Tolerance of a Combination of Milbemycin Oxime and Praziquantel in Breeding and Lactating Bitches After Repeat Dosing

Rudolf Schenker, PhD[1]
Robert Cody, M. Agr. Sc. H. Dip. Tox[1]
Günther Strehlau, Dr. rer.nat[1]
Pablo Junquera, Dipl. Biol., PhD[2]

[1]*Novartis Animal Health Inc.*
Basel, Switzerland
[2]*Vetparcs GmbH*
Zürich, Switzerland

KEY WORDS: milbemycin oxime, praziquantel, safety, dog, pregnancy, lactation

ABSTRACT

A total of 34 confirmed pregnant Beagle bitches were randomly allocated to 2 treatment groups. Group 1 with 15 bitches was treated with placebo tablets, and Group 2 with 19 bitches was treated with medicated tablets containing a combination of milbemycin oxime and praziquantel (MILBEMAX®) at no less than the highest recommended dose rate once weekly during pregnancy and lactation. The bitches and their pups were submitted to periodic observations, including clinical assessments, weighing, and blood analysis for hematology and clinical chemistry. In addition, the reproductive performance of each individual bitch was assessed. There were no differences in reproductive performance between Group 1 and Group 2 in terms of length of pregnancy (mean, 64.3 and 64.8 days, respectively), number of pups born (mean, 5.9 and 5.8 respectively), number of pups alive at Day 4 after birth (mean, 4.5 and 4.7, respectively), number of pups weaned at Day 56 after birth (mean, 4.1 in each group), and congenital abnormalities (3 in each group). A few hematology and clinical chemistry parameters showed significant differences between both treatment groups. However, the recorded values were within the normal range for each parameter and were not associated with any clinical signs. Differences in average weight of the pups between the groups were due to litter size and gender distribution. These results demonstrate that the medicated tablets investigated containing a combination of milbemycin oxime and praziquantel administered once weekly were well tolerated by bitches and their pups during pregnancy and lactation.

INTRODUCTION

Milbemycin oxime is a macrocyclic lactone with high efficacy against a number of helminth parasites of dogs such as heartworms,[1-3] hookworms,[4-9] roundworms,[9] and whipworms.[7,10] The safety of milbemycin oxime for dogs has been broadly investigated in numerous studies that have shown it is well tolerated by adult dogs, pregnant bitches, and pups.[11] In one particular study, bitches were treated at 1.5 mg milbemycin oxime per

kg body weight daily from mating until weaning. Neither the bitches nor their offspring showed signs of adverse drug effects. The safety of praziquantel for dogs has also been studied in several toxicity and tolerability investigations,[12] and many years of use of this active ingredient in numerous countries have confirmed that this compound can be used safely in dogs. The individual commercial products are also approved for use in pregnant bitches in the European Union, the United States, and numerous other countries. The present investigation was performed to confirm the safety of the combination product for bitches and their offspring during pregnancy and lactation when used at the highest recommended dose rate.

MATERIALS AND METHODS

Animals

Forty-one Beagle bitches from a research colony age 30.2-92.8 months and weighing 9.8-21.8 kg were assigned to the study. Four proven Beagle stud dogs were used but were not part of the test system. Each bitch and stud dog was identified by a unique number tattooed in the ear and by a subcutaneously implanted microchip. Born pups were identified by a subcutaneously implanted microchip. Each bitch was acclimatized in the study unit for at least 7 days prior to the beginning of the study.

Bitches were examined by a veterinary surgeon 7 days before the beginning of the trial. Only healthy animals as assessed by the veterinary examination and by the results of the hematology and clinical chemistry analysis of samples collected 7 days before the beginning of the trial were included in the study. Stud dogs were examined by a veterinary surgeon prior to being used in the study. No animal used in the study was treated with any anthelmintic in the 3 months prior to being enrolled in the study.

