

Comparison of the hypothalamic–pituitary–adrenal axis in MDR1-1 Δ and MDR1 wildtype dogs

Katrina L. Mealey, DVM, PhD, DACVIM, DACVCP, John M. Gay, DVM, PhD, DACVPM, Linda G. Martin, DVM, MS, DACVECC and Denise K. Waiting, LVT

Abstract

Objective: To evaluate the hypothalamic–pituitary–adrenal (HPA) axis in MDR1-1 Δ (dogs with the MDR1 mutation associated with ivermectin sensitivity) and MDR1 wildtype dogs.

Design: Prospective study.

Setting: Institutional vivarium.

Animals: Seven healthy Collie dogs.

Measurements: MDR1 genotyping was used for allocation of dogs to 1 of 2 groups: dogs homozygous for the wildtype MDR1 allele (MDR1 wildtype) and those homozygous for the MDR1-1 Δ mutation (MDR1 mutant). Blood samples were obtained for determination of cortisol and adrenocorticotropin hormone (ACTH) concentrations under basal conditions, before and after ACTH administration, and before and after dexamethasone administration.

Main results: Significant differences were identified between the MDR1 mutant and MDR1 wildtype groups. Basal plasma cortisol concentrations and cortisol concentrations after ACTH administration were significantly lower in MDR1 mutant dogs as compared with MDR1 wildtype dogs. Plasma ACTH concentrations after dexamethasone administration were significantly lower in MDR1 mutant dogs as compared with MDR1 wildtype dogs.

Conclusions: Results suggest that P-glycoprotein (P-gp) plays a role in regulation of the HPA axis. Furthermore, it appears that the HPA axis in MDR1 mutant dogs that lack P-gp is suppressed compared with MDR1 wildtype dogs. This finding may explain some clinical observations in breeds known to harbor the MDR1 mutation including Collies, Shelties, Australian Shepherds, and others. There is a clinical impression that many of these dogs have worse outcomes in response to stress and, at times, respond poorly to appropriate therapy. HPA axis suppression, secondary to the MDR1 mutation, could result in a relative adrenal insufficiency (RAI) state during times of stress or illness. Further studies are required to determine the relationship between the MDR1 genotype and RAI.

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Introduction

P-glycoprotein (P-gp), the product of the MDR1 or ABCB1 gene, is a crucial component of the blood–brain barrier, protecting the brain from many potentially toxic xenobiotics.¹ P-gp functions as an ATP-dependent drug transporter that is expressed on the luminal membrane of brain capillary endothelial cells where it transports a variety of substrates from the brain tissue back into the

capillary lumen. P-gp appears to be highly homologous, but not identical, among mammalian species. Drugs that are known substrates for canine P-gp include ivermectin, loperamide, vincristine, vinblastine, and doxorubicin.^{2–5} Over 50 drugs have been shown to be substrates for human and murine P-gp^{6,7} and, because of the high degree of homology of P-gp between species, it is suspected that these same drugs are substrates for canine P-gp. Individuals that lack P-gp, as occurs in herding breed dogs with the MDR1-1 Δ mutation, are highly susceptible to neurotoxicosis after routine doses of P-gp substrate drugs such as ivermectin and loperamide.^{8–11} This susceptibility underscores the importance of P-gp in limiting exposure of the brain to certain compounds.

From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA.

Address correspondence and reprint requests to:

Katrina L. Mealey, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6610.

E-mail: kmealey@vetmed.wsu.edu

Exogenous substances are not the only substrates for P-gp. In rodents, endogenous hormones including corticosterone are substrates for P-gp suggesting that P-gp may have a role in regulating their plasma concentrations.¹² Results from recent studies support this contention. In rodent studies, P-gp was shown to restrict access of corticosterone and cortisol to the brain.¹² Furthermore, *abcb1ab* (-/-) double knockout mice that lack P-gp have a suppressed hypothalamic–pituitary–adrenal (HPA) axis compared with wildtype mice.¹³ Collectively, these results suggest that P-gp normally limits the concentration of cortisol and corticosterone at the hypothalamus and pituitary blunting feedback inhibition of the HPA axis. If this premise is correct, then higher concentrations of cortisol and corticosterone would be expected to reach the hypothalamus and pituitary in animals that lack P-gp resulting in greater feedback inhibition of the HPA axis and low endogenous cortisol levels (Figure 1). Consequently, dogs such as herding breed dogs with the MDR1-1Δ mutation would be expected to be predisposed to relative adrenal insufficiency (RAI).

