

# Repeat Dose Tolerance of a Combination of Milbemycin Oxime and Praziquantel in Breeding and Lactating Queens

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## ABSTRACT

Two groups of 15 queens each were treated with placebo or with medicated tablets containing a combination of milbemycin oxime and praziquantel (Milbemax<sup>®</sup>) at at least the highest recommended dose rate once weekly during pregnancy and lactation. The queens in each group and their kittens were submitted to periodic controls, including clinical assessments, weighing, and blood analysis for hematology and clinical chemistry. The reproductive performance of each individual queen also was assessed. The results demonstrate that the investigated tablets containing a combination of milbemycin oxime and praziquantel are well tolerated by queens and their kittens during pregnancy and lactation.

## INTRODUCTION

Milbemycin oxime—a mixture of milbemycin A3 and A4 oximes—is a macrocyclic lactone that, alone or in combination with

praziquantel, has been shown to be highly effective against several parasites of cats such as heartworms,<sup>1-3</sup> various other roundworms,<sup>4-6</sup> and mites.<sup>7</sup> Praziquantel is a cestocidal with known efficacy against various parasitic tapeworms but not against roundworms.<sup>8</sup> Its combination with milbemycin oxime also has been shown to be efficacious against this group of worms.<sup>9</sup>

The safety of milbemycin oxime for cats has been broadly investigated in several studies that have shown it is well tolerated by both adult cats and kittens. In 2 separate studies of adult cats and kittens 2 to 3 weeks of age, respectively, animals were treated at 1, 3, and 5 times the minimum recommended dose rate of 2 mg/kg at an interval of 15 days during 90 days.<sup>10</sup> The tolerability of milbemycin oxime administered once at 10 times the recommended dose rate of 2 mg/kg also was investigated in adult cats and kittens.<sup>10</sup> In addition, the safety of praziquantel has been studied in several toxicity and tolerability investigations on breeding cats and kittens,<sup>11</sup> and many years of use of this active ingredient in many countries have confirmed the favor-

able experimental results. The present investigation was performed to confirm the safety of milbemyacin oxime in combination with praziquantel when administered to queens during pregnancy and lactation.

## **MATERIALS AND METHODS**

### **Animals**

Thirty European mixed-breed queens from a specific pathogen free (SPF) colony aged between 19.6 and 75.2 months and confirmed pregnant on pregnancy Day 32 ( $\pm 2$  days) were used for the study. At removal from the SPF colony, all queens were vaccinated against feline calicivirus, feline rhinotracheitis virus, and feline panleukopenia virus. Three proven tomcats were used for mating but were not part of the test system. No animal used in the study had been treated with any anthelmintic in the 3 months prior to the start of the study. Each queen and tomcat was identified by a number tattooed in the ear and by a subcutaneously implanted microchip. Each kitten born was identified by a subcutaneously implanted microchip.

The day of enrolment for queens was the day their last (pre-experimental) litter was weaned. On this day, all animals were examined by a veterinary surgeon. Only healthy animals were included in the study. Each queen had breeding records indicating that there were at least 3 kittens weaned in at least 2 of the previous 3 litters and that no congenital malformations were identified in any of those kittens. A blood sample used as baseline was collected from each queen for hematology and clinical chemistry analysis within one week prior to the start of the study.

Prior to and after mating, up to 15 queens were group housed in pens measuring at least  $5.2 \times 2.4 \text{ m}^2$  (length  $\times$  width). Litter boxes containing wood shavings were provided in each pen. For mating purposes, up to a maximum of 5 queens were housed in a mating pen with their assigned tomcat. After mating had been confirmed, each queen was exposed to the tomcat for an additional 5 days and was then returned to group housing with the other mated queens.

After littering, each queen was permanently housed alone with her litter in a wire mesh maternity cage measuring  $1.2 \times 0.57 \times 0.85 \text{ m}^3$  (length  $\times$  width  $\times$  height) with the floor covered with rubber matting. Standard commercially available diets were fed to queens and kittens at the recommended rates.

