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Comparative therapeutic effect of toltrazuril, sulphadimidine and amprolium on *Eimeria bovis* and *Eimeria zuernii* given at different times following infection in buffalo calves (*Bubalus bubalis*)

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Abstract

We compared the therapeutic effect of three anticoccidial drugs (toltrazuril, sulphadimidine and amprolium) in buffalo (*Bubalus bubalis*) calves experimentally infected with *Eimeria bovis* (*E. bovis*) and *E. zuernii* oocysts (3×104 oocyst/calf). Buffalo calves (1.5-4 month old, 70-kg body weight) were randomly allocated into 3 groups (9 calves each). Group T was experimentally infected with oocysts and treated with toltrazuril (20 mg/kg BW twice orally at a 1-week interval). Group S was experimentally infected with oocysts and treated with sulphadimidine (125 mg/kg injected IM followed by half dose for 4 successive days). Group A was experimentally infected with oocysts and treated with sulphadimidine (125 mg/kg injected into 3 group) to represent timing of the drug administration: 1st day of coccidia infection (FD), onset of clinical signs of coccidiosis (CC), and onset of oocyst shedding into the faeces (OS). Clinical signs, body-weight gain (BWG) and number of oocysts per gram feces (OPG) were monitored daily for 35 days post-infection (DPI). The OPG were reduced (but the BWG was not different) in the T calves compared to S and A calves. Within the same group, treatment from the 1st day of infection reduced the OPG and increased the BWG compared to the later treatment timings.

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1. Introduction

Bovine coccidiosis is an infectious disease affecting calves all over the world resulting in considerable economic losses each year to the beef and dairy industries (Daugschies and Najdrowski, 2005). The disease can be produced by several *Eimeria* species, of which *E. bovis* and *E. zuernii* are the most pathogenic.

Immunity to coccidiosis produced by infection with *E. bovis* apparently involves both humoral (Faber et al., 2002) and cellular responses (Speer et al., 1985). Calves that recover from infection are either solidly immune from reinfection (species specific) or can be infected without showing clinical disease (Sanger et al., 1959).

Several anticoccidial drugs are effective in treatment and control of coccidiosis in both beef calves (Miner and Jensen, 1976; Benz and Ernst, 1979; Mundt et al., 2005) and buffalo calves (Sanyal et al., 1985). These drugs included toltrazuril (Epe et al., 2005), sulphadimidine (Svensson, 1998) and amprolium (Sanyal et al., 1985; Slater et al., 1970). However, the comparative therapeutic efficacy of these drugs on *E. bovis* and *E. zuernii* infection in buffalo calves was not yet investigated.

Therefore, we compared the chemotherapeutic effect of three commonly used anticoccidial drugs in Egypt (toltrazuril, sulphadimidine, and amprolium) on treatment and control of experimental infection of *E. bovis* and *E. zuernii* in buffalo calves. The comparative assessment was based on the clinical signs, the number of oocyst per gram feces (OPG) and the body-weight gain (BWG).

2. Material and methods

2.1. Experimental design

The experimental design was approved by the local committee of the Faculty of Veterinary Medicine, Benha University, Egypt and conformed to the guidelines of National Institute of Health (NIH) of Egypt. A total of 27 native-breed buffalo calves 1.5–4 months old with 72.5 kg average body weight were used in this study (Table 1). These buffalo calves were obtained from a local market at Moshtohor, Qaluobia, Egypt. Buffalo calves were adapted for 2 weeks through which the

Table 1

Experimental design of our Eimeria infection-and-treatment study in Egyptian buffalo calvs (3 calves per subgroup, 27 calves total)

Group/name	Timing subgroup	Treatment timing	Treatment reference
T Toltrazuril-treated group	First day (FD)	From the First day	Rudetskii (1982)
(20 mg/kg bw orally twice	Clinical coccidiosis (CC)	Once clinical signs are seen	Khahra et al. (1983)
at a 1-week interval)	Oocyst shedding(OS)	Once oocyst shedding is seen	Krotenkov et al. (1990)
S Suphadimidine-treated group (125 mg/kg injected IV followed by half dose (IM) for 4 successive days)	First day (FD) Clinical coccidiosis (CC) Oocyst shedding(OS)	From the First day Once clinical signs are seen Once oocyst shedding is seen	Rudetskii (1982) Khahra et al. (1983) Krotenkov et al. (1990)
A Amprolium-treated group	First day (FD)	From the First day	Rudetskii (1982)
(50 mg/kg orally for 7	Clinical coccidiosis (CC)	Once clinical signs are seen	Khahra et al. (1983)
successive days)	Oocyst shedding(OS)	Once oocyst shedding is seen	Krotenkov et al. (1990)

All calves received 3×10^4 oocysts.

animals were fed a ration composed of concentrates, crushed barely, wheat bran, soybean meal with free access to fresh water. Buffalo calves were reared in individual partitions under hygienic condition on a clean concrete floor previously disinfected with 10% ammonia solution. Fecal samples were examined daily for 2 weeks and were free from coccidial oocysts. The buffalo calves were randomly allocated into 3 groups, each of which was classified into 3 subgroups using an online randomizer program (www.randomizer.org). We cleaned the partitions twice daily and disinfected them with 10% ammonia solution throughout the experiment to avoid reinfection. In addition, buffalo calves were not allowed to graze to avoid reinfection and the feeding was restricted to partitions where feed and water troughs were constructed and located to avoid contamination.