RAI is characterized by inadequate production of cortisol in relation to an increased physiological demand during periods of stress such as critical illness.^{14,15} Human patients with RAI have a reduced capacity to cope with critical illness and these patients have been shown to have a poorer outcome than patients with a normal HPA axis.¹⁴ Interestingly, some veterinarians have described Collies as ‘wimpy’ or as ‘not participating in their own recovery’ because some individuals of this breed have had poorer outcomes or have not responded as well as dogs of other breeds with similar illnesses.^a These anecdotal observations are consistent with the hypothesis that dogs with the MDR1-1Δ mutation have a blunted HPA axis compared with MDR1 wildtype dogs. Therefore, the purpose of this study was to investigate HPA system regulation in MDR1-1Δ and MDR1 wildtype dogs utilizing dexamethasone suppression and adrenocorticotropin hormone (ACTH) stimulation testing.

Materials and Methods

Animals

All aspects of this study were approved by the Institutional Animal Care and Use Committee. Seven Collie dogs were studied ranging in age from 1 to 8 years of age. Dogs were determined to be healthy on the basis of results from physical examination, a complete blood count, serum biochemistry panel, and urinalysis. Three dogs that were homozygous for the MDR1 wildtype genotype (1 male, 2 females) and 4 dogs that were homozygous for the MDR1-1Δ mutation (2 males,

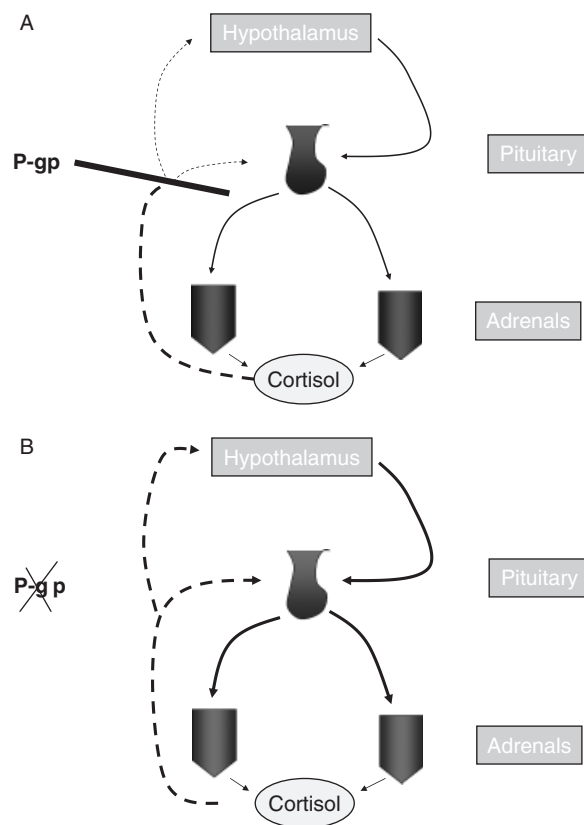


Figure 1: Illustration of the hypothalamic–pituitary–adrenal (HPA) axis in wildtype dogs (A) and MDR1 mutant dogs (B). In wildtype dogs, P-glycoprotein (P-gp) is present at the blood–brain barrier and limits entry of cortisol into the brain further limiting cortisol’s feedback inhibition of corticotrophin releasing hormone (CRH) secretion and adrenocorticotropin hormone (ACTH). In MDR1 mutant dogs, P-gp is not present at the blood–brain barrier, therefore, greater concentrations of cortisol are present within the brain enabling cortisol to exert feedback inhibition of CRH, ACTH, and, ultimately, cortisol secretion. Solid lines indicate stimulatory effects (with bolder lines indicating greater stimulation) while dashed lines indicate inhibitory effects (with bolder lines indicating greater inhibition).

