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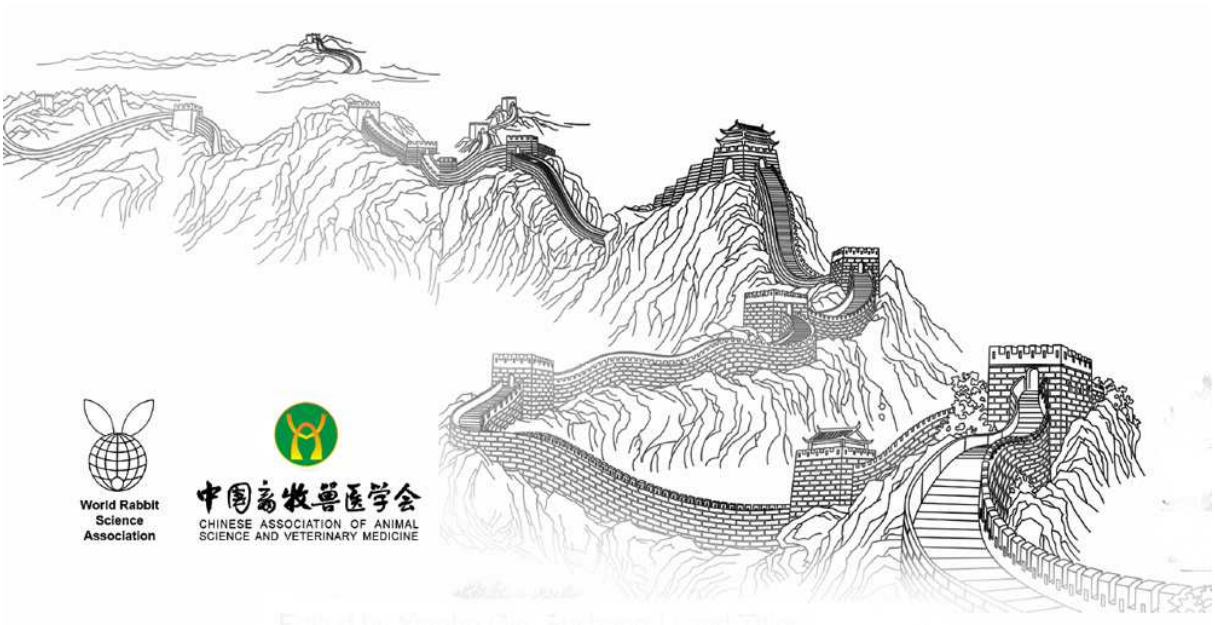

PROPHYLACTIC AND THERAPEUTIC EFFICACY OF PONAZURIL AGAINST RABBIT COCCIDIOSIS

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ABSTRACT

Prophylactic administration of anticoccidial drugs is still the main approach for the control of rabbit coccidiosis. In this study, we tested the prophylactic and therapeutic effect of ponazuril in rabbits. Administration of 20 mg/kg for prophylaxis or 30 mg/kg for therapeutics was effective in reducing oocyst output and preventing weight loss. No side effect was observed on production performance in prophylactic administration with dosage of 10 mg/kg or 30 mg/kg, while 60 mg/kg caused reduction of weight gain. Ponazuril in 30 mg/kg was comparable to that of diclazuril in 1mg/kg, reduced up to 87% oocyst output in naturally infected rabbits. These results together suggest that ponazuril is a potential anticoccidial drug for rabbit coccidiosis.

Key words: ponazuril; rabbit coccidiosis; oocyst; *Eimeria*

INTRODUCTION

Coccidiosis, caused by the infection of apicomplexan parasites of the genus *Eimeria*, is the major parasite disease of rabbits and responsible for the huge economic losses in rabbit industry (Jing et al., 2012; Schlolaut et al., 2013). Currently, prophylactic administration of anticoccidial drugs in feed is the main approach for the control of rabbit coccidiosis as no vaccine currently available (Pakandl, 2009).

Ponazuril, a triazinone derivative related to toltrazuril, was initially approved for the treatment of equine protozoal myeloencephalitis (EPM) (Lindsay et al., 2000; MacKay et al., 2008). In recent studies, ponazuril was tested for the treatment of coccidiosis of rats, prairie dogs (*Cynomys ludovicianus*) and lamas (*Lama glama*) (Billeter et al., 2005; Gardhouse and Eshar, 2015; Prado et al., 2011). Thus, in this study, ponazuril was tested as a potential candidate for the preventing or treating rabbit infected with eimeria parasites.

MATERIALS AND METHODS

Drugs, animals and parasites

Ponazuril and diclazuril, in the form of active pharmaceutical ingredient, were purchased from Widely Chemical Technology Co. Ltd (Wuhan, Hubei).