From the time of enrolment, each bitch was housed individually in a pen measuring approximately 1.9 m × 2.4 m. In each pen there was a whelping box measuring approximately 0.75 m × 2.0 m. An infrared lamp was introduced at the time of whelping to provide a supplementary source of heat. For mating, the stud dog was brought to the bitch's pen. Standard commercial food was provided at the recommended rates. Pups were offered pup food from 28 days postpartum onwards. They were weaned at 56 days of age. On the evening prior to the day of weaning, all diet was removed from the pups' pen. Potable water was available ad libitum via nipple drinkers and water bowls.

The pups were vaccinated against *Bordetella bronchiseptica* at 3 weeks of age and against canine distemper virus, canine adenovirus-2, canine parvovirus, and canine parainfluenza virus at 6 weeks of age.

Treatments

Eighteen bitches were allocated to Group 1 to be treated with placebo, and 23 bitches were allocated to Group 2 to be treated with medicated tablets containing a combination of 12.5 mg milbemycin oxime and 125 mg praziquantel (MILBEMAX®, Novartis Animal Health, Switzerland). Placebo tablets were of the same shape and size as the medicated tablets. Bitches were assigned to the 2 groups at random, as they became available, using random order numbers derived from Fisher and Yates tables. Each bitch was treated at weekly intervals until the litter of pups had been weaned at 56 days of age. The number of tablets to be administered to each bitch was calculated on the basis of the animal's weekly body weight. The tablets were administered by mouth. Dosing was carried out at the same time each week. Each bitch was observed for successful intake of the tablets immediately after dosing and during the following hour.

Assessment of Tolerability

Each bitch was weighed prior to feeding on the day before treatment with medicated or placebo tablets. General health observations were carried out daily for each bitch until

her last blood sampling, and for each stud dog until all the bitches allocated to him had been confirmed pregnant. The parameters assessed included behavior, salivation, miosis, mydriasis, nervous signs, and feces. Blood samples were taken on study Day -7, 28 days after first administration of medicated or placebo tablets, on 1st, 4th and 7th administration days during pregnancy, on 1st and 4th administration days after whelping, and 1 week after the last administration day during lactation. The 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, phosphorus, calcium, gamma glutamyl transpeptidase, and cholesterol) using usual routine procedures and equipment.

Diagnosis of pregnancy was carried out 28 days and 35 days following the first confirmed mating, using an ultrasound scanner fitted with a linear probe. The following aspects of reproductive performance were recorded for each bitch: length of pregnancy, number of pups born, number of pups alive on postpartum Days 1 and 4, number of pups weaned, presence of any congenital abnormalities, and occurrence of dystocia.

Each pup was weighed after birth and then weekly until weaning on Day 56 postpartum. A veterinary examination of each pup was carried out after birth and on the day of weaning. General health observations were carried out on each pup once daily from birth until weaning. Examination included behavioral abnormalities, feeding ability, nervous signs, and salivation. On the days on which a bitch was treated with medicated or placebo tablets, clinical observations were performed on each pup of her litter. The parameters assessed included behavior, salivation, miosis, mydriasis, nervous signs, and feces. A blood sample was collected from each pup on the day of weaning. Blood samples were analyzed for routine hematology and clinical chemistry parameters as previously described for the bitches. Pups that were aborted, stillborn, or died before weaning were subjected to a postmortem examination by a veterinary surgeon.

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

Statistical Analysis

No statistical analysis of the clinical assessments was performed since all animals (bitches and pups) were found normal at all time points with only 2 exceptions (see results). Observed body weights and corresponding changes from baseline were analyzed using a repeated measures analysis of variance for examining 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 the differences between both treatment groups were examined using the Mann-Whitney U-test. Changes after treatment were tested for both groups (Wilcoxon test). Statistical comparisons of test item and placebo group with respect to individual hematology or clinical chemistry parameters observed at specific times were performed as well. The influence of treatment on reproductive performance was analyzed by Mann-Whitney U-tests. For congenital abnormalities, Fisher's exact probability test was applied to compare groups. The influence of treatment on the reproductive performance in each bitch was analyzed by determining arithmetic and geometric means, median, standard deviation, and minimum and maximum. Parametric tests were not performed as the parameters did