2 females) were included in the study. Genotyping was performed at a commercial laboratory.^b

Sample collection and hormone assays

For each dog, baseline ACTH values were measured on 3 occasions and baseline cortisol values were measured on 5 occasions. ACTH stimulation and dexamethasone suppression studies were performed once. Studies were performed such that collection of basal (prestimulation or presuppression) samples occurred at approximately 08:30 hours (± 30 minutes). Blood was collected by jugular venipuncture. Blood was injected into EDTA tubes for cortisol determination. For ACTH determination, blood was injected into prechilled EDTA tubes

with a protease inhibitor, 100 μ L aprotinin, added. Plasma was harvested by centrifugation at 4 °C. Plasma was transferred to prechilled cryovials and frozen at -80 °C until analyzed. Cortisol and ACTH concentrations were determined by a commercial laboratory^c using methodology validated for the dog as previously described.¹⁶ A washout period of at least 2 weeks was allowed between each study.

ACTH stimulation testing

ACTH (1 μ g/kg) was administered intravenously (IV) (cephalic vein). Blood was collected before (time 0) and 1 hour after ACTH administration for determination of plasma cortisol concentrations.

Dexamethasone suppression testing

Three different doses of dexamethasone (0.05, 0.01, and 0.001 mg/kg) were used. Dexamethasone was injected IV (cephalic vein). Blood was collected before (time 0), 4, and 8 hours after dexamethasone injection for determination of cortisol and ACTH concentrations.

Statistics

Data were entered into a spreadsheet^d and statistical analyses were performed using a commercial statistics program.^e Because of repeated sampling over fixed times after multiple levels of stimulation, repeated measures ANOVA models with nested subjects as random factors were employed. Standardized residuals were plotted to detect departures from normality. Greenhouse–Geisser epsilon values were calculated for the repeated measures to determine if *F*-test degrees of freedom required reduction for deviations from compound symmetry and were adjusted accordingly. A critical value of 0.05 was used as the threshold of statistical significance and results are presented with standard error of the mean (SEM) as an indication of data variability.

Results

For each dog, baseline ACTH values were measured on 3 occasions and baseline cortisol values on 5 occasions. A statistically significant association was observed between genotype and cortisol levels ($F_{1,5} = 7.17$, $P = 0.044$), but not for ACTH ($P = 0.24$). Mean basal cortisol concentrations in MDR1 mutant dogs were 39.3 ± 6.18 versus 61.2 ± 5.35 nmol/L SEM for MDR1 wildtype dogs. Figure 2 shows a box and whisker plot of basal cortisol concentrations in MDR1 mutant and MDR1 wildtype dogs. For ACTH stimulation testing, MDR1 mutant dogs displayed significantly lower overall mean plasma cortisol concentrations after ACTH administration than MDR1 wildtype dogs (181.5 ± 11.0

