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Therapeutic Evaluation of Common Used Anti-coccidials in Buffalo Calves Against *Eimeria* Spp. and Integrated Strategy Management for Control of *Eimeria* Spp. Infection

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ABSTRACT

Treatment was evaluated; Amprolium showed a reduction in oocyst counts by an average of 96.46 percent, whereas Toltrazuril resulted in a slightly higher average reduction of 97.34 percent which showed Toltrazuril was deemed more efficacious than Amprolium. Species identification confirmed the presence of three Eimeria species (*E. bovis, E. auburnesis, E. albamensis*) by conventional and molecular methods. Integrated control strategies, including improved farm management, frequent fecal sample screening, and regular use of anti-coccidials, were advised and assessed across three organized farms. Results showed a fifty percent decrease in mortality rates and improvement in body condition scores (BCS) in the study period, advocating these strategies effectively managing coccidiosis with improvement of health and economic sustainability. Present study underscores the significance of regular monitoring and tailored interventions in managing parasitic infections in buffalo calves, with special to better calf welfare and farm productivity of livestock owners.

HIGHLIGHTS

- We have identified and categorized the abstract, reducing the speculative elements.
- We have condensed the introduction by eliminating redundant or less informative sections.

Keywords: Coccidiosis, buffalo calves, therapeutic management, integrated strategy management

Buffaloes in India represent approximately 57% of the global population and play a significant role in the country's milk production, earning them the moniker "black gold of India" due to the presence of world-champion milk-producing buffalo germplasm. A primary challenge to buffalo husbandry is calf diarrhea, which can be attributed to bacterial, viral, or parasitic etiologies. Among the parasitic causes, coccidiosis, engendered by *Eimeria* spp., is notably impactful in the context of calf diarrhea. The infection precipitated by coccidia leads to enteritis across various species, though clinical manifestations vary in accordance with the pathogen's specific pathogenesis. The economic determinants stemming from these conditions significantly contribute to morbidity and mortality rates within pure germplasm populations. Coccidiosis is recognized as a principal enteropathogen in neonatal buffalo calves, with a prevalence observed across virtually all buffalo-rearing regions. It is predominantly observed in young calves, though older animals typically develop robust immunity following infection. Bovine coccidiosis constitutes a globally prevalent infectious disease among calves, inflicting considerable annual economic losses upon the beef and dairy industry. Coccidiosis emerges

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as a critical protozoal infection within cattle and buffalo populations, leading to bloody diarrhea in calves. As the condition exacerbates, the discharge progresses to include bloody fluid, blood clots, and liquid feces. Complications such as secondary infections, notably pneumonia, are prevalent. Affected animals exhibit signs of emaciation, dehydration, emaciated weakness, and lethargy, alongside a rough coat, sunken eyes, and drooping ears. These clinical manifestations contribute to a decline in the animal's overall health and productivity, culminating in significant economic losses for farmers. Hence to avoid these losses, the present work of integrated strategies for the management of coccidia infection on organised farms was planned. Owing to the losses caused by coccidiosis, particularly in calves which causes heavy morbidity and mortality and economic losses of livestock owners. It is, therefore, essential to know the current status of coccidiosis in buffalo calves in and around the Mumbai region and its therapeutic evaluation by commonly used anti-coccidials. Hence, the present study was designed with the following broad objectives: that study therapeutic management of clinical coccidiosis by using toltrazuril and amprolium in buffalo calves on organized farms and evaluate different management strategies for integrated control of *Eimeria* spp. in buffalo calves on organized farms.

