experimentally prepared vaccine was studied in calves under one month of age. A group of 20 cattle calves under one month of age were purchased from the local market and reared under standard manage mental conditions. Calves on day 6 were equally divided into four groups. The detail for each group is as under:

Group-I: Vaccine A was given at the dose rate of 1 ml per calf orally.

Group-II: Vaccine B was given at the dose rate of 1 ml per calf orally.

Group-III: Vaccine C was given at the dose rate of 1 ml per calf orally.

Group-IV: Placebo

### Collection of Sera

Humoral immune response of calves was studied for two months. Blood samples were collected from jugular vein. Blood was allowed to clot at room temperature. Serum was separated and stored in aliquot of 1 ml at -20°C till further use. Serum samples from the immunized calves of each group (n=5) were collected at day 7, 14, 21, 28, 35, 42, 49, 56 post vaccination.

### Humoral Immune Response

Indirect haemagglutination (IHA) test was performed to assess the antibody titer in collected sera [8]. Briefly the procedure was as follow:

Sheep erythrocytes were sensitized with glutaraldehyde and sonicated antigen followed the method of Tokuda and Warrington [11] with modifications. The sensitized erythrocytes were finally resuspended in Phosphate Buffered Saline (PBS) to make 1.5% suspension. A two fold serial dilution of the serum samples was made with PBS. Equal volume (0.05 ml) of sensitized erythrocytes (1.5%) was added in each well of the microtitre plates. The plates were tapped to ensure even mixing of erythrocytes and incubated at 37°C for 90 minutes. The degree of haemagglutination in each well was recorded in comparison with control. The highest dilution producing HA was recorded by observation suspended aggregates as positive and button formation as negative.

### Biological Challenge

On day 15 post-vaccination, all the calves in group I, II, III and IV were challenged by 20,000 sporulated oocysts of species *Eimeria bovis*. The faecal samples were collected weekly upto day 42 post-vaccination. Numbers of oocysts per gram of faeces were calculated from each challenged group weekly till day 42. Clinical Sign were also recorded.

### Results and Discussion

#### Vaccine

**Safety Test:** All the vaccines were checked for presence of any live parasites and were found free. Vaccines were also cultured on blood agar and were free of any bacterial contamination.

#### Immune Response of Experimental Vaccines

The geometric mean antibody titers of vaccinated and non-vaccinated groups by Indirect Haemagglutination test (IHA) are presented in Table 1. The highest Antibody titer (512) was recorded in group (A and B) on day 35. Antibody titer (362) on day 35 in groups C was lower as compared to group A and B.

Indirect Haemagglutination test (IHA): Antibody titer (geometric mean titer) was higher in calves vaccinated with vaccine I, II when compared to vaccinated group C given vaccine III. No significant difference was observed between group A & B (vaccinated with vaccine I & vaccine II) but significant difference was noted when compared with group C given vaccine III (un-sonicated sporulated oocysts) (Table 1).

#### Biological Challenge

The geometric mean antibody titers of vaccinated and non-vaccinated groups after challenge by Indirect Haemagglutination test (IHA) are presented in Table 2. The highest antibody titer (447.5) was recorded in group A and B on day 28. In group C the highest (388.0) antibody titer was observed on day 21. Antibody titer in group C was lower as compare to group A and B. Results of the challenge experiments revealed that vaccine I & II gave maximum protection as compared to vaccine III.