2.2. Clinical examination

Each calf was examined clinically every day according to Blood and Radostits (1989). The clinical examination was conducted by a veterinarian from the veterinary teaching hospital of Faculty of Veterinary Medicine, Benha University, Egypt who was blinded to the experimental groups.

2.3. Preparation of inoculum

We raised the oocysts of *E. bovis* and *E. zuernii* in five experimentally inoculated buffalo calves for propagation and preparation of inocula as described by Joyner et al. (1966). The oocysts were obtained using special sieves after centrifugation and sedimentation according to Fayer and Hammond (1967). After washing with distilled water for four times, the oocysts were kept in 2.5% potassium–dichromate solution in glass dishes at room temperature to enhance sporulation. We examined the dishes daily until 90–95% sporulation was obtained. Prior to inoculation, the sporulated oocysts were washed four times by using distilled water. We used a hemocytometer for oocysts counting. The total number of sporulated oocysts was estimated by multiplication of the obtained number by the dilution factor. We inoculated the dose of sporutaled oocysts (3×10^4) via stomach tube to all experimental animals.

2.4. Fecal samples

We collected the fecal samples (collected directly from the anus) from all buffalo calves daily for 35 days (the period of our experiment) for counting the number of oocysts in 1 g of feces (OPG).

2.5. Microscopic examination of fecal samples

Microscopic examination of the fecal samples was conducted daily. Oocysts were separated from faeces through mortaring (using screens of meshes 400 μ m, 200 μ m, and 63 μ m). After centrifugation and flotation with saturated-salt solution, samples were taken from the top over a cover slide and then examined under the microscope.

2.6. Oocyst count

We counted the oocysts in positive samples by using McMaster technique after Benz and Ernst (1979). Counting was conducted by a veterinarian blinded to the experimental design at the lab of Animal Medicine Department, Faculty of Veterinary Medicine, Benha University.

2.7. Therapeutic drugs

We compared the efficacies of three anticoccidial drugs. These drugs included:

Toltrazuril 2.5% (Bycox) solution (Marcyrl Pharmaceuticals Industries, El-Abour City, Egypt) was given orally twice at a 1-week interval at a dose rate of 20 mg/kg BW according to Pilarczyk et al. (1999).

Sulphadimidine 33.3% (Amoun Pharmaceutical Industries, Egypt) was injected intravenously at a dose rate of 125 mg/kg bw, followed by half of this dose I/M for 4 successive days according to Kpahra and Jasmer (1986).

Amprolium 20% (Egyptian Co. for chemicals and pharmaceuticals (ADWIA) S.A.E. 10th of Ramadan City, Egypt) was given orally daily at a dose rate of 50 mg/kg bw for 7 successive days according to Sanyal et al. (1985).

2.8. Body-weight gain

The body weight was recorded before infection (initial body weight) and 35 days post-infection. The BWG was calculated by subtracting the initial body weights from body weight at 35 DPI. We used the Kingship weighing instrument, model GRW-600 (capacity of 600 kg \times 50 g) (Cairo Vet. Company for Vet. Products) in weighing the buffalo calves. Each calf was weighed three times at each occasion and the average was used in data analysis.

2.9. Statistical analysis

Sigma stat software (Sigma stat 2.03 for windows; SPSS Inc., Chicago, IL) was used to conduct a two-way analysis of variance (ANOVA) (Schuler et al., 2004) to test the simultaneous effects of drug and timing on the OPG on the first day that oocysts were detected and at the end of our experiment, and on BWG by the end of the experiment. Data were assumed to be normally distributed. Differences between means were assessed by a multiple-comparison procedure (Holm-Sidak test). Results were expressed as mean \pm standard error (S.E.). We used alpha = 0.05.

3. Results

The calves in the three groups had initial body weights of mean (minimum–maximum): group T 73 kg (68–80 kg); group S 72 kg (66–77 kg); and group A 72 kg (65–80 kg).