not meet the assumption of normal distribution. All tests were carried out at the 5% level of significance ($P < 0.05$). To estimate and quantify the relationship of body weight in dependency of litter size, gender, and time, the weight records of the pups in the placebo group were further analyzed by applying a model of linear analysis of covariance with class influence factors litter size and gender and covariate time. This model is appropriate to describe the observed data; R2, the measure of the proportion of total variation determination explained by the model, is about 92%.

RESULTS

Bitches

A total of 7 bitches did not become pregnant, 3 in Group 1 (placebo) and 4 in Group 2 (medicated) ($P = 1.000$). They were excluded from the subsequent evaluation and analysis of the results. All clinical assessments of the bitches resulted in normal findings except for 2 bitches who showed loose feces, 1 in Group 1 (at Day 1 after the first treatment) and 1 in Group 2 (at Days 31 and 38 after the first treatment). Table 1 shows summary statistics of individually calculated areas under the curve for weight profiles divided by the corresponding period length for both treatment groups during the different phases of the study: no significant difference was found between the groups. In the pre-study evaluation, the investigated hematology and clinical chemistry parameters showed no significant difference between treatment groups. During pregnancy and lactation, the following hematology and clinical chemistry parameters showed significant changes from baseline (Wilcoxon signed rank test) in both treatment groups: white and red blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, lymphocytes, neutrophils, alkaline phosphatase, aspartate aminotransferase, total protein, albumin, globulin, cholesterol, creatinine, glucose, urea, calcium, and phosphorous. In addition, chloride, potassium, and prothrombin time changed significantly from baseline only in Group 1 and total bilirubin and alanine aminotransferase only in Group 2. Table 2 shows all the hematology and clinical chemistry parameters for which a significant difference between both treatment groups was found during the study. The remaining parameters showed no significant difference between both treatment groups. Regarding the reproductive performance, congenital abnormalities were recorded for the litter of 6 bitches, 3 in each of treatment groups. Dystocia was found in 2 bitches, 1 in each of the treatment groups. Table 3 summarizes the results for the remaining parameters considered to assess reproductive performance. No significant difference between the treatment groups

Table 1. Summary Statistics for Body Weights of Bitches Treated With Placebo (Group 1) or With Medicated Tablets (Group 2).

Phase	Type of Analysis	Group	N	Mean/Median*	SD	*P*-Value
Pregnancy	AUC without change from baseline	1	15	16.84 (G)	3.93	0.6773
		2	19	17.50 (G)	4.20	
	AUC with change from baseline	1	15	1.02 (M)	0.34	0.1106
		2	19	1.40 (M)	0.63	
Lactation	AUC without change from baseline	1	15	15.32 (G)	3.76	0.8698
		2	19	15.35 (G)	3.95	
	AUC with change from baseline	1	15	-1.39 (M)	1.22	0.3342
		2	19	-0.63 (M)	1.50	

AUC = area under the curve; G = geometric mean; M = median.
*In kg.

was found for any of the reproductive performance parameters investigated.

Pups

A total of 59 pups died before weaning, 26 (14 males and 12 females) in Group 1 and 33 (17 males and 16 females) in Group 2. Fifty-three of these pups died between Days 1 and 7, 1 on Day 10, 1 on Day 14, and 1 on Day 30 postpartum. Eight pups were cannibalized (3 in Group 1 and 5 in Group 2); 5 were stillborn (1 in Group 1 and 4 in Group 2), 2 were neglected by the bitch (all in Group 1), 2 had a cleft palate and were euthanized (1 in each group), 2 had blood in the abdominal cavity (1 in each group), 2 had abnormal kidneys (1 in each group), 2 were a runt (1 in each group), 1 was mummified (Group 1), 1 had a hydrocephalus and was euthanized (Group 1), and 1 was septicemic (Group 2). The postmortem examinations failed to remark any abnormalities in the remaining 33 pups that died before weaning.

