

# Comparison of the Efficacy of Two Commercial Coccidicidal Compounds on Experimentally Infected Dogs

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## Abstract

The aim of the present study was to compare the coccidicidal efficacy of two commercial compounds for artificially infected dogs. Eighteen coccidia-free dogs, male and female aged between 2 and 4 months old, were infected each with 20,000 *Cystoisospora oocysts* cultured under laboratory conditions. When the puppies showed high counts of oocysts per gramm (OPG) (McMaster method), they were divided into 3 groups (G). G1 was treated with a compound named One<sup>®</sup> (Lab. Bio Zoo) containing 150 mg of toltrazuril/tablet, administered daily/5days dosing 1 tablet per 10 kg b.w. G2 received Giacoccide<sup>®</sup> (Pet's Pharma) containing 250 mg of sulfadimetoxine and 165 mg of dimetridazole dosing 1 tablet per 10 kg b.w. twice a day for 10 days. G3 served as an untreated control. The puppies were coprologically monitored on Days 0, 3, 5, 10, 15, 20 and 25 to determine the percentage of OPG. Efficacy was measured based on the OPG reduction on treated animals relative to the untreated control. The results indicated an efficacy for compound One of 78.4%, 100%, 100%, 100%, 100% and 100%, for Days 3, 5, 10, 15, 20 and 25, respectively and for Giacoccide 40.6%, 45.5%, 47.4%, 65.9%, 90.4% and 92.7%, for Days 3, 5, 10, 15, 20 and 25, respectively. No statistical difference was observed on the weight of the treated puppies ( $p < 0.764$ ), but the control group was statistically different to the treated ones ( $p < 0.014$ ). It was concluded that compound One showed greater efficacy than Giacoccide for the treatment of canine coccidiosis in artificially infected dogs.

## Keywords

Efficacy, Coccidicides, Experimentally Infected Dogs

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## 1. Introduction

Coccidia are obligate intracellular protozoan parasites commonly found in the gastrointestinal tract of dogs and cats [1]-[3]. The most commonly diagnosed infections in dogs are *Cystoisospora* (synonym *Isospora*) *canis* and *Cystoisospora ohioensis*. This is a typical disease of puppies and kittens <6 months old and parasite recurrence is rare in animals >1 year of age [2].

To control this disease, trimethoprim sulfonamide, furazolidone and amprolium have been frequently used [4]. In addition, sulfadimethoxine is the only drug that has been approved for the treatment of coccidiosis in dogs and cats [5], but since sulfonamides are coccidiostatic, a low level of persistent infection is possible after treatment [1].

More recently, toltrazuril (TZR), 1-methyl-3-[3-methyl]-4[4-(trifluoromethylsulfanyl) phenoxy] phenyl-1,3,5-triazinane-2,4,6-trione, is currently the treatment used by many clinicians for *Cystoisospora* spp. infections in small animals. Since these drugs are coccidiocidal they may be superior to other drugs for the treatment for coccidiosis [6]. Daughschies *et al.* (2000) [7] have mentioned that ponazuril (toltrazuril sulphone) when administered to all at-risk puppies and kittens on entrance to shelters may aid in the control of coccidiosis.

The aim of the present study was to compare the coccidicidal efficacy of two commercial compounds for dogs artificially infected with *Cystoisospora canis* and *C. ohioensis*.

## 2. Material and Methods

### 2.1. Study Location

The present study was carried out at the Experimental Unit of the Parasitology Department, of the FMVZ-UNAM.

### 2.2. Animals

Two to four months old stray-dogs of both sexes were selected for inclusion in this study. They were recruited from a municipal animal shelter in Mexico City. At reception, all dogs were vaccinated against distemper, parvovirus and rabies (Vaccine Puppy Fence L5)<sup>®</sup> and they were dewormed against nematodes and cestodes with Panacur-Plus<sup>®</sup> (Intervet) containing 500 mg fenbendazol and 25 mg praziquantel. The puppies were fed with pelleted food (Dog Chow puppies) and water was provided *ad libitum*.

### 2.3. Experimental Compounds

Compound A: One<sup>®</sup> (Laboratorios Bio Zoo), containing 150 mg toltrazuril/tablet.

Compound B: Giacoccide<sup>®</sup> (Pet's Pharma) containing 250 mg of sulfadimetoxine and 165 mg of dimetridazole formulated as tablets.