3.1. Clinical signs

All infected groups showed various degrees of diarrhea manifested by soiling of tail and hind quarter with sometimes tenesmus, loss of appetite and dehydration. The clinical signs were ameliorated at different periods after onset of treatment with toltrazuril, sulphadimidine and amprolium (Table 2).

3.2. Fecal examination

We first found faecal oocyst on day 13 post-infection.

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Table 2

Group	Subgroup	Clinical signs	First day of appearance post-infection (median)	Days post-infection by which signs were no longer seen			
T Toltrazuril	First day (FD)	-Mild diarrhea -Decreased appetite	19	26 days post-infection.			
	Clinical coccidiosis (CC)	-Diarrhea -Inappetence	9	14-15 days post-infection			
	Oocyst shedding (OS)	-Severe diarrhea -Bloat -Anorexia -Dehydration -Restlessness	9	21–23 days post-infection			
S Suphadimidine	First day (FD)	-Diarrhea -Inappetence	20	27 days post-infection			
	Clinical coccidiosis (CC)	-Diarrhea -Anorexia	9	13-17 days post-infection			
	Oocyst shedding (OS)	-Severe diarrhea -Depression -Off food -dehydration	9	23-26 days post-infection			
A Amprolium	First day (FD)	-Diarrhea	22	28 days post-infection			
A Ampionum	Clinical coccidiosis (CC)	-Diarrhea -Off food -Restlessness	9	16–19 days post-infection			
	Oocyst shedding (OS)	-Severe diarrhea -Dehydration	9	23-30 days post-infection			

Clinical signs after infection with Eimeria of 27 Egyptian buffalo calves (3 calves per drug-timing sub-group)

3.3. Oocyst count

The mean OPG in the toltrazuril group was lower than the means in the other two drug groups on both days 13 and 35 post-infection. Moreover, treatment from the first day (FD) of infection significantly reduced (P < 0.05) the OPG compared to treatment after the clinical coccidiosis (CC) and compared to treatment after oocyst shedding (OS) in feces. Additionally, there was a significant interaction between drug and timing on day 13 (Table 3).

3.4. Body-weight gain

The BWG at day 35 was higher when calves were treated on the first day of infection (rather than waiting to observe clinical signs of oocyst shedding) but did not differ between drugs (Table 4). On the other hand, the BWG was not significantly affected by the drug used and there was no interaction between drug and timing.

4. Discussion

The signs the calves showed (dullness, anorexia, diarrhoea, dehydration and loss of body weight starting) were also observed by Jonic et al. (1988); Muirhead (1989); Carlos et al. (1999) and Bangoura and Daugschies (2007). Diarrhea and inappetence were the main clinical signs in

Table 3

The oocysts per gram feces in buffalo calves (*n* = 9 calves per drug and 3 calves per drug-timing subgroup (FD, CC, OS)) at Qaluobia (Egypt) experimentally infected with *E. bovis* and *E. zuernii* and treated with toltrazuril (T), sulphadimidine (S) and amprolium (A) at different timing [first day (FD), clinical coccidiosis (CC) and oocyst shedding (OS)]

DPI	Group	Group										Statistical analysis (two-way factorial ANOVA)							
	Toltrazuril (T)			Suphadimidine (S)		Amprolium (A)			Treatment (groups)			Treatment timing (subgroups)			Treatment × timing				
	FD	CC	OS	FD	CC	OS	FD	CC	OS	d.f.	F	Р	d.f.	F	Р	d.f.	F	Р	
13 DPI [*] Mean S.E.	72 ^a 14.4	429 ^b 54.9	179 ^c 14.4	182 ^a 14.4	690 ^b 49.1	280 ^c 14.4	189 ^a 7.2	791 ^b 49.1	292° 14.4	2	32.9	<0.001	2	200.9	<0.001	4	5.2	0.006	
35 DPI ^{**} Mean S.E.	47 ^a 7.5	10 ^a 5.1	213 ^b 133.3	211 ^a 52.5	76 ^a 2.3	512 ^b 1.3	340 ^a 7.5	220 ^a 2.3	600 ^b 2.0	2	29.1	<0.001	2	40.0	<0.001	4	1.6	0.212	

Different superscripts indicate significant difference between means within groups.

* The error MS and DF are 2976.15 and 18, respectively.

