Jurnal Veteriner Juni 2011 Vol. 12 No. 2: 77-82
ISSN : 1411 - 8327

Efficacy of Piperazine Dihydrochlloride Against *Toxocara Vitulorum* in Buffalo Calves

(EFIKASI PIPERAZIN DIHIDROKLORIDA TERHADAP CACING TOXOCARA VITULORUM PADA PEDET KERBAU)

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ABSTRACT

A study was conducted to evaluate the efficacy of piperazine dihydrochloride against natural infection of *Toxocara vitulorum* in buffalo calves. In the first trial 60 based on fecal ascarid egg count and body weight naturally infected calves were, allocated into four groups. Three groups (groups B, C and D) were given piperazine dihydrochloride per os at dose levels of 200, 300 and 500 mg per kg body weight, respectivelythe remain group is non-treated controls. Piperazine treatment reduced egg excretion levels at 98 to 99 per cent within 7, 14 and 21 days after treatment. Depite no significant differences were found among the three doses, highest egg count reductions were observed in calves given the drug 300 mg per kg bodyweight. The second trial were conducted using 10 additional calves. Piperazine significantly reduced T vitulorum egg excretion by 93% within 7 days post the treatment.

Key words: buffalo calves, piperazine, *Toxocara vitulorum*

ABSTRAK

Penelitian telah dilakukan untuk mengetahui efikasi piperazin dihidroklorida terhadap infeksi alamiah *Toxocara vitulorum* pada pedet kerbau. Pada percobaan pertama, 60 ekor pedet kerbau yang secara alami terinfeksi parasit dibagi menjadi empat kelompok masing-masing terdiri dari 15 ekor pedet berdasarkan kesetaraan berat badan dan jumlah telur ascarid dalam tinja (EPG). Tiga kelompok (Kelompok B, C dan D) diobati dengan piperazin dihidroklorida per oral dengan dosis masing-masing 200, 300 dan 500 mg/kg BB, sedangkan kelompok A bertindak sebagai kontrol tanpa pengobatan. Pengobatan dengan dengan piperazin ternyata mampu menurunkan jumlah telur ascarid dalam tinja sebanyak 98–99 % pada hari ke-7, 14 dan 21 setelah pengobatan. Meskipun tidak ditemukan perbedaan yang nyata dari tingkat penurunan jumlah telur diantara ketiga kelompok pengobatan, tingkat rataan tingkat penurunan jumlah telur pada pedet yang diobati dosis 300 mg/kg BB lebih tinggi dibandingkan kedua kelompok lainnya. Pada percobaan kedua, 10 ekor pedet kerbau dibagi menjadi dua kelompok masing-masing 5 ekor; Kelompok T yang diobati dengan dosis 300 mg/kg BB dibandingkan dengan Kelompok kontrol (K). Dari pengamatan jumlah cacing dewasa yang dilakukan pada saat nekropsi 7 hari pasca pengobatan, efikasi piperazin terhadap stadium dewasa T. vitulorum mencapai 93%. Tidak ditemukan efek samping pasca pengobatan sehingga jenis obat ini aman dipakai pada pedet kerbau.

Kata-kata kunci: pedet kerbau, piperazine, *Toxocara vitulorum*

INTRODUCTION

Toxocara vitulorum (Neoascaris vitulorum) is a common parasite of cattle and buffalo calves in tropical and subtropical regions (Mahieu and Naves 2008, Asif Raza *et al.,* 2007; Srikitjakarn *et al.,* 1987). The most important source of infection for calf is the infective larvae passing through the milk of the dam. The larvae will undergone two molting in the alimentary tract of the definitive host (calves) before becoming adult worm in the duodenum of the calf. First eggs shed in feces when calf aged between 16-28 days old. The ingestion of infective eggs by calves over 6 months old seldom results in patency. The larvae migrating to the tissues where they stored; In female animals, larvae resume their development in late pregnancy allows further

transmammary transmission (Roberts 1993). Prevalence of the parasite infection has been reported being very high in buffalo calves in Asian countries (e.g. Satrija *et al.,* 1996; Roberts 1993; Srikitjakarn *et al.,* 1987). The parasite affects calves in their very young age and cause significant villus atrophy, particularly in the duodenum (Neves and Starke-Buzetti, 2005). Such morphological abnormalities cause adverse effects on feed digestion and absorbsion in the affected region leading to slower body growth rate. The infection may even cause significant economic losses due to morality among calves in the infected flocks (Srikitjakarn *et al.,* 1987; Satrija *et al.,* 1996). Considering role of swamp buffaloes as the prime source of draught power in rice fields and as a source of meat in Indonesia, *Toxocara vitulorum* control program should become a part of the animal health management in rural area of Indonesia.