The records of the 140 pups that reached weaning (62 and 78 pups from Groups 1 and 2, respectively) were included in the evaluation of the results regarding pup safety. The gender distribution of these pups resulted in 46.8% and 52.6% female and 53.2% and 47.4% male in Groups 1 and 2, respectively. This difference was of no statistical significance ($P = 0.6099$). The regular clinical assessments for these pups failed to show any abnormalities regarding salivation, miosis, mydriasis, and nervous signs. The average litter size resulting for these weaned pups was 4.3 and 5.2 pups for Groups 1 and 2, respectively.

Table 4 shows the mean weights of the pups from Day 4 postpartum until weaning. From Day 32 postpartum until weaning, the pups in Group 1 showed significantly higher weights than those in Group 2. A further analysis of these weight records by litter size and gender showed that within the same litter size, males were significantly heavier than females, and for the same gender, pups from smaller litters were significantly heavier than those from smaller litters (Table 5). In addition, the model of linear analysis of covariance with class influence factors litter size and gender, and covariate time applied to the weights of pups from the placebo group indicated a strong monotonic relationship between litter size and body weight for both genders: male pups had higher weight gains than female pups within the same litter size, and the higher the litter size, the slower the growth rates and corresponding weight estimates at specific times (Table 6).

Table 7 shows all the hematology and clinical chemistry parameters for which a significant difference between the pups from both treatment groups was found during the study. The remaining parameters showed no significant difference between both treatment groups.

DISCUSSION

As expected, significant differences from baseline were recorded for several hematological and clinical biochemistry parameters in both treatment groups. Such changes are not surprising considering the substantial physiological and metabolic changes that are associated with pregnancy and lactation, and have been described to occur for a number of parameters (eg, hematocrit, leukocytes, immunoglobulins, cholesterol, proteins, glucose, etc) during the normal course of pregnancy and lactation.[13] The scope of this study did not allow further exploration of the few parameters that changed from baseline only in 1 group but not in the other. Nevertheless, the mean values for all these parameters remained within the normal range for the corresponding parameters, the differences between the groups were mostly smaller than the individual deviations from the average within the same treatment group, and these changes were not associated with any clinical signs of illness.

A few hematology and clinical chemistry parameters showed significant differences between bitches (Table 2) and

Table 2. Summary Statistics for Hematology and Clinical Chemistry Parameters Showing Significant Differences Between Bitches Treated With Placebo (Group 1) or With Medicated Tablets (Group 2).

Phase	Parameter	Analysis	Group	N	Mean/Median*	SD	*P*-Value
Pre-mating	Esinophils (%)	Test result†	1	15	1.27 (G)	0.83	0.0440
			2	19	0.74 (G)	0.60	
Pregnancy	Potassium (mmol/L)	Test result‡	1	15	4.44 (G)	0.43	0.0151
			2	19	4.08 (G)	0.32	
	Prothrombin time 2 (s)	Test result‡	1	15	10.83 (G)	0.99	0.0038
			2	19	12.05 (G)	0.97	
	Prothrombin time 3 (s)	Test result‡	1	15	11.05 (G)	1.11	0.0451
			2	19	11.95 (G)	0.83	
	Potassium (mmol/L)	Average AUC without change from baseline	1	15	4.52 (G)	0.33	0.0264
			2	19	4.27 (G)	0.29	
	Lymphocytes (%)	Average AUC without change from baseline	1	15	-1.43 (M)	2.81	0.0358
			2	19	3.97 (M)	4.93	
	Lymphocytes (× 10^3/µL)	Average AUC without change from baseline	1	15	0.16 (M)	0.49	0.0374
			2	19	0.40 (M)	0.60	
Lactation	Neutrophils (%)	Test result§	1	15	75.30 (G)	5.65	0.0304
			2	19	70.33 (G)	5.59	
	Lymphocytes (%)	Test result¶	1	15	19.51 (G)	3.96	0.0058
			2	19	25.01 (G)	5.70	
	Total protein (%)	Test result¶	1	15	66.04 (G)	3.38	0.0123
			2	19	61.70 (G)	4.21	
	Globulin (g/L)	Test result¶	1	15	34.03 (G)	2.33	0.0067
			2	19	31.01 (G)	2.99	
	Lymphocytes (%)	Average AUC without change from baseline	1	15	21.37 (G)	3.26	0.0344
			2	19	24.43 (G)	5.24	
	Globulin (g/L)	Average AUC without change from baseline	1	15	31.93 (G)	3.08	0.0344
			2	19	29.97 (G)	2.95	
	Lymphocytes (%)	Average AUC with change from baseline	1	15	-2.43 (M)	3.90	0.0108
			2	19	3.47 (M)	5.71	