Potable water was available ad libitum via a water bowl in pens and cages. The microbial quality of the water was analyzed weekly for total microbial count and coliform count. Daily environmental temperature ( $16^\circ \text{C}$  to  $24^\circ \text{C}$ ) and humidity readings (36% to 70%) were taken in the unit where the queens were housed.

### **Treatments**

Fifteen queens each were allocated at random to either Group 1 to be treated with placebo or Group 2 to be treated with medicated tablets containing a combination of 16-mg milbemyacin and 40-mg praziquantel (Milbemax<sup>®</sup>, Novartis Animal Health Inc., Basel, Switzerland). Random order numbers derived from Fisher and Yates tables were used for allocation. Placebo tablets had the same shape, color, and weight as the medicated tablets. Each queen was treated according to the following dose recommendation: 1 tablet for animals  $\leq 3.9 \text{ kg}$  body weight; 1.5 tablets for animals 4.0–5.9 kg body weight; 2 tablets for animals 6.0–7.9 kg body weight corresponding to a maximum of 4.0 mg milbemyacin and 10 mg praziquantel per kg body weight. On the day of treatment, the animal was offered a quarter of a tin of wet diet prior to dosing. After it was consumed, the test item or the placebo was administered. One hour later, the queen was offered the remainder of her daily diet. Each tablet was administered by mouth. The tablets were placed at the back of the animal's tongue to facilitate swallowing. A small volume of water was administered to encourage swallowing. Each queen was observed for successful intake of the tablets immediately after dosing and 1 hour later. Each queen was treated at weekly intervals beginning 1 day after her previous (pre-experimental) litter had been weaned

and continuing until her next litter of kittens was weaned at 55 days of age.

### **Assessment of Tolerability**

All the queens were weighed prior to feeding on the day prior to each treatment. General health observations were carried out once daily for each queen until her last blood sampling (see later), and for each tomcat until all the queens allocated to him had been confirmed pregnant. In addition, on each day of treatment, each queen was clinically assessed prior to treatment and at 1, 2, 4, and 7 hours after treatment. Observed parameters included behavior, salivation, miosis, mydriasis, nervous signs, and feces. Blood samples were collected from queens on the first administration day after mating, on the fourth and seventh administration days during pregnancy, on the first and fourth administration days after littering, and 1 week after the last administration day during lactation. Blood samples were analyzed for routine hematology parameters (red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, white blood cell count, percent differential white blood cell count, platelet count, and prothrombin time) and clinical chemistry (urea, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total protein, albumin, globulin, total bilirubin, glucose, sodium, chloride, potassium, phosphorous, calcium, gamma glutamyl transpeptidase, and cholesterol) using standard routine procedures and equipment. Each queen was fasted from the evening prior to blood sampling. A pregnancy diagnosis was performed on all queens  $32 \pm 2$  days after the first confirmed mating using an ultrasound scanner fitted with a linear probe ( $\geq 5$  MHz). The reproductive performance of each queen was assessed by recording the following parameters: length of pregnancy (interval between day of first observed mating and day the last kitten was born), number of kittens born, number of kittens alive (1 and 4 days old), number of kittens weaned (55 days old), presence of

any congenital abnormalities, and occurrence of dystocia.

Each kitten was weighed at 4 days of age and then at weekly intervals until Day 56. A veterinary examination of each kitten was carried out the day after littering and on the day after weaning (56 days old). General health observations were carried out on each kitten once daily starting on Day 2. Each kitten was individually examined for abnormalities of behavior, feeding ability, nervous signs, and salivation. In addition, after the kittens had reached 28 days of age, on the day of treatment of the queen, all the kittens in her litter were clinically assessed prior to treatment of the queen and 2, 4, 7, and 24 hours after treatment. The parameters assessed included behavior, salivation, miosis, mydriasis, and nervous signs. As the kittens were group housed, the feces of individual kittens could not be assessed. Blood samples were collected from kittens on the day after weaning. Each kitten was fasted from the evening prior to blood sampling. Blood samples were analyzed for hematology and clinical chemistry as previously described for the queens. All dead kittens that were not cannibalized by their queens were subject to a thorough post-mortem examination.