** The error MS and DF are 6899.67 and 18, respectively.

Table 4

The body-weight gain of buffalo calves (n = 9 calves per drug and 3 calves per drug-timing subgroup (FD, CC, OS)) at Qaluobia (Egypt) experimentally infected with *E. bovis and E. zuernii* and treated with toltrazuril (T), sulphadimidine (S) and amprolium (A) at different timing [first day (FD), clinical coccidiosis (CC) and oocyst shedding (OS)]

Groups	Toltrazuril (T)			Suphadimidine (S)			Amprol	ium (A)		Statistical analysis (two-way factorial ANOVA)								
	FD	CC	OS	FD	CC	OS	FD	CC	OS	Treatment (groups)		Treatment timing (subgroups)			Treatment \times timing			
										d.f.	F	Р	d.f.	F	Р	d.f.	F	Р
Mean [*] S.E.	12 ^a 0.72	10 ^b 1.4	8 ^c 0.90	10 ^a 0.35	10 ^a 0.60	9 ^b 0.09	10 ^a 0.06	9 ^b 0.23	8 ^c 0.52	2	1.75	0.20	2	14.17	< 0.001	4	1.13	0.37

Different superscripts indicate significant difference between means within groups.

^{*} The error MS and DF are 1.33 and 18, respectively.

subgroups treated from the first day of infection and these signs appear later (by inspection) than in calves with delayed treatment. The association between the delayed appearances of signs to treatment from first day is unexplained and therefore, further investigations are required to establish this association. Radostits et al. (2007) stated that coccidiostats must be given early in the life cycle of the coccidia to suppress the development of life cycle of the parasite.

Also by inspection, buffalo calves seemed to recover slightly earlier in group T than in the other groups. Treatment with toltrazuril was effective also in beef calves (Mundt et al., 2003, 2005; Staschen, 2004).

We first found faecal oocyst on day 13 post-infection. Hermosilla et al. (1999) also found that the prepatent period of *E. bovis* was 12 days and that shedding was from day 25 to day 35 post-infection.

Our results (Table 3) suggest that toltrazuril is more effective than sulphadimidine and amprolium in reducing the number of excreted oocyst in experimentally infected buffalo calves. Similar findings were also obtained by Emanuel et al. (1988). Sulphadimidine was more effective than amprolium in suppressing the *E. bovis* and *E. zuernii* oocyst production. However, Sanyal et al. (1985) found the opposite for *E. bareillyi* in buffalo calves. It is noteworthy that the later study was conducted on calves experimentally infected with *E. bareillyi*, which could be different in pathogenicity from *E. bovis* and *E. zuerni* that were used for experimental infection in our study. The preliminary anatomic locations of the coccidian we used are the caecum, colon, and distal ileum (Gregory and Catchipole, 1990) whereas *E. bareillyi* tend to be located in the upper jejunum (Pande et al., 1971).

Our results suggested that treatment after appearance of clinical coccidiosis (CC) induces faster recovery than treatment after oocyst shedding (OS)-similar to Mundt et al. (2003). Moreover, Radostits et al. (2007) stated that the susceptibility to coccidiostats varied with the different stages of life cycle of Eimeria. When the drug is given during the drug-susceptible stage (before 13–15 days) it produces its desirable effect. However, drugs given after 16–17 days appear to be less effective.

Given the significant early interaction between the drug and timing of the treatment, we suggested that treatment with toltrazuril from first day of treatment is the most effective way to overcome eimeriosis in buffalo calves as demonstrated by reduction of the OPG. Mundt et al. (2003) and Staschen (2004) reported that treatment of coccidiosis in calves by toltrazuril during the prepatent period (metaphylactic treatment) produces high anticoccidial effect. In addition, Pilarczyk et al. (1999) demonstrated a very low coccidia infection after baycox administration. The effectiveness of toltrazuril may be attributed to its effect against all intracellular stages (schisogony and gamogony) of eimeriosis (Balicka-Ramisz, 1999). Therefore, we recommend that in rearing buffalo calves, treatment by toltrazuril (20 mg/kg BW orally twice at a 1-week interval) from the first day of expected exposure to eimeria species.

Treatment from the first day of infection produced higher BWG than treatment given after observing signs or oocysts shedding on the 35th DPI. This could be explained by the observation that the duration of diarrhea in subgroups CC and OS was longer and more severe than subgroup FD. Because diarrhea hinders the absorption of food from coccidia-induced damaged intestinal mucosa (Greiner et al., 1984; Mundt et al., 2005), this diarrhea can lead to weight loss and emaciation (Greiner et al., 1984; Svensson, 1998). Moreover, the anorectic effects of coccidiosis coupled with depressed intestinal absorption would markedly reduce feed efficiency in calves. On the other hand, comparison between the three drugs in regard to the BWG did not reveal a significant change, although there was a significant change in the OPG among the three drugs. Radostits et al. (2007) reported that severely affected calves did not quickly regain the body weight losses which occurred during the clinical case of coccidiosis.

In conclusion, toltrazuril was more effective than sulphadimidine and amprolium in suppressing *E. bovis* and *E. zuernii* oocysts shedding in buffalo calves. We recommend treating from the first day of suspected exposure rather than waiting for clinical signs or oocyst shedding to be observed, because treatment from the first day of infection lowered oocyst shedding and improved body-weight gain 35 days after infection.

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