*Mean or median. G = geometric mean; M = median.
†Blood sampled after the last administration before mating.
‡Blood sampled after the first treatment during pregnancy.
§Blood sampled after the second treatment during lactation.
¶Blood sampled after the third treatment during lactation.

Table 3. Reproductive Performance Parameters of Bitches Treated With Placebo (Group 1) or With Medicated Tablets (Group 2).

Parameter	Group	N	Mean (A)	SD	*P*-Value
Length of pregnancy (days)	1	15	64.3	3.2	0.5286
	2	19	64.8	3.0	
Number of pups born	1	15	5.9	2.6	0.9302
	2	19	5.8	2.7	
Number of pups alive Day 1	1	15	5.0	2.5	0.5173
	2	19	5.5	2.8	
Number of pups alive Day 4	1	15	4.5	2.4	0.7663
	2	19	4.7	2.7	
Number of pups weaned Day 56	1	15	4.1	2.6	1.0000
	2	19	4.1	3.1	
Number of dead pups	1	15	1.8	1.8	0.5880
	2	19	1.7	2.6	

Table 4. Mean Weights of Pups Born to Bitches Treated With Placebo (Group 1) or With Medicated Tablets (Group 2).

Day Post Partum	Group	N	Mean (G)	SD	*P*-Value
4	1	62	440.5	86.77	0.4967
	2	78	434.5	96.96	
11	1	61	683.7	170.78	0.3144
	2	78	652.1	176.35	
18	1	62	905.1	297.24	0.2754
	2	78	849.1	255.15	
25	1	62	1110.5	414.26	0.0817
	2	78	1006.5	327.18	
32	1	61	1381.3	505.88	0.0445*
	2	78	1233.2	438.93	
39	1	62	1734.0	658.37	0.0106*
	2	78	1492.9	565.78	
46	1	56	2088.5	835.54	0.0028*
	2	78	1741.8	695.35	
53	1	62	2359.7	982.52	0.0104*
	2	78	2011.8	833.41	
56	1	60	2417.9	1019.5	0.0093*
	2	74	2067.8	843.1	

*Significant ($P < 0.05$).

between pups (Table 7) from both treatment groups at various time points during the study. Nevertheless, the values recorded remained within the normal range for all parameters, and the differences between groups were mostly smaller than the individual deviations from the average within the same group. These differences can be explained by the strong variability of the individual metabolic and physiological responses of bitches to pregnancy and lactation and of pups to the processes of early development. None of these changes were associated with clinical signs of illness or with impairments of the reproductive performance of the bitches or with normal development of the pups.