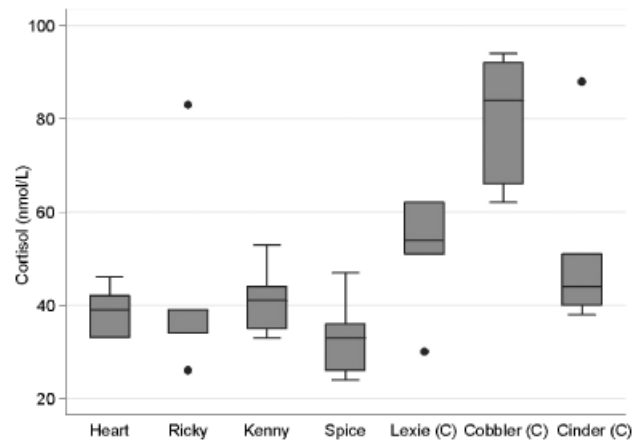


Figure 2: Box and whisker plot of basal plasma cortisol samples in MDR1 mutant dogs (mutant; $n = 4$ dogs, 3 samples per dog) and MDR1 wildtype dogs (control; $n = 3$ dogs, 3 samples per dog). Basal plasma cortisol concentrations are significantly lower in MDR1 mutant dogs ($*P = 0.044$) as compared with MDR1 wildtype dogs. Box, interquartile range (IQR); line, median; whiskers, $1.5 \times$ IQR; dots, data points greater than $1.5 \times$ IQR.

vs. 226 ± 12.7 nmol/L SEM, $F_{1,5} = 7.02$, $P = 0.045$), but the degree of response (i.e., slope) was not different ($P = 0.216$) (Figure 3).

For dexamethasone suppression testing, statistically significant differences in plasma cortisol concentrations between MDR1 mutant and wildtype dogs in response to dexamethasone administration were not detected at any dexamethasone dose (Figure 4). At the 4- and 8-hour time points after 0.05 mg/kg of dexamethasone

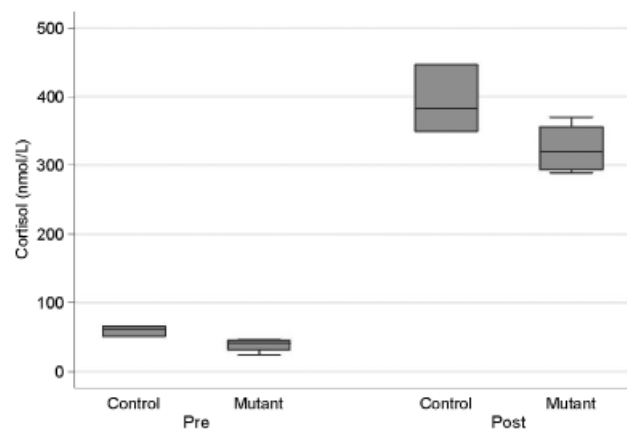


Figure 3: Box and whisker plot of plasma cortisol concentrations in MDR1 wildtype dogs (control; $n = 3$ dogs) and MDR1 mutant dogs (mutant; $n = 3$ dogs) immediately before (pre) and 1 hour after (post)-intravenous administration of 1 μ g/kg synthetic adrenocorticotropin hormone (ACTH). The mean plasma cortisol concentration after ACTH administration was significantly lower in MDR1 mutant dogs ($*$) than in MDR1 wildtype dogs ($P = 0.045$).

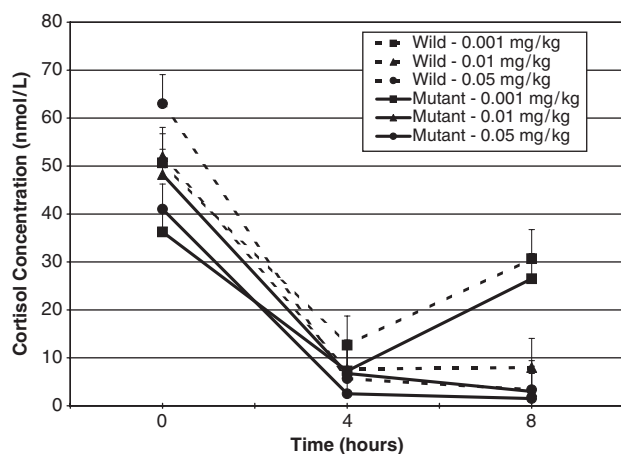


Figure 4: Mean (SEM) plasma cortisol concentrations in MDR1 wildtype dogs (wild; $n = 3$) and MDR1 mutant dogs (mutant; $n = 4$) before (time 0), 4, and 8 hours after dexamethasone administration. Dexamethasone was administered at 3 different doses with at least a 2-week washout period between dosing. Statistically significant differences in plasma cortisol concentrations between MDR1 mutant and wildtype dogs in response to dexamethasone administration were not detected.