New Zealand White rabbits, coccidia-free, were purchased from Beijing Xinglong Laboratory Animal Factory. All rabbits were fed ad libitum. After 30 day old, these rabbits were housed individually in wire-floored cages. For trials in naturally infected rabbits, sixty rabbits were randomly selected from populations raised in a farm (Benyue Rabbit Farm, Hubei Province of China).

Oocysts derived from a naturally infected rabbit were used in this study. *E. intestinalis*, *E. magna* and

E. perforans were three major species in these mixed oocysts. Oocysts were propagated and maintained in the laboratory using the methods described elsewhere (Long et al., 1976, Coudert et al. 1995).

Experimental design
To test the prophylactic and treatment effect of ponazuril, eighteen rabbits of 30-day-old were divided into 6 groups of 3 animals each group. Each rabbit in the five groups was inoculated with 1×10^5 sporulated oocysts according to a previous study using mixed occysts of *E. flavescens*, *E. intestinalis*, *E. magna*, *E. perforans* and *E. stiedai* for testing the efficacy of toltrazuril against coccidiosis in rabbits (Peeters et al. 1986). While the last group of animal were orally inoculated with PBS as control. Two groups of animals were fed with 10 mg/kg and 20 mg/kg ponazuril, respectively, 7 day prior to parasite inoculation; while another two groups of animals were treated with 15 mg/kg and 30 mg/kg ponazuril, respectively, 7 days after parasite inoculation. The fifth group was only inoculated with oocysts but with no drug in the feed.

In the second trial of testing the effect of ponazuril on the performance of rabbit, four groups of rabbits (n=4) were fed with 0, 10, 30 and 60 mg/kg ponazuril, respectively, but without parasite inoculation.

In the third trial, ponazuril was used for the treatment of naturally infected rabbits in a small rabbit farm in Hubei Province. Sixty rabbits of 30-day-old were randomly divided into two groups of 30 rabbits. The infection status of these animals was confirmed by fecal ooycst counting before the supplementary of drugs. Rabbits in one group were treated with ponazuril in feed (30 mg/kg) and those in another group were treated with diclazuril in feed (1mg/kg). Clinical sign and death was recorded each day. Body weight gain and feed consumption was monitored every seven days during the assay. Fecal oocyst counting were performed at the first day and then every week.

Statistical analysis
SPSS 19.0 software was used in statistical analysis, each group of rabbits weight gain. Food consumption rate and total oocyst output were analyzed by One sample *t* text, and the intergroup comparison of treatment results was carried out using Duncan's multiple ange test.

RESULTS AND DISCUSSION

We first confirmed that ponazuril was effective against rabbit coccidiosis (Table 1). Oocyst output was significantly inhibited in rabbits with 10 mg/kg supplementation at day 21-28 after the infection; while very few oocyst shed during the whole trial in the group with 20 mg/kg supplementation. For the therapeutic use of ponazuril, dosage with 30 mg/kg significantly reduced the oocyst output from the second week after infection; and no oocyst was detected from day 21 to 28 after infection. No diarrhea was observed in medicated rabbits; while anorexia and severe growth depression was found in infected and non-medicated rabbits.

The influence of the ponazuril on zootechnic performance of uninfected animals was summarized in table 2. The average weight gain show on significant difference between no-medicated group and groups with drug administration. We found that rabbits in 60 mg/kg group showed symptom of anorexia, depressed and weight loss, but without diarrhea.

In the trial conducted in HuBei Benyue Farm, no oocyst was detected in natural infected rabbits after medication with ponazuril in 30 mg/kg or diclazuril in 1 mg/kg at day 21, indicating that ponazuril was effective in eliminating the coccidia infection.

Table 1 Experiments of the dosage of ponazuril against rabbit coccidia

Groups (n=3)	Oocyst dosage	Death of animal	Medication period	Oocysts shed (10^6)				Weight gain (g/day)	Daily feed consumption (g)	FCR
				Day 1-7	Day 8-14	Day 15-21	Day 21-28			
Non-infected Control	-	0	–	0	0	0	0	49.26±5.16^{a}	171.42	3.48
Infected control	10^5	1	–	7.6	880	27	0.44	26.69±6.89^{d}	120	4.49
With 10 mg/kg	10^5	0	D 1-28	0	8.4	2.6	0.03	38.99±5.25^{bc}	157.14	4.03
With 20 mg/kg	10^5	0	D 1-28	0	0.6	0.08	0	42.37±7.52a^{b} c	151.42	3.57
With 15 mg/kg	10^5	1	D 8-28	7.2	36.2	2.44	0.06	36.78±4.57^{c}	148.57	4.04
With 30 mg/kg	10^5	0	D 8-28	5.2	29.7	0.19	0	46.31±4.77^{ab}	171.42	3.7

Note: Data was analyzed using Duncan's multiple range test for significant difference. Means ± SD with different superscript are significantly different. ($P < 0.05$).