The study was blinded by ensuring that the persons responsible for administering the tablets to the queens were not involved in performing the general health observations, veterinary examinations, clinical assessments, body weights, and blood samplings during the study.

No statistical analysis of clinical assessments was performed, as all animals (queens and kittens) were found normal at all timepoints. Observed body weights of queens and kittens and corresponding changes from baseline were analyzed using a repeated measures analysis of variance to examine the influence of time and treatment group. Individual average values over time (with and without change from baseline) were determined for hematology and clinical chemistry parameters, and differences between placebo and medicated groups

**Table 1.** Summary Statistics for Clinical Chemistry Parameters Showing Significant Differences Between Queens Treated With Placebo (Group 1) or With Medicated Tablets (Group 2) During Pregnancy and Lactation.

Phase	Parameter	AUC Analysis	Group	N	Mean (A)	SD	P-Value
Pregnancy	Chloride (mmol/L)	With change from baseline	1	15	120.01	0.87	0.0310
			2	15	118.79	2.19	
	Total protein (g/L)	Without change from baseline	1	15	1.26	4.11	0.0279
			2	15	-1.82	2.99	
	Globulin (g/L)	Without change from baseline	1	15	2.03	3.31	0.0181
			2	15	-0.86	3.16	
Lactation	Total protein (g/L)	With change from baseline	1	15	-2.33	4.13	0.0424
			2	14	-5.54	4.22	

were examined using the Mann-Whitney U-test, both for queens and kittens. For queens, changes after treatment were tested for both groups (Wilcoxon test). Statistical comparisons of the test item and placebo groups with respect to individual hematology or clinical chemistry parameters were performed as well. The influence of treatment on reproductive performance parameters in each queen was analyzed using the Mann-Whitney U-test. For congenital abnormalities, Fisher's exact probability test was applied to compare groups. The level of significance was  $\alpha = 5\%$ ; all tests were performed 2-sided.

## RESULTS

### Queens

Pre-study evaluation of all hematology and clinical chemistry parameters showed no significant difference between Group 1 (placebo) and Group 2 (medicated). During pregnancy, both groups showed significant changes from baseline (Wilcoxon signed rank test) for leukocytes, hemoglobin, hematocrit, albumin, alanine aminotransferase, cholesterol, and urea. Additionally, Group 1 also showed differences from baseline for red blood cell count, globulin, creatinine, chloride, and phosphorous, whereas Group 2 showed additional differences from

baseline for mean corpuscular volume, sodium, and total bilirubin.

Regarding the differences between both treatment groups during pregnancy, the analysis of average area under the curve (AUC) applied to the hematology and clinical chemistry parameters resulted in only chloride showing small differences (Table 1).

During lactation, both groups showed significant changes from baseline (Wilcoxon signed rank test) for red and white blood cell counts, neutrophils, eosinophils, mean corpuscular volume, hemoglobin, hematocrit, globulin, gamma-glutamyl-transpeptidase, total protein, urea, creatinine, and calcium. Additionally, Group 1 also showed differences from baseline for leukocytes, alkaline phosphatase, cholesterol, and phosphorous, whereas Group 2 showed additional differences from baseline for sodium, albumin, and total bilirubin. Average AUC analysis with change from baseline indicated only 1 significant difference between both groups for the total protein (Table 1).

No clinical signs suggesting abnormal behavior, feces, or salivation, or presence of miosis, mydriasis, or nervous signs were observed between both groups during pre-mating, pregnancy, and lactation. The summary of body weight statistics is shown in

**Table 2.** Summary Statistics for Body Weight of Queens Treated With Placebo (Group 1) or With Medicated Tablets (Group 2) During Pregnancy and Lactation.