Groups	GMT								
	0 days	7 days	14 days	21 days	28 days	35 days	42days	49 days	56 days
Group-A	-	181.0	362.0	512.0	512.0	512.0	362.0	362.0	362.0
Group-B	-	181.0	362.0	512.0	512.0	512.0	362.0	362.0	362.0
Group-C	-	128.0	256.0	362.0	362.0	362.0	256.0	256.0	256.0
Group-D	-	-	-	-	-	-	-	-	-

**Table 1:** Geometric mean Antibody titer by Indirect Haemagglutination (IHA) test against *E. bovis* in calves vaccinated with sonicated sporocyst vaccine (un-challenged). **Group A:** Supernatant from sonicated sporulated oocyst; **Group B:** Sediment from sonicated sporulated oocyst; **Group C:** Un-sonicated sporulated oocysts; **Group D:** Non-vaccinated group

Groups	Challenge	post challenge									
	0 days	7 <sup>th</sup> days of Vaccination	15 <sup>th</sup> days of Vaccination	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days
Group-A	-	181.0	362.0	415.9	415.9	445.7	445.7	362.0	362.0	362.0	362.0
Group-B	-	181.0	362.0	445.7	445.7	445.7	445.7	415.9	415.9	415.9	415.9
Group-C	-	128.0	256.0	388.0	388.0	388.0	315.2	315.2	315.2	315.2	315.2
Group-D	-	-	-	39.4	48.5	32.0	32.0	32.0	32.0	32.0	32.0

**Table 2:** Geometric mean Antibody titer by Indirect Haemagglutination (IHA) test against *E. bovis* in calves vaccinated with sonicated sporocyst vaccine (challenged). **Group A:** Supernatant from sonicated sporulated oocysts; **Group B:** Sediment from sonicated sporulated oocysts; **Group C:** Un-sonicated sporulated oocysts; **Group D:** Non-vaccinated group

Animals in group A were found absolutely normal. They were active and healthy. Their feed and water intake was normal. Their feces were normal. No mortality was recorded in this group. After challenge, oocysts appeared in feces on 3rd week (post challenge) showing 600 oocyst per gram of feces which gradually increased from 700-900 OPG on 4<sup>th</sup> week (post challenge). Oocyst number per gram of feces decreased to 700 at the end of 6<sup>th</sup> week.

Animals in group B were also found normal. They were active & healthy. Their feces were normal. No mortality was recorded in this group after challenge. Oocysts appeared in feces on 21 days (post challenge) showing 900 oocyst per gram of feces which gradually decreased to 850 on 4<sup>th</sup> week (post challenge). Oocyst number per gram of feces decreased to 700 at the end of 6<sup>th</sup> week.

Animals in group C were also found normal. They were active & healthy. Their feed & water intake was normal. Their feces were normal. No mortality recorded in this group after challenge. Oocysts appeared in feces on 21 days (post challenge) showing 750 oocysts per gram of feces which gradually increased from 800 to 1050 on 4<sup>th</sup> week (post challenge). Oocyst number at the end of 6<sup>th</sup> week was 850.

On day 18 post challenge, change in the behavior of animals was observed. They were all depressed. Diarrhea was observed in all animals. Oocysts appeared in feces were 18500 which gradually increased to 24050 at 4th week post challenge. Oocyst number per gram of feces decreased to 18050 at 6 week (post challenge) (Table 3).

Groups	Vaccinated			Days post challenge					
	0 day	7 days	14 Days	7 days	14 days	21 days	28 days	35 days	42 days
Group-A	-	-	-	-	-	850	900	750	700
Group-B	-	-	-	-	-	900	850	700	700
Group-C	-	-	-	-	-	1050	950	900	850
Group-D	-	-	-	-	-	20,000	24,050	20,050	18,050

**Table 3:** Post vaccination challenge response of calves (oocysts count). Group A: Supernatant from sonicated sporulated oocyst; Group B: Sediment from sonicated sporulated oocyst; Group C: Un-sonicated sporulated oocysts; Group D: Non-vaccinated group

Statistically, mean oocysts count of group A was  $800 \pm \text{S.E.}45.64$ , of group B  $787.50 \pm \text{S.E.} 51.53$  and C was  $937.5 \pm \text{S.E.} 42.69$  Whereas mean antibody titer of group D (non-vaccinated group) was  $20537.50 \pm \text{S.E.}1260.5$ .

Statistically, there was non-significant difference among group A and group B. But no significant difference A Vs D, B Vs D, C Vs D, B Vs C groups was noted.