No significant difference was recorded between both treatment groups for the parameters related to the reproductive performance of the bitches. The global mortality of pups before weaning was comparable in both treatment groups and corresponds with what is known from the literature.[14] Nevertheless, in the placebo group, more pups died from bitches with a large litter size than in the medicated group. As a consequence of this rather coincidental fact, the proportion of pups from smaller litters after weaning became higher in the placebo group than in the medicated group. In fact, the average litter size shifted from 5.9 for the born pups to 4.3 for the weaned pups in the placebo group, and from 5.8 to 5.2, respectively, in the medicated group. The gender proportion in both treatment groups also shifted from the born pups to weaned pups, but not as strongly as litter size. In the placebo group, the percentage of males was 53.4% at birth and 53.2% at weaning compared with 48.6% and 47.4%, respectively, in the medicated group. As a consequence, among the weaned pups, males and those born in smaller litters were overrepresented in the placebo group when compared with the medicated group.

Table 5. Average Weights at Weaning by Litter Size and Gender for All Pups Born During the Study.

Pups/Litter	Sex	N	Weight (g) at Weaning
1 to 3	F	11	3376.7
	M	7	3754.9
4 to 6	F	26	2443.4
	M	40	2879.0
7 to 9	F	33	1719.7
	M	23	1891.7

Table 6. Daily Weight Gains and Weights at Days 32 and 56 Postpartum for the Weaned Pups of Bitches Treated With Placebo Tablets as Predicted by a Model of Linear Analysis of Covariance With Class Influence Factors Litter Size and Gender, and Covariate Time.

Sex	Litter Size Category*	Weight Gain (g/day)	Predicted Weight (g) at Day 32	Predicted Weight (g) at Day 56
Females	1	54.4	1730.2	3036.3
	2	64.7	2456.7	4009.8
	3	59.0	20.71.4	3487.1
	4	58.5	1934.3	3338.3
	5	45.7	1649.0	2746.5
	6	37.8	1326.3	2232.7
	8	27.5	1053.1	1712.5
	9	9.7	697.6	931.5
Males	1	94.1	3030.0	5287.7
	3	62.2	2246.9	3739.8
	4	62.7	089.9	3595.2
	5	49.5	1721.1	2908.4
	6	46.3	1620.9	2732.1
	8	43.6	1483.4	2528.9
	9	14.0	831.1	1167.2

*Missing litter size categories: Not enough numbers for accurate calculation. Results of litter size with only 1 or 2 pups are less reliable, since the data situation is sparse for these extreme cases.

The evolution of the body weights of the pups remained comparable for both treatment groups until Day 25 postpartum. However, from Day 32 until weaning, the pups in the placebo group became significantly heavier than those in the medicated group. On the other side, the analysis of the development of the body weights during the study clearly shows that litter size and gender were the determinant factors for the final weight of the pups: For the same litter size, males were substantially heavier than females, and for the same gender, the pups born in a smaller litter were heavier than those born in a larger one (Tables 4 and 5). This is consistent with the common experience reported for dogs[13,14] and also explains the higher average body weight of the weaned pups in the placebo group from Day 32 postpartum onwards. In this group, males were overrepresented (53.2% compared with 47.4% in the medicated group) and the average litter size was also smaller (4.3 compared with 5.2 in the medicated group). Consequently, the difference between the treatment groups in the average body weight of pups from Day 32 postpartum until weaning is most likely to have been caused by these coincidental facts and not by the medication.

Based on these results it can be concluded that MILBEMAX® tablets can be safely administered to bitches during pregnancy and lactation.

Table 7. Summary Statistics for Hematology and Clinical Chemistry Parameters Showing Significant Differences Between Pups Born to Bitches Treated With Placebo (Group 1) or With Medicated Tablets (Group 2).

Parameter	Group	N	Mean (A)	SD	*P*-Value
Mean corpuscular volume (fl)	1	62	67.64	2.31	00197
	2	78	66.47	2.91	
Platelet count (×10³/µL)	1	62	373.02	127.62	0.0016
	2	78	453.38	134.31	
Alanine aminotransferase (u/L)	1	62	34.23	14.05	0.0001
	2	78	46.61	25.40	
Urea (mmol/L)	1	62	2.20	1.21	0.0001
	2	78	2.77	1.06	
Total protein (g/L)	1	62	46.81	4.03	0.0282
	2	78	45.53	3.65	
Albumin (g/L)	1	62	27.62	2.21	0.0370
	2	78	26.95	1.96	
Cholesterol (mmol/L)	1	62	5.05	1.07	0.0001
	2	78	3.75	0.96	
Chloride (mmol/L)	1	62	108.89	2.47	0.0006
	2	78	111.03	3.82	
Aspartate aminotransferase (u/L)	1	62	34.23	14.05	0.0001
	2	78	46.61	25.40	