MATERIALS AND METHODS

The study was carried out in and around the Mumbai region in organized buffalo farm the present study was designed to note the anthelmintic efficacy of two drugs, namely Amprolium, and Toltrazuril in buffalo calves. The buffalo calves belonging to different categories viz. age, sex, and breed were screened randomly for the period of one year starting from April 2023 to January 2024, during which 254 faecal samples of buffalo calves were examined. A total of 254 fecal samples were collected from buffalo calves showing diarrhea or dysentery and healthy from the college farm (LFC) Aarey colony and around the Mumbai region. Samples were collected either immediately after defecation or directly from the rectum. Examination of fecal samples done by concentration Technique (Floatation method) The fecal samples of buffalo calves were found positive for *Eimeria* spp. Infection, were processed for the number of oocysts per gram of the feces to determine the severity of infection. Therapeutic trials were also conducted to evaluate anti-coccidials drugs Amprolium and Toltrazuril for their clinical efficacy in infected buffalo calves with fecal samples positive for *Eimeria* spp. Oocyst was used for the comparative study (n =18), which were grouped into two groups consisting of nine calves in each group T1 and group T2. Group T1 involved nine calves who were treated with a single drug, Amprolium and group T2 involved nine calves who were treated with Toltrazuril formulation orally for five days. Positive samples were then analyzed by Stoll's egg counting technique for OPG (oocyst per gram) count before treatment (day 0) and after that on 7^{th} and 14^{th} day post-treatment. The therapeutic efficacy of the drugs calculated on the basis of the number of animals found free of infection as determined by fecal sample examination and reduction in OPG count of the feces of the group following formula.

$$\text{Percent Efficacy of drug} = \frac{\text{Mean OPG before treatment} - \text{Mean OPG after treatment}}{\text{Mean OPG of the group before treatment}}$$

RESULTS AND DISCUSSION

Efficacy of Amprolium against *Eimeria* spp. infection in buffalo calves

The T1 group underwent treatment with Amprolium oral powder I.P suspension, administered orally at a dose of 20 mg/kg body weight. Within this cohort of five calves, a complete resolution of oocyst counts (100%) was achieved in two individuals 21 days following treatment initiation. Additionally, a reduction in oocyst counts ranging from 93% to 94% was observed in the remaining four calves after a 21-day treatment period. The aggregate average diminution in oocyst counts for this group was determined to be 96.46%. Despite demonstrating efficacy in managing *Eimeria* spp. Infections, Amprolium oral powder I.P suspension was found to be less effective as compared to the Toltrazuril-based treatments evaluated in the present study.

Efficacy of Toltrazuril against *Eimeria* spp. infection in buffalo calves

The T2 group received treatment with BAYCOX 2.5% oral solution, administered at a dosage of 15 mg/kg body

Table 1: Details of anti-coccidials used in present studies

Sl. No.	No of calves	Trade Name	Active ingredient	Dose rate	Route of administration
1	12	Amprolium Oral Powder I.P (Vetiquinol Pharmaceuticals Ltd)	Amprolium	20 mg/kg b. wt	Orally
2	12	Baycox (Toltrazuril 2.5% oral solution)	Toltrazuril	15 mg/kg b. wt	Orally

Table 2: Factorial experiment (ANOVA) table showing analysis of treatment groups for comparison

ANOVA Table

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Replications	8	505185.185	63148.148	4.508	0.000
Treatments	11	110616574.074	10056052.189	717.944	0.000
Factor A	2	63036296.296	31518148.148	2250.214	0.000
Factor B	3	29063240.741	9687746.914	691.649	0.000
A × B	6	18517037.037	3086172.840	220.335	0.000
Error	88	1232592.593	14006.734	—	—
Total	107	—	—	—	—

Treatment undertaken for treating coccidiosis and their performance

Sl. No.	Average	SE	CD
Treatment 0	2241.667[a]	33.64	At 5%=55.428
Treatment 1	658.333[b]	31.35	At 1%=73.449
Treatment 2	586.111[c]	10.25	At 1%=76.345

weight. Within this group, composed of nine calves, a complete elimination (100%) of oocyst counts was observed in two individuals 21 days' post-treatment. While the remaining animals demonstrated a drastic decrease in oocyst counts ranging from 94% to 95% after the same treatment duration. The mean reduction in oocyst count across the entire T2 group was calculated to be 97.34%. Consequently, BAYCOX 2.5% oral solution was found to surpass Amprolium in efficacy against *Eimeria* spp. Infections in this study.

Treatment 1(Amprolium) and Treatment 2 (Toltrazuril) showed the effect on 7th day of the experiment onwards and reduced the oocyst output significantly as compared to their respective day 0 and compared to T0 (Control group).