### 2.4. Oocysts Production for Infection

Oocysts from *Cystoisospora* spp. were cultured in 2% potassium dichromate, were initially obtained from naturally infected puppies. They were refrigerated until used.

### 2.5. Infection of Puppies

Each animal was infected with approximately 20,000 oocysts of *Cystoisospora* spp. given in 1 ml of water by means of a plastic 5 ml syringe. Nine days after infection the dogs showed high numbers of OPG counted by the McMaster method.

### 2.6. Identification of Puppies

Each animal was identified by a collar numbered tag and also by anatomical features.

### 2.7. Procedure of the Study

On Day 0 (treatment day) dogs were divided into 3 groups (G) of 6 animals each. They were proportionally distributed according to their number of OPG.

G1 received compound One administered every 24 hours for 5 days dosing 1 tablet/10kg b.w.  
 G2 received Giacoccide dosing 1 tablet/10kg b.w. every 12 hours during 10 days.  
 G3 served as an untreated control.

## 2.8. Fecal Analysis

The puppies were individually caged and were coprologically examined on Days 0, 3, 5, 10, 15, 20 and 25. The oocysts present were counted to discover the individual or group burden of coccidia in the experimental animals.

## 2.9. Efficacy Measurement

The compound's efficacy was assessed as a percentage of the OPG reduction on the treated puppies relative to the OPG counts in the untreated control using the formula described by [8] where:

$$\% \text{ Efficacy} = \frac{\text{Number of oocysts on the control group} - \text{Number of oocysts on the treated group}}{\text{Number of oocysts on the control group}} \times 100.$$

## 2.10. Statistical Analysis

The obtained data was submitted to the Kruskal-Wallis Rank test to determine possible statistical differences between the experimental groups. It was carried out using the S.A.S packet (2004).

## 3. Results

The obtained results can be seen in a general form in **Tables 1-3**.

**Table 1.** Copromicroscopic analysis (OPG) before and after treatment with two commercial compounds in puppies artificially infected with *Cystoisospora* spp.

Groups (n = 6)	Animal No.	Sex	Weight (kg)	Dose (tablet)	Tx* Days after treatment						
					0	3	5	10	15	20	25
One 1 Tablet/ 10 kg/5days	1	H	5	1/2	5200	0	0	0	0	0	0
	2	H	2.5	1/4	13,100	3850	0	0	0	0	0
	3	H	5	1/2	10,250	4200	0	0	0	0	0
	4	H	5	1/2	11,250	1600	0	0	0	0	0
	5	H	2.5	1/4	6200	800	0	0	0	0	0
	6	H	2.5	1/4	3850	50	0	0	0	0	0
<b>Total No. of OPG in the group</b>					<b>49,850<sup>a</sup></b>	<b>10,500<sup>a</sup></b>	<b>0<sup>a</sup></b>	<b>0<sup>a</sup></b>	<b>0<sup>a</sup></b>	<b>0<sup>a</sup></b>	<b>0<sup>a</sup></b>
Giacoccide 1 tabl/10kg twice/day/10days	7	H	3	1/2	6950	4200	3750	2200	2100	850	500
	8	H	3.5	1/2	8500	1600	2400	3400	2800	350	400
	9	H	8	1	6300	6200	5800	6800	1400	0	0
	10	H	6	3/4	1800	1750	3100	3200	2650	250	100
	11	M	5	1/2	22,700	13,650	8900	7350	5200	2750	1700
	12	M	6	3/4	2200	1450	2500	2600	2400	450	850
<b>Total No. of OPG in the group</b>					<b>48,450<sup>a</sup></b>	<b>28,850<sup>b</sup></b>	<b>26,450<sup>b</sup></b>	<b>25,550<sup>b</sup></b>	<b>16,550<sup>b</sup></b>	<b>4650<sup>b</sup></b>	<b>3550<sup>b</sup></b>
Untreated control	13	H	3	---	10,800	8300	9650	10,800	16,400	1800	100
	14	H	3.5	---	6600	14,050	12,800	20,200	17,950	16,800	37,200
	15	H	6	---	8400	60,500	18,400	6400	550	650	1450
	16	H	5.5	---	8200	1560	2800	1350	450	550	2200
	17	H	4	---	8200	5200	3900	1600	800	800	450
	18	M	8	---	6400	5650	1850	850	950	800	400
<b>Total No. of OPG in the group</b>					<b>48,600<sup>a</sup></b>	<b>95,260<sup>b</sup></b>	<b>49,400<sup>b</sup></b>	<b>41,200<sup>b</sup></b>	<b>37,100<sup>b</sup></b>	<b>21,400<sup>b</sup></b>	<b>41,800<sup>b</sup></b>

OPG = oocysts per gramm. \*Treatment. <sup>a,b</sup>Different letter indicate statistical difference (p < 0.05).