was administered, plasma ACTH concentrations fell below the limit of quantitation and so statistical comparisons were not performed. However, a significant interaction ($F_{1,5} = 7.99$, $P = 0.037$) was detected between genotype (MDR1 status) and ACTH plasma concentrations. Means for the MDR1 mutant dogs receiving 0.01 mg/kg dexamethasone dose were significantly lower (12.67 ± 1.47 pg/mL SEM) than MDR1 wildtype dogs (22.89 ± 1.70 pg/mL SEM) receiving that dose of dexamethasone (Figure 5). At the lowest dexamethasone dose (0.001 mg/kg), a statistically significant difference in plasma ACTH concentrations was not detected between groups.

Discussion

P-gp functions as an ATP-dependent drug efflux pump that is capable of transporting substrate drugs against a concentration gradient from the intracellular to the extracellular space.¹⁷ It is expressed on the luminal aspect of brain capillary endothelial cells where it functions to limit brain penetration of substrate xenobiotics including the endogenous hormones, corticosterone, and cortisol.^{1,17,18} Studies in rodents have shown that P-gp limits access of corticosterone to the brain.¹² Further studies were performed in genetically engineered *abcb1ab* double knockout mice that are phenotypically similar to MDR1 mutant dogs since both lack P-gp. Because P-gp normally limits access of endogenous corticosteroids to the brain, it was hypothesized that lack of P-gp might profoundly influence HPA axis regula-

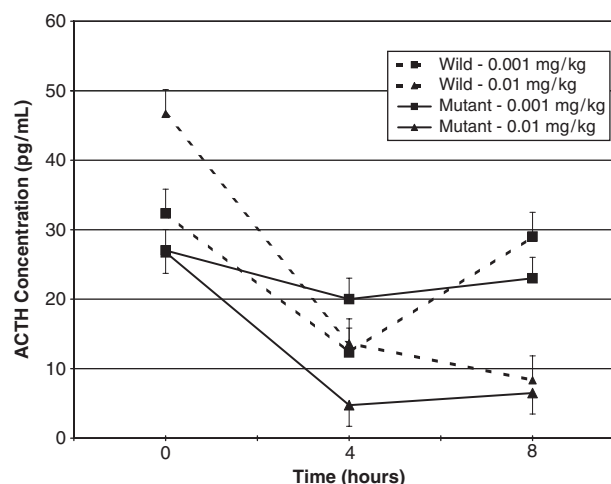


Figure 5: Mean (SEM) plasma adrenocorticotropic hormone (ACTH) concentrations in MDR1 wildtype dogs (wild; $n = 3$) and MDR1 mutant dogs (mutant; $n = 4$) before (time 0), 4, and 8 hours after dexamethasone administration. A significant interaction ($P = 0.037$) was detected between genotype (MDR1 status) and ACTH plasma concentrations. Dexamethasone was administered at 2 different doses with at least a 2-week washout period between dosing.

tion by allowing higher brain concentrations of endogenous corticosteroids to suppress corticotrophin releasing hormone (CRH) secretion. Studies were performed in the *abcb1ab* double knockout mice to test this hypothesis. Compared with wildtype mice, *abcb1ab* ($-/-$) double knockout mice have lower plasma cortisol concentrations under basal conditions and under conditions of stress.¹³ The knockout mice also have downregulated CRH mRNA expression in the hypothalamic paraventricular nucleus compared with wildtype mice suggesting a sustained suppression of the HPA system.¹³ Because MDR1 mutant dogs are phenotypically similar to *abcb1ab* ($-/-$) double knockout mice with respect to brain penetration of other P-gp substrates (e.g., ivermectin, loperamide), it was suspected that MDR1 mutant dogs might show evidence of chronic HPA axis suppression.