Treatment 1(Amprolium) on day 14th and Treatment 2 (Toltrazuril) on day 21st showed the lowest oocysts output. Hence, it can be said that T1 treatment gives earlier best results as compared to T2 treatment.

In the current investigation, the efficacy of two anti-coccidials agents, namely Amprolium and Toltrazuril, was assessed against infections caused by *Eimeria* spp., revealing effectiveness rates of 96.46% and 97.34%, respectively. Comparative analysis indicated that Toltrazuril exhibited superior efficacy in combating *Eimeria* spp. Infections when compared with Amprolium. Corroborating these findings, Navkar *et al.* (2022) conducted a study to evaluate the comparative efficacy of specific drugs in the management of coccidial infections in buffalo calves. Their results demonstrated a significant reduction in oocyst per gram (OPG) counts following the initial week of treatment across all groups under study. Notably, the decline in OPG counts was more pronounced in groups receiving Toltrazuril compared to those treated with Amprolium. Furthermore, the duration required to achieve a zero OPG count was longer in the Toltrazuril group, with the eradication of oocysts occurring by the fourth week of treatment. In contrast, groups treated with

Amprolium achieved a zero OPG count by the second or third week. These observations suggest that Toltrazuril is more efficacious in reducing Coccidia oocysts from buffalo calves compared to Amprolium; that conclusion strongly supports the outcomes of a study conducted by Zapa *et al.* (2022) and earlier research by Ghanem *et al.* (2006), both of which observed similar trends in the efficacy of these anti-coccidial drugs.

For integrated control of coccidiosis in buffalo calves at organised farms two-way strategies were applied

1. Best management to prevent the infection in calves.
2. Regular use of anti-coccidial after fecal examination.

Management

Calves pens were kept totally dry by following several tricks viz. frequent removal of faeces, urine and un-utilised feed + to keep the shed mostly dry use of lime powder as per requirement. It has enabled the maintaining the shed dry so that sporulation of discharged oocysts has been reduced and infection rate came down significantly. Coccidiosis disease is described as 'disease of management'. In farm better management lower the rate of coccidiosis. Utmost care has been taken to nurture the said principle at farm.

Use of anticoccidials

A frequent faecal examination was conducted to note the infection of *Eimeria* in calves. As and when *Eimeria* oocysts were detected to the OPG level > 100; administration of anti-coccidials was undertaken. It has prevented the coccidiosis.

Assessment of integrated control approach

It has been assessed in two ways i.e., reduction in mortality rate and overall body condition of the calves. After the implementation of the above two strategies, the overall body condition normal score has been improved drastically and mortality rate has come down to almost normal. This practice was undertaken throughout experimental period. From this small experiment, it can be learned that, as much management is improved on the farm, it reflects in the reduction of incidence of coccidiosis on the farm, hence recommended.

1. Body condition score (BCS 1): indicated emaciated, BCS 2 indicate thin, BCS 3 average, BCS 4 fatty and BCS 5 Obese, in Murrah buffalo calves were agreed with the previous worker Anitha *et al.* (2011) and Reddy and Alapati (2013).
2. Mortality % is worked on death occurred on account of coccidiosis with total calf strength on the particular farm.

The table 3 presents data on the assessment of integrated control strategies across three organized farms over a period from April 2023 to January 2024. The assessment focuses on two main parameters: mortality percentage (the proportion of buffalo calves) and body condition score (a qualitative measure of the health or condition of the farm's buffalo calves, likely on a scale where a higher number indicates better condition). Here's a breakdown of the trends and key points observed in the table 3.

Mortality

Across the months, all three farms show a general trend of decreasing mortality percentages. This suggests that the integrated control strategies being implemented may be effective in reducing losses over time.

Farm 1 started with a 25% mortality rate in April 2023 and noted a decrease to 11% by January 2024 whereas farm 2 shows a similar pattern, starting from 20% mortality in April 2023 and decreasing to 8% by January 2024, indicating the most significant reduction among the three farms.

Farm 3 begins with a 27% mortality rate, the highest among the three farms in April 2023, and decreases to 11% by January 2024.