**Table 2.** Efficacy percentage of two coccidicidal compounds in artificially infected puppies.

Groups (n = 6)	Tx	Days after treatment (%)					
	0	3	5	10	15	20	25
1 One	0	100	100	100	100	100	100
	0	52.4	100	100	100	100	100
	0	48.1	100	100	100	100	100
	0	80.2	100	100	100	100	100
	0	90.1	100	100	100	100	100
	0	99.3	100	100	100	100	100
Efficacy/group (%)	<b>0<sup>a</sup></b>	<b>78.4<sup>a</sup></b>	<b>100<sup>a</sup></b>	<b>100<sup>a</sup></b>	<b>100<sup>a</sup></b>	<b>100<sup>a</sup></b>	<b>100<sup>a</sup></b>
2 Giacoccide	0	48.1	53.7	72.8	74	89.5	93
	0	80.2	70.3	58	65.4	95.6	95
	0	23.4	28.4	16	82.7	100	100
	0	78.4	61.7	60.4	67.2	96.9	98.7
	0	0	0	9.2	35.8	66	97.9
	0	82.1	69.1	67.9	70.3	94.4	89.5
Efficacy/group (%)	<b>0<sup>a</sup></b>	<b>40.6<sup>a</sup></b>	<b>45.5<sup>b</sup></b>	<b>47.4<sup>b</sup></b>	<b>65.9<sup>b</sup></b>	<b>90.4<sup>b</sup></b>	<b>92.7<sup>b</sup></b>

<sup>a,b</sup>Different letter indicates statistical difference (p < 0.05).

**Table 3.** Difference on the weight gain of treated puppies artificially infected with *Cystoisospora* spp.

Groups (n = 6)	Animal No.	Weight (kg)		
		Day 0	Day 25	Weight difference
1 One	1	5	6	1
	2	2.5	3.5	1
	3	5	7	2
	4	5	6.5	1.5
	5	2.5	3.5	1
	6	2.5	3.5	1
	<b>Total weight/group</b>	<b>22.5</b>	<b>30</b>	<b>7.5</b>
	<b>Average weight/animal</b>	<b>3.7<sup>a</sup></b>	<b>5<sup>b,c</sup></b>	<b>1.25<sup>b,c</sup></b>
2 Giacoccide	7	3	3	0
	8	3.5	4.5	1
	9	8	12	4
	10	6	7	1
	11	5	6.5	1.5
	12	6	7	1
	<b>Total weight/group</b>	<b>31.5</b>	<b>40</b>	<b>8.5</b>
	<b>Average weight/animal</b>	<b>5.2<sup>a</sup></b>	<b>6.6<sup>c</sup></b>	<b>1.41<sup>c</sup></b>
Untreated control	13	3	3	0
	14	3.5	4	1
	15	6	7	1
	16	5.5	6	0
	17	4	4	0
	18	8	8	0
	<b>Total weight/group</b>	<b>30</b>	<b>32</b>	<b>2</b>
	<b>Average weight/animal</b>	<b>5<sup>a</sup></b>	<b>5.3<sup>a</sup></b>	<b>0.33<sup>a</sup></b>

<sup>a,b,c</sup>Different letter indicates statistical difference (p < 0.05).

### 3.1. Coprological Analysis

The G1 treated with One (toltrazuril) was initiated on Day 0 with a total of 49,850 OPG with a minimum of 3850 and a maximum of 13,100 OPG, an average/animal having of 8308 OPG.

On Day 3 after treatment the OPG percentage decreased 79% and for the 5th day after treatment the percentage of OPG reduction was 100% (**Table 1**).

The G2 treated with Giacoccide (sulfadimetoxine and dimetridazole) showed a gradual percentage of OPG initiating with 48,450 OPG in the group with a minimum of 1800 OPG and a maximum of 22,700 OPG, with an average/animal of 8075 OPG.

On the 10th day after the treatment this group decreased the OPG 52.7% (25,550 oocysts) and decreased the oocyst production down to 3550 OPG (92.6%) for Day 25 after treatment (**Table 1**).