Phase	Period	Group	N	Mean (A) (kg)	SD	P-Value
Pre-mating	Pre-treatment (Day -1, 0)	1	15	3.29	0.38	0.3699
		2	15	3.49	0.54	
	Post-treatment (Day >0)	1	15	3.57	0.26	0.6672
		2	15	3.69	0.38	
Phase	Type of Analysis	Group	N	Mean (A) (kg)	SD	P-Value
Pregnancy	AUC without change from baseline	1	15	4.13	0.32	0.1646
		2	15	4.38	0.46	
	AUC with change from baseline	1	15	0.55	0.21	0.4807
		2	15	0.58	0.16	
Lactation	AUC without change from baseline	1	15	3.58	0.37	0.2134
		2	15	3.79	0.49	
	AUC with change from baseline	1	15	-0.44	0.31	0.5338
		2	15	-0.49	0.27	

Table 2. No significant differences in body weights were found between the treatment groups during the pre-treatment and the post-treatment phases. The same applies to the differences in body weight during pregnancy and lactation, as well as to the weight increases during pregnancy and the weight decreases during lactation.

All reproduction parameters measured showed similar results for both treatment groups and the small differences were not significant (Table 3). Dystocia did not occur.

### Kittens

The analysis of the clinical assessments and of the body weights revealed no significant differences between the kittens from both treatment groups. Regarding the evaluation of the hematology and clinical chemistry parameters, small although significant differences between Groups 1 and 2 were found only for alanine aminotransferase

(46.11 and 40.61 U/L, respectively), glucose (6.19 and 6.09 mmol/L, respectively), sodium (149.52 and 149.14 mmol/L, respectively), and phosphorous (2.14 and 2.04 mmol/L, respectively).

### DISCUSSION

Within each treatment group, differences from baseline were recorded during pregnancy for several hematology and clinical chemistry parameters. These differences can be considered as associated with the substantial metabolic changes inherent to pregnancy and lactation. The fact that during these phases a few parameters changed from baseline in the placebo group but not in the medicated group or vice versa could not be further investigated. However, most of these changes from baseline were smaller than the deviations from the average observed between single animals in the

**Table 3.** Summary Statistics for Reproductive Performance of Queens Treated With Placebo (Group 1) or With Medicated Tablets (Group 2) During Pregnancy and Lactation.

Parameter	Group	N	Mean (A)	SD	P-Value
Length of pregnancy (days)	1	15	64.1	1.1	0.6815
	2	15	63.8	1.8	
Kittens born (n)	1	15	5.5	1.5	0.7044
	2	15	5.5	1.9	
Kittens alive, Day 1 (n)	1	15	5.1	1.5	0.8992
	2	15	4.7	2.1	
Kittens alive, Day 4 (n)	1	15	4.8	1.3	0.7998
	2	15	4.6	2.3	
Kittens weaned, Day 55 (n)	1	15	4.7	1.3	1.0000
	2	15	4.5	2.2	
Dead kittens (n)	1	15	0.7	1.2	0.3736
	2	15	1.1	1.6	

same group. In addition, they fell within the normal range observed for these parameters and they can be explained by the natural variability of the response of individual animals to pregnancy and subsequent lactation.

The only significant differences between the treatment groups identified during the trial concerned a few clinical chemistry parameters: chloride, globulin, and total protein during pregnancy, and total protein during lactation (Table 1). The recorded differences were of a similar magnitude as the ones observed between individual animals within the same treatment group and were not associated with clinical signs of potential adverse drug reactions. In addition, they were not associated with any impairment of the reproductive performance of the queens, or of normal development in the kittens.

## CONCLUSION

In view of these results, it can be concluded that the treatment of queens during pregnancy and lactation with medicated tablets containing a combination of milbemycin

oxime and praziquantel administered once weekly at at least the highest recommended dose rate was well tolerated by both the queens and their kittens.

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