Combination between hygiene of pens and deworming remains the best approach to control *Toxocara vitulorum* infection (Roberts 1993). The classical anthelmintic compound piperazine has been known to be effective against roundworm (Ascarid) in domesticated animals. Piperazine is commercially available in its simple salts including piperazine adipate, citrate, phosphate, sulfate, tartrate, and dihydrochloride (Roberson 1982). In Indonesia, piperazine is one of the cheapest and easily found anthelmintic used for farm animals (DITJENAK-ASOHI 2000). Therefore it is of important to evaluate the use of piperazine as drug of choice for toxocarosis in buffalo calves.

The present study was designed to observe the efficacy of piperazine dihydrochloride against *T. vitulorum* in naturally infected young buffaloes. This piperazine formulation has been demonstrated to have a very high efficacy against *Ascaris suum* in naturally infected pigs (Steffan *et al*., 1988).

RESEARCH METHODS

Animals

A total of seventy buffalo calves aged between 21-45 days old were used in the present study. They were all naturally infected with *T. vitulorum* as indicated by excretion of ascarid eggs in their faeces (EGP>5000). They were selected from flocks of buffaloes belonging to small holder farmers in Cirebon Regency, West Java. During the study, the calves were reared together with their dams in buffalo pens that situated in village communal lands.

The Anthelmintic

A granular formulation of piperazine dihydrochloride (Ascarex D- Akzo Nobel Surface Chemistry, Sweden) was used in this study. According to the manufacturer the product contained 53 per cent piperazine base. The compound was diluted with tap water to a concentration of 10% (v/v) immediately before dosing of the calves.

Experimental design

The present study was carried out in two consecutive trials according to the following experimental designs:

Trial 1

Sixty naturally *T. vitulorum* infected calves were randomly allocated into four groups each consist of 15 calves (8 males and 7 females calves). The groups were also comparable in the term of ascarid egg excretion levels (EPG), and body weights. Calves of groups B, C and D were each treated with a single dose of piperazine dihydrochloride at dose levels of 200, 300 and 400 mg per kg bodyweight, respectively. Calves in group A served as non-treated controls. The drug was administered orally using a drencher. Effect of various piperazine dihydrochloride dosages on the parasite was monitored fecal egg count (EPG) on one day before the treatment and day 1, 7, 14, and 21 after the treatment. The clinical condition of the animals were examined weekly throughout the experiment.

Trial 2

Ten calves were divided into two equal groups (3 male and 2 female) designed comparable with respect to sex, bodyweight and egg excretion levels. One group (group C) was left untreated (controls), whereas animal in group T were treated with a single dose of piperazine dihydrocloride at the dose level of 300 mg per kg body weight per os. Fecal samples were taken on day 1,2,4, and 6 of the treatment. At day 7 the animals were slaughtered for post mortem *T. vitulorum* worm counts.

Total Fecal Parasites Egg Count

Faecal eggs count were estimated using a modified McMaster technique, and counts were expressed as numbers of egg per gram faeces (EPG). At slaughter, the small intestine was

removed from carcass. This organ was cut open examined according to standard techniques for recovery of helminths and pathological examinations (Hansen and Perry, 1994). All *T. vitulorum* measuring more then 1 cm in length were isolated immediately and stored in screw capped plastic jars in physiological saline solution until counting procedures were under taken.

Calculation and Statistical Analysis

The egg count per gram faeces (EPG) and the worm counts were transformed to the natural logarithm of (EPG+20) and (worm count +1), respectively, prior the calculation of geometric mans (GEM) and percentage of efficacy and fecal egg count reduction (Wood *et al.,* 1995).

The geometric mean of EPG was calculated according to the formula: GEM= exp [1/n Σ In(EPG + 20)] -20

The geometric mean of post mortem worm counts was calculated according to the formula: GEM = exp [1/n Σ ln(worm count + 1)] – 1

The percentage (%) of reduction in fecal egg count was calculated as follows:

Reduction (per cent) = (GEM of EPG before treatment - GEM of EPG after treatment) x (GEM of EPG before treatment)$^{-1}$ x 100

The percentage (%) efficacy of the drug against *T. vitulorum* was estimated on he basis of the geometric mean (GEM) numbers of worm recorded:

Efficacy (per cent) = (GEM worm numbers in control group – GEM worm numbers in treated group) x (GEM numbers in controls)$^{-1}$ x 100

Comparison were made between the control and the treated groups by one-way analyses of variance and the T method for multiple comparison (Trial 1). Furthermore, student tests were performed to analyses significant differences between the control and the treated group in trial 2.