Table 2 Influence of continuous medication with ponazuril on performance of rabbits

Groups(n=4)	Death of animal	Weight gain (g/day)	Daily feed consumption(g)	FCR
without drug	0	50±4.72^{a}	155.27	3.10
With 10 mg/kg	1	34.52±6.27^{bc}	142.77	4.13
With 30 mg/kg	0	40.47±4.12^{ab}	150.76	3.72
With 60 mg/kg	0	26.34±7.19^{c}	140.63	5.33

Table 3 Field experiments of ponazuril against rabbit coccidiain in a rabbit farm

Groups (n=30)	Death of animal	dosage	OPG (10^4)				Weight gain (g/day)
			Day 1	Day 7	Day 14	Day 21	
Ponazuril	0	30 mg/kg	7.93	3.18	0.82	0	36.33±3.21^{a}
Diclazuril	0	1 mg/kg	8.46	11	1.04	0.05	35.09±7.33^{a}

Two shortcomings of ponazuril were observed from this study. Compared to diclazuril, it is at high cost using ponazuril at a dosage of 10 mg/kg or even higher. The palatability of ponazuril should also be taken into consideration as the phenomenon that obvious wasting of feed by rabbits was found in the high dosage group (60 mg/kg). These two prominent problems should be solved before the commercializing of ponazuril as drug for the control rabbit coccidiosis.

CONCLUSIONS

In summary, ponazuril can protect of rabbits against coccidia infection. However, more research should be performed to explore the application of ponazuril in the control of rabbit coccidiosis.

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Prophylactic and Therapeutic Efficacy of Ponazuril Against Rabbit Coccidiosis

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Introduction

Management of rabbit coccidiosis is still a big challenge for the rabbit industry

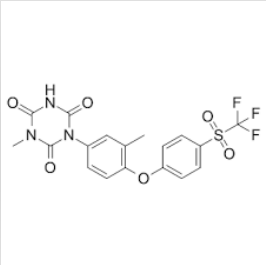
- high morbidity and mortality
- no vaccine
- drugs available are few

Locality	Examined no.	Positive no.	Prevalence (%)
North China	200	68	34.0
South China	120	31	25.8
Southwest China	120	75	62.5
Northwest China	20	14	70.0
Northeast China	20	13	65.0

Jing et al., Parasitol Res. 2012

Introduction

- **Ponazuril**
- Is a triazinone derivative related to toltrazuril;
- was used for the treatment of equine protozoal myeloencephalitis (EPM) and coccidiosis.



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- **Ponazuril supplemented in feed was medicated to the rabbits with or w/t coccidia infection.**
- **Body weight gain, feed consumption and fecal oocyst number were measured weekly in three trials.**

Results

Trial 1 Ponazuril in the concentration of up to 30 mg/kg in feed does not influence the growth of rabbits

Groups (n=4)	Weight gain (g/day)	Daily feed consumption (g)	FCR
w/t drug	50±4.72^{a}	155.27	3.10
10 mg/kg	34.52±6.27^{bc}	142.77	4.13
30 mg/kg	40.47±4.12^{ab}	150.76	3.72
60 mg/kg	26.34±7.19^{c}	140.63	5.33

Results

Trial 2 Ponazuril is effective against coccidia infection in rabbits

Groups (n=3)	Oocysts shed (10^6)				Weight gain (g/day)	Daily feed consumption (g)	FCR
	Day 1-7	Day 8-14	Day 15-21	Day 21-28			
Non-infected Control	0	0	0	0	49.26±5.16^{a}	171.42	3.48
Infected control	7.6	880	27	0.44	26.69±6.89^{d}	120	4.49
With 10 mg/kg	0	8.4	2.6	0.03	38.99±5.25^{bc}	157.14	4.03
With 20 mg/kg	0	0.6	0.08	0	42.37±7.52^{abc}	151.42	3.57
With 15 mg/kg	7.2	36.2	2.44	0.06	36.78±4.57^{c}	148.57	4.04
With 30 mg/kg	5.2	29.7	0.19	0	46.31±4.77^{ab}	171.42	3.70

Results

Trial 3 Ponazuril is also effective in naturally infected rabbits

Groups (n=30)	dosage	OPG ($X10^4$)				Weight gain (g/day)
		Day 1	Day 7	Day 14	Day 21	
Ponazuril	30 mg/kg	7.93	3.18	0.82	0	36.33±3.21
Diclazuril	1 mg/kg	8.46	11	1.04	0.05	35.09±7.33

Conclusion

- Ponazuril can protect rabbits against coccidia infection.
- More work should be done to explore the application of ponazuril in the control of rabbit coccidiosis.

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