The statistical analysis indicated that on Day 0 there was no difference among groups but there was observed a statistical difference from the 3rd day ( $p < 0.034$ ); Day 5 ( $p < 0.002$ ); Day 10 ( $p < 0.0027$ ); Day 15 ( $p < 0.002$ ); Day 20 ( $p < 0.003$ ) and Day 25 ( $p < 0.004$ ).

When comparisons were made with the treated groups, on Day 3 there were no statistical differences ( $p > 0.11$ ); but from Day 5 up to 25 there was found a statistical difference ( $p < 0.0074$ ).

When comparisons were made with the group treated with compound One against the control one, significant differences were encountered from the 3rd day up to 25 ( $p < 0.01$ ). However, a comparison from Giacoccide versus the control showed no difference ( $p < 0.15$ ).

G3 always remained with high OPG numbers initiating with 48,600 OPG, followed by 95,260, 49,400, 41,200, 37,100, 21,400 and 41,800 OPG/group for Days 0, 3, 5, 10, 15, 20 and 25, respectively (**Table 1**).

### 3.2. Percentage of Efficacy

G1 (treated with One-tablet) exerted an efficacy percentage of 78.4% on Day 3 after treatment.

From Day 5 on, the percentage of OPG reduction reached 100% (**Table 2**).

G2 showed on Day 3 posttreatment 40.6% of efficacy, gradually increasing its coccidicidal activity to percentages of 45.5%, 47.4%, 65.9%, 90.4% and 92.7%, for Days 5, 10, 15, 20 and 25 posttreatment, respectively (**Table 2**).

The statistical analysis demonstrated that the percentage of efficacy between groups on Day 3 showed no difference; however, from Day 5 on there was found a statistical difference ( $p$ -value = 0.002778); on Day 10 ( $p$ -value = 0.002778); on Day 15 ( $p$ -value = 0.002778); on Day 20 ( $p$ -value = 0.009622) and for Day 25 ( $p$ -value = 0.009622).

### 3.3. Weight Difference

G1 initiated on Day 0 with puppies between 2.5 and 5 kg, (average 3.7 kg/animal). For Day 25 (end of the study) the average final weight was of 5 kg, with a weight difference of 1.25 kg/animal (**Table 3**).

G2 initiated on Day 0 with weights from 3 to 8 kg, (average 5.2 kg/animal), ending with a mean weight of 6.6 kg/animal. The mean difference of weight on this group was 1.41 kg/animal.

For G3 the mean weight/animal was 5 kg and for Day 25 the average weight recorded was 5.3 kg/animal. The weight gain obtained for this group was 0.33 kg/animal. The statistical analysis indicated a weight gain among groups ( $p < 0.03802$ ).

When the treated groups (One and Giacoccide) were compared, the Kruskal-Wallis rank test showed a statistical difference among groups ( $p < 0.024$ ).

In addition there was a statistical difference between compound One with the control group ( $p < 0.014$ ), but not with compound One and Giacoccide ( $p < 0.764$ ).

With regard to the degree of infection of OPG and the efficacy of both compounds for male and female, it was not possible to measure this parameter since only 3 out of 18 were male (**Table 1**).

## 4. Discussion

To control coccidiosis in dogs other drug regimens have been used with some success; these include trimethoprim-sulfa (30 - 60 mg/kg of trimethoprim daily for 6 days in animals—4 kg; or 15 - 30 mg/kg trimethoprim daily for 6 days in animals—4 kg) and a variety of protocols using amprolium alone or in combination with sul-

fadimethoxine [5].

In the United States, the Companion Animal Parasite Council reports that prevalence rates for *Isospora* spp. infection in dogs and cats can vary from 3% to 30% ([www.capevet.org](http://www.capevet.org)). In a study of 1,199,293 fecal samples from dogs in the United States, 4.4% contained *Isospora* spp. oocysts [9]. In environments with heavy infections, treatment of all animals in contact with other animals, particularly puppies and kittens, could be considered.

In Mexico epidemiological studies on the presence of Coccidiosis in dogs are very scarce but it is a fact that a considerable number of puppies are infected with *Cystoisospora* spp. and we did not have any problem to find positive dogs to coccidia for inclusion in the trial.

In our study due to the great reproducibility of OPG in the puppies it was amazing to be able to integrate groups with similar numbers of oocysts which by the way facilitated the evaluation of compounds under study.