Body Condition score

The BCS buffalo calves were measured by hand palpation for fat cover with hand over 4 important locations on the calves' body *viz* backbone (top line or spine), ribs, hips bones (pins and hooks), and tail head, which ranges from one to five number scale. There's an improvement in body condition scores across all farms over the period. This improvement aligns with the decrease in mortality percentages, further suggesting the positive impact of the integrated control strategies.

Table 3: Assessment of integrated control strategies on organised farm by farmers questioner

Months	Farm 1		Farm 2		Farm 3	
	Mortality %	**Body condition score**	**Mortality %**	**Body condition score**	**Mortality %**	**Body condition score**
April 2023	25	1	20	1	27	1
May 2023	25	1	20	1	27	1
June 2023	20	1	19	1	27	1
July 2023	30	1	25	1	25	1
Aug. 2023	25	1	25	2	22	1
Sept. 2023	18	2	21	2	18	2
Oct. 2023	16	3	15	3	17	3
Nov. 2023	15	3	12	3	15	3
Dec. 2023	11	3	10	4	13	3
Jan. 2024	11	4	8	4	11	4
Overall	**19.6**	**2**	**17.50**	**2.2**	**20.2**	**2**

Farm 1 and Farm 3 both improved from a body condition grade of 1 in April 2023 to 4 by January 2024.

Farm 2 starts at the same point but reaches a body condition grade of 4 by December 2023, one month earlier than the other two farms, and maintains this in January 2024. Additionally, it's the only farm to achieve a body condition grade of 4, indicating slightly more effective or efficient implementation of control strategies or possibly better initial conditions for improvement.

The present study suggests a BCS of 3-4 was ideal for better management of buffalo calves; the table indicates a positive trend in both decreasing mortality rates and improving body condition scores from April 2023 to January 2024 across all three farms on an increased BCS 1 to BCS 4 i.e. emaciated to average good. These trends suggest that the integrated control strategies implemented on these farms are effective in improving the health and sustainability of their buffalo calves. Farm 2, in particular, shows the most substantial improvement in both categories, suggesting it might have adopted the most effective strategies or had conditions more conducive to rapid improvement.

A body condition score (BCS) system in rearing buffalo calves is a subjective grading method for the evaluation of energy release of dairy animals Anitha*et al.* (2011). The present finding was totally tally with garnsworthy 1988. The scoring system (BCS) has been developed by other workers like Lowman *et al.* (1976) used a 0 to 5 scale in beef cattle. Whereas Edmonson *et al.* (1989) developed a score for HF cows, Sarjan and Alapati 2013, a new body condition (BCS) score was developed in a 1-5 scale using 0.5 increments in examining 8 skeletal check points. Similar study carried out by Sarjan Rao *et al.* (2002), and Anitha *et al.* (2005) used this score chart for scaling the crossbred cows in India. The similar benchmarks used by these authors were applied while determining the BCS in the present study.

From the above study, it can be firmly said that:

1. To save the lives of buffalo calves, it is essential to frequently examine the dung samples at least till they attain the age of 6 months, and upon detection of any parasitic stages, immediate therapy shall be administered.
2. While dealing with the health and management of buffalo calves in organized farms coccidiosis shall be taken seriously without any negligence.

As and when *Eimeria* oocysts are detected to the OPG level > 100; administration of anti-coccidials shall be undertaken. It will help to prevent mortality and economic losses of the farmer occurring on account of coccidiosis.

CONCLUSION

It may be concluded that the efficacy study observed that Amprolium oral powder, administered at 20 mg/kg, effectively reduced *Eimeria* spp. Oocyst counts in buffalo calves, with an average reduction of 96.46% post-

treatment. The Toltrazuril solution, administered at 15 mg/kg, was highly effective in treating *Eimeria* spp. reduction in oocyst count to 97.34 percent.

The present study suggests a BCS of 3-4 was ideal for better management of buffalo calves, indicating a positive trend in both decreasing mortality rates and improving body condition scores. These trends suggest that the integrated control strategies implemented on these farms are effective in improving the health and sustainability of buffalo calves.

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