REFERENCES

1. Grieve RB, Frank GR, Stewart VA, Parsons JC, Belasco DL, Hepler DI: Chemoprophylactic effects of milbemycin oxime against larvae of *Dirofilaria immitis* during prepatent development. *Am J Vet Res* 1991;52:2040-2042.
2. Tagawa M, Okano S, Hayashi Y, Kusano K: Prophylactic effect of milbemycin oxime against *Dirofilaria immitis* infection in dogs: optimum dose and administration time. *J Vet Med Sci* 1994;55:693-694.
3. Tagawa M, Hara Y, Ejima H, Hayashi Y, Kusano K: Prophylactic efficacy of milbemycin oxime against multiple infection of dogs with *Dirofilaria immitis*. *J Vet Med Sci* 1994;56:779-780.
4. Bowman DD, Johnson RC, Hepler DI: Effects of milbemycin oxime on adult hookworms in dogs with naturally acquired infections. *Am J Vet Res* 1990;51:487-490.
5. Bowman DD, Lin DS, Johnson RC, Hepler DI: Effects of milbemycin oxime on adult *Ancylostoma caninum* and *Uncinaria stenocephala* in dogs with experimentally induced infections. *Am J Vet Res* 1991;52:64-67.
6. Wade CG, Mercer SH, Hepler DI, Craig TM: Effect of milbemycin oxime against *Ancylostoma caninum* in dogs with naturally acquired infection. *Am J Vet Res* 1991;52:951-953.
7. Blagburn BL, Hendrix CM, Lindsay DS, Vaughan JL, Hepler DI, Wright JC: Efficacy of milbemycin oxime against naturally acquired or experimentally induced *Ancylostoma* spp and *Trichuris vulpis* infections in dogs. *Am J Vet Res* 1992;53:513-516.
8. Niamatali S, BhopaleV, Schad GA: Efficacy of milbemycin oxime against experimentally induced *Ancylostoma caninum* and *Uncinaria stenocephala* infections in dogs. *J Am Vet Med Assoc* 1992;9:1385-1387.
9. Reinemeyer CR, Faulkner CT, Assadi-Rad AM, Burr JH, Patton S: Comparison of the efficacies of three heartworm preventives against experimentally induced infections with *Ancylostoma caninum* and *Toxocara canis* in pups. *J Am Vet Med Assoc* 1995;206:1710-1715.

10. Horii Y, Otsuka Y, Tateishi M, Makimura S, Kusano K: Anthelmintic efficacy of milbemycin oxime against Trichuris vulpis in dogs. J Vet Med Sci 1998:60;271-272.
11. US Food and Drug Administration Center for Veterinary Medicine. New Animal Drug Application 140-915 for milbemycin oxime tablets; 1990. Available at www.fda.gov/cvm/FOI/1370.htm. Accessed February 10, 2006.
12. Shmidl JA, Cox DD, McCurdy HD, Kohlenberg ML: Summary of safety evaluations for praziquantel in dogs. *Vet Med Small Anim Clin* 1981;76:692-697.
13. Feldman EC, Nelson RW: *Canine and Feline Endocrinology and Reproduction*. St. Louis: WB Saunders; 2004:788-790.
14. Widmann-Acanal B: Rasseneffekte auf Fortpflanzungs- und Welpenabgangsrate bei Hunden unter gleichzeitiger Berücksichtigung rassenbedingter Dystokiedispositionen bei einigen Hunde- und Katzenrassen. PhD Thesis, Tierärztliche Hochschule Hannover 1992.