Myoung-Seok *et al.*, (2010) [10] have mentioned that toltrazuril (TZR) was very well absorbed through the gastrointestinal tract and rapidly metabolized to TZR\_SO and TZR\_SO<sub>2</sub> providing sufficient levels to obtain therapeutic effects for the control of coccidiosis.

Our study on its own shows clearly defined reduction patterns of oocysts.

In the case of G1 (compound One) the presence of *Isospora* oocyst is absent from the 3rd day on after treatment, thus indicating a high percentage of efficacy exerted by toltrazuril.

However, with regard to this compound various protocols have been tested under experimental and field conditions for canine and feline *Cystoisospora* spp. infections [1] [6] [7] [11]-[13]. The drug appears to be well tolerated even in very young animals [11] [12] [14], but depending on the protocol used, infection and oocyst shedding might not be completely eliminated and repeated re-infection can occur [1].

In the case of G2 (Giacoccide) the shedding oocysts was present for the 25 days of the study even though at the end the number of oocysts present was very low.

Here it is important to note that the efficacy gradually increased but at the end this group reached only a 92.7% reduction.

Moreover, the OPG reduction was also noted in the control group. As is well documented coccidiosis is generally self-limited, and most healthy puppies and kittens will clinically eliminate infections without therapy. Therefore it is unknown if a certain percentage of efficacy obtained at the end of the study could also partially be due to this spontaneous elimination of oocysts by the puppies. However, administration of treatment can speed the resolution of clinical disease and may lessen environmental contamination and the potential for infecting other contact animals.

Various puppies generally showed diarrhea which was undoubtedly generated by the inoculated oocysts; however, some days after treatment the puppies became normal recovering their hydric balance, and started to gain weight.

With regard to the administration calendar, toltrazuril has the advantage of daily dosification/5days, while giacoccide has to be administered twice a day/10days, requiring more time and effort of the veterinary practitioner. Certainly it causes longer stress for the puppy and is time consuming for the veterinarian.

Here it is also important to mention the combined effect of compound One since it contains a wide antiparasitic spectrum. This compound is formulated with fenbendazol (nematicide), praziquantel (cestodicide) plus toltrazuril (coccidicide). In the case of Giacoccide it is formulated only with sulfadimetoxine and dimetridazole providing only an anti-protozoan effect. However, since the aim of this study was to evaluate only the coccidial effect of both compounds the data relative to other active antiparasite ingredients incorporated to the former compound is mainly complementary information.

Since sporulated *Cystoisospora* spp. oocysts are extremely resistant to routine disinfection protocols, additional measures, such as the control of transport hosts thorough environmental cleaning with either 10% ammonia left in contact with contaminated surfaces for at least 10 min or with steam, treatment of all animals and prophylactic treatment of incoming animals might be required in shelters, kennels or catteries, or places where there are recurrent problems [1]. Complete removal requires that contaminated surfaces be left in contact with 10% ammonia for at least 10 min or steam cleaned [1]. In any case, a more practical goal might be to reduce fecal oocyst counts and environmental contamination to minimal levels rather than aim for a "sterile" environment.

Finally it is also important to mention that at the end of the study the physical condition of the treated puppies was notably good, the hair looking lively and the skin a little bit greasy, all dogs being happy and playful.

On the contrary, puppies from the control group the diarrhea was absent at the end of the study, but they also

showed a sad appearance with some degree of dehydration attributed to the previous coccidia infection.

This study had a number of limitations. Small group size limited the statistical interpretation of data and it is possible that larger group sizes could have more accurately represented the proportions of animals that were treatment failures in each of the groups. Furthermore, the random allocation of animals to treatment groups might have resulted in more uniform groups with regard to bodyweight, fecal oocyst count and fecal consistency score. In particular, the fecal oocyst count at enrollment varied widely among treatment groups, which could have affected subsequent fecal oocyst counts and our interpretation of the response to treatment as well. Variations in fecal consistency scoring upon entrance could also have affected perceived response to treatment. Additional research concerning the use of toltrazuril at different dosages is required to determine a protocol that can safely and reliably eliminate infection in dogs.

## 5. Conclusion

It is concluded that compound One formulated with toltrazuril is more efficacious than Giacoccide containing sulfadimetoxine and dimetridazole for the removal of Coccidia infections in experimentally infected puppies.

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