The present study reports the immunizing effect of inactivated sporulated oocyst and inactivated sonicated vaccines against bovine coccidiosis. Indirect haemagglutination (IHA) test was developed for detecting antibodies to coccidian. Serum antibody levels in calves were measured against soluble oocyst (sporulated) antigen.

IHA antibody titer was significantly higher ( $P < 0.05$ ) in calves vaccinated with inactivated sonicated vaccines as compared to the calves vaccinated with inactivated sporulated vaccines.

Results of the challenge experiments indicated that the inactivated sonicated vaccine gave protection to the challenge calves as immune calves contained high level of antibodies that resisted heavy dose of challenge. Disease was observed in non-vaccinated calves post challenge whereas vaccinated remained healthy.

The oocyst count per gram of feces was significantly higher ( $P < 0.05$ ) in control group C (unvaccinated) as compared with the vaccinated groups. Similar findings were also reported by Hammond et al [12] who conducted ten experiments using a total of 84 young male Holstein-Friesian calves. In each of seven experiments, 4 to 6 of 10 to 12 calves were immunized with 25,000 to 60,000 oocysts, In three experiments, a total of 13 calves was immunized with 0.5 to 1.0 million oocysts. About 1 month after the immunizing inoculation, all of the calves were given 0.5 to 1.0 million oocysts. In nine experiments the number of merozoites in the caecal contents was determined for the 13th day through the 18th day after inoculation by daily samples through cannulas. The mean for 14 non-immunized calves was 401, 300/ml while for 12 calves immunized with 25,000 to 60,000 oocysts it was 53,000. The corresponding figures for the four non-immunized calves and the eight immunized with 0.5 or 1.0 million oocysts were 179,000 and 1,000. At necropsy, few schizonts were found in the small intestine in the immunized calves than in the non-immunized calves killed 14 to 16 days after inoculation. At examination of histological sections of large intestine, the immunized calves were found markedly lower percentage of infected epithelial tissue than non-immunized calves.

A total of 2000 oocysts of mixed species (Predominantly *E.bovis*) applied on each of five consecutive days did not protect calves against a massive challenge infection with  $2 \times 10^5$  oocysts [13]. On the contrary,  $5 \times 10^4$  oocysts of *E. bovis* induced protection against a double dose reinfection 4 weeks after the primary infection [14].

Attempts to produce a herd-specific vaccine by radiation of oocysts isolated from the feces were made. However, vaccination with radiated oocysts (250 Gy, Co-60) resulted in Patent infection and induced only partial protection [15]. Cattle remained exposed to infection throughout their entire life and moderate to low oocyst excretion may be observed even in adult cows [16] and thus it is obvious that protection by the immune system does not necessarily confer sterile immunity.

Active (Species specific) immunity, both humoral and cellular, develops rapidly after first antigen contact, its intensity being dependent on the number of oocysts ingested. However, no absolute

protection is achieved and even older animals can excrete [10]. Similar findings were also observed by Hughes et al. [17] who investigated cellular and humoral responses following gavage inoculation of 6 week old calves with 35,000-40,000 oocysts of *Eimeria bovis*. At 3-4 days intervals for 40 days after inoculation (DAI) blood was taken and assessed for serum IgG against merozoites and sporozoites of *Eimeria bovis*. The results indicated (a) that sporozoites and merozoites share antigens recognized by serum IgG, (b) that there was no episode of marked immunosuppression during acute infection and (c) that cellular immunity was probably more important in resistance against reinfection than humoral in immunity. Snoep and Potters [18,19] suggested that older, immune cattle might serve to reduce the quantity of oocysts of *E.alabamensis* on contaminated pasture. Pastures should be drained and wet areas fenced off to avoid the accumulation of infective oocysts in the immediate environment of the animals. Turnout to clean pastures (i.e. not grazed by calves in the same or the previous year) will also considerably reduce the risk of Coccidiosis.

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