RESULTS AND DISCUSSION

Trial 1

Geometric mean of EPG counts of buffalo calves before and after the treatment are shown in Table 1. Average pre-treatment (day 0) fecal egg counts of groups A, B, C and D were 50494, 53084, 55251, and 53617 EPG, respectively. The non treated control group (A) maintained, with some fluctuations, the initial high egg counts on days 7, 14 and 21 post treatment. In contrast,

Table 1. Geometric mean of ascarid egg counts (EPG) and fecal egg count reduction of the buffalo calves before and after piperazine dihydrochloride treatments

Group	Fecal egg counts/EPG (Min-Max) **			
	Day 0*	Day 7	Day 14	Day 21
A (Control)	50,494[a] (16,300-262,800)	84,100 [a] (29,800-412,800)	73,110 [a] (53,200-305,000)	10,077 [a] (0-185,000)
B (200 mg/kg BW)	53,084 [a] (15,800-271,800)	1121 [b] (0-271,800)	245 [b] (0-153,000)	61 [b] (0-20,600)
EPG reduction (%)		97.9	99	99
Group C (300 mg/kg BW)	55,251 [a] (5000-209,400)	809 [b] (0-93,600)	453 [b] (0-570,000)	114 [b] (0-260,400)
EPG reduction (%)		99	99	99
D (500 mg/kg BW)	53,617 [a] (10,900-263,400)	2081 [b] (0-116,200)	1690 [b] (0-65,600)	503 [b] (0-197,600)
EPG reduction (%)		96	99	99

*Average of EPG from day -1 and 0

**Different superscript in each sampling dates indicate a significant different (P< 0.01)

the piperazine treated groups (groups B, C and D) showed a significant decline already on day 7. From day 7 onwards, average EPG values were less than 4 per cent of the pre-treatment values of these groups, and differed significantly from these as well from those of group A ($P<0.01$). Fecal egg count reduction in all piperazine the treated groups reached 99% from day 14 after the treatment. Despite no statistically significant differences were found among groups B, C and D, the highest egg count reductions were observed in calves group receiving piperazine dihydrochloridire at dose level of 300 mg per bodyweight.

At the start of the experiment, most of the animal showed clinical signs of toxocarosis in the form of gray-colored light scouring or constipation, anorexia and poor growth. Following piperazine treatment, the clinical conditions of group B, C and D calves improved promptly, and no clinical signs of parasitic gastroenteritis were observed from day 7 onwards. In contrast, there were persistent soft faeces to watery diarrhea among calves in group A, until the end of the trial.

High fecal egg count reduction in the present experiment is similar to the result of Islam *et al.,* (2005) who demonstrated 100% fecal egg count reduction when treating naturally *T. vitulorum* infected calves with 200 mg piperazine citrate/kg bw. However, Baruah *et al*., (1980) and Akhtar *et al*., (1982) found only 82 to 88 per cent egg count reduction in calves given piperazine at 88 to 220 mg/kg bodyweight. Such discrepancy may partly be due to variability of piperazine compounds used in those studies. The antiparasitic activity of various salts of piperazine depends almost solely on the piperazine base (Roberson 1982). Among various piperazine salts, dihydrochloride salt is known to have the highest concentration of piperazine base (50-53%), whereas the lowest concentration is found in the citrate salt (35%). Other commonly found piperazine salts such as adipate and phosphate contain 37 and 42 % of piperazine base, respectively.

Some calves in piperazine treated group remained excreting substantial number of ascarid eggs after the treatment. Such animal may harbored a heavy parasite burden which could not be expelled by a single treatment. Therefore, it is necessary to give a second additional treatment five to seven days later in an attempt to depress the EPG below clinical level. Once should be kept in mind that piperazine treatment may be ineffective when given to calves less then 21 days old because the drugs is not effective against immature parasite (Roberts 1989). To overcome this problem the treatment should be given twice, i.e. animals at age 21 days and repeated treatment at age between 25 to 28.

Trial 2

At the start of the trial, mean ascarid egg counts (group C) two experimental groups were 25,316 and 29,418 EPG (group T), respectively. The EPG values of the non-treated control remained elevated until the end of the trial (Figure 1). Following piperazine treatment animals in group T exhibited a certain, yet not statistically significant decline already on days 2 and 4. Only one out of five group C calves remained excreting ascarid eggs in the feces on day 6, and the mean EPG values of the treated and non-treated animal became significantly different ($P< 0.05$). The percentage egg count reduction in animals treated with 300mg/kg BW of piperazine dihydrochloride was up to 99.8%. Expulsions of adult worm in the feces were observed on two and three days after treatment. dihydrochloride

At post mortem, *T. vitulorum* burdens in the control calves varied from 3 to 114 adult worms with a mean of 10.5, whereas a mean worm count of 0.6 was found in the treated calves. With exception of one calves that harbored 13 adult worm, no worm was found in calves of Group T (Table 2). Gross pathological examination revealed extensive oedema in association with ptechiae until hemorrhages on

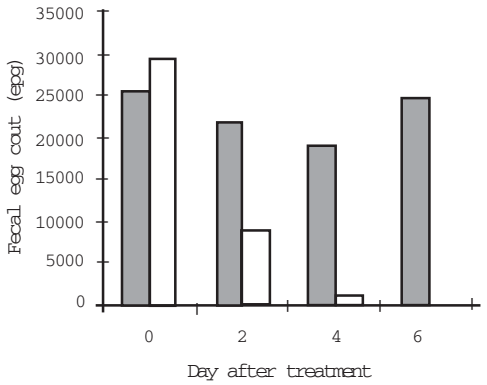

Figure 1. Geometric mean of ascarid egg counts (EPG) of the buffalo calves before and after treatment with 300 mg/kg BW piperazine dihydrochloride. ■ C □T

Table 2. Number of adult *T. vitulorum* recovered *post mortem* in the small intestine of buffalo calves of group K and T on day 7 after treatment with 300 mg/kg BW piperazine dihydrochloride

Group	Calf				Number of adult *T. vitulorum* recovered *post mortem*
	No	Age (days)	Sex	Body Weight (kg)	
Non treated Control / K	K1	32	F	43	5
	K2	29	F	37	3
	K3	30	M	37	17
	K4	35	M	41	3
	K5	32	M	41	114
Geometric mean					10.5
Piperazine treated/ T (300 mg/kg BW)	T1	29	M	41	13
	T2	30	F	36	0
	T3	32	M	44	0
	T4	35	F	37	0
	T5	35	M	35	0
Geometric mean					0.7*
Efficacy (%)					(93)

*Significantly different (P< 0.05) from controls (Group K)

small intestinal mucosa of the control calves. Small intestine of the treated calves showed some oedema and slight hyperemia. Such pathological changes probably associated with significant increases of the population of mast cells, eosinophils, lymphocytes, and Goblet cells in the duodenum, jejunum and ileum during the peak of the infection (Neves and Starke-Buzetti, 2005).

Results of the present may be the first experimental documentation of the efficacy of piperazine dihydrochloride against *T.vitulorum* in buffalo calves in Indonesia. Postmortem worm count results revealed the efficacy of the drug against patent infection of *T.vitulorum* was 93 per cent when given as a single dose of 300 mg/ kg BW. WAAVP standard categorize anthelmintics with efficacy between 90 to 98 % as effective against the target parasite (Wood *et al.,* 1995). Previous study showed efficacy of this compound against *Asaris suum* in pig reached 99 to 100 per cent (Steffan *et al*., 1988). A consistently high efficacy of piperazine dihydrochloride, in its new granular formulation, against ascarid in various host species may be explained by improved drug quality and proper individual dosing of the animals.

Clinical finding observed in the experimental calves at the pre-treatment period are similar with previously described toxocarosis signs (Akhtar, 1982, Roberts, 1993). Diarrhoea recorded in heavily infected animals may be a result of irritation of the surface of intestinal mucosa. Rapid egg count reduction observed 4 day after treatment onwards was coincided with expulsion of adult worms at day 2 to 3 after treatment. Expulsion of the worms facilitated improvement and disappearance of the clinical sign of toxocarosis.

Mode of action of piperazine against ascarid has been extensively studied *in vitro* using *Ascaris suum* models. Piperazine acts as a GABA agonist of low on nematode muscle extra-synaptic GABA receptors (Martin *et al.,* 1996). It causes hyper polarization of nerve membrane and flaccid paralysis of the nematode, leading to the worm expulsion by normal peristalsis (Del Castillo *et al.,* 1964).

CONCLUSION

In conclusion, single dose treatment with 300 mg/kg BW of piperazine dihydrochloride is effective against potent *T. vitulorum* infection in buffalo calves. It is suggested that the treatment should be given to calves twice : first between at age 21 days and repeated at age 25 to 28 days.

ACKNOWLEDGMENTS

The authors wish to thanks Akzo Nobel Surface Chemistry, Sweden for the financial support of this study. We are grateful to Cirebon Regency Livestock Services for technical assistance during the study.

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