The present data suggest that lack of P-gp function at the blood-brain barrier in MDR1 mutant dogs results in altered activity and regulation of the HPA axis. Similar to results from rodent studies, mean basal cortisol concentrations in MDR1 mutant dogs (39.3 ± 6.18 nmol/L SEM) were significantly lower than in MDR1 wildtype dogs (61.2 ± 5.35 nmol/L). It is presumed that this apparent suppression of systemic cortisol concentrations in MDR1 mutant dogs results from greater cortisol feedback on hypothalamic paraventricular neurons in these dogs as compared with wildtype dogs that have normal P-gp function. The fact that MDR1 mutant dogs

had lower plasma cortisol concentrations, even after ACTH stimulation, suggests that there is chronic suppression of cortisol production. This observation raises questions as to the ability of MDR1 mutant dogs to respond to stress.

Results of dexamethasone suppression tests from the present study differed somewhat from results reported for *abcb1ab* (-/-) double knockout mice. In both MDR1 mutant dogs and *abcb1ab* (-/-) double knockout mice, plasma concentrations of ACTH were significantly lower than in their wildtype counterparts. However, while plasma concentrations of cortisol were suppressed at lower dexamethasone doses in *abcb1ab* (-/-) double knockout mice as compared with wildtype mice, there was not a statistically significant difference in plasma cortisol concentrations after dexamethasone administration in MDR1 mutant versus wildtype dogs.

It is not clear why ACTH concentrations following dexamethasone suppression differ in MDR1 mutant dogs as compared with wildtype dogs while cortisol concentrations do not. One can speculate that differences in plasma cortisol concentrations may have been observed if plasma samples had been obtained at later times. The higher ACTH concentrations in MDR1 wildtype dogs may have resulted in greater plasma cortisol concentrations after the last sample time point (i.e., 8 hours after dexamethasone administration). It is also possible that greater subject numbers would have demonstrated a significant difference in plasma cortisol concentrations. There was also greater variability for plasma cortisol concentrations than for plasma ACTH concentrations. Whether this difference may have been due to variability among the subjects or variability within the cortisol assay as compared with the ACTH assay is not known. However, despite the variability of plasma cortisol concentrations, the number of subjects was adequate to demonstrate significant differences in HPA axis regulation for several other parameters tested including resting cortisol concentrations, post-ACTH cortisol concentrations, and greater suppression of ACTH secretion after dexamethasone administration.

Collectively, results of this study provide evidence that the HPA axis in MDR1 mutant dogs is suppressed as compared with wildtype dogs. Similar to *abcb1ab* (-/-) double knockout mice, MDR1 mutant dogs lack P-gp function. Consequently, there is increased penetration of corticosteroids into the central nervous system, resulting in more pronounced negative feedback inhibition of stress hormone secretion. While this excessive negative feedback inhibition in MDR1 mutant dogs does not appear to interfere with basal physiologic function in these dogs, it is reasonable to speculate that HPA axis function may be inadequate in situations such

as acute illness, resulting in RAI or an RAI-like phenomenon.

RAI is a syndrome characterized by insufficient production of cortisol in relation to an increased demand during periods of severe stress, particularly in critical illnesses such as sepsis or septic shock.^{14,19} The syndrome is presumed to be associated with altered function of the HPA axis. Insufficient stress hormone synthesis appears to be a transient phenomenon in human RAI patients since life-long replacement of corticosteroids (as would be essential in patients with true hypoadrenocorticism) is not necessary.¹⁹ From a clinical perspective, it is extremely important to recognize septic patients with RAI because these patients tend to carry a worse outcome if untreated. Treatment, which consists of low ('physiologic') doses of corticosteroids, appears to reduce morbidity and mortality rates, particularly in septic patients.²⁰ Although RAI is well documented in critically ill human patients,²⁰⁻²² relatively less is known about adrenal dysfunction in critically ill veterinary patients. However, research models of sepsis and hemorrhagic shock suggest the presence of RAI in animals.^{21,22} There are also a small number of clinical studies that suggest RAI or RAI-like syndromes in certain populations of veterinary patients.^{f,g,h}

Interestingly, herding breed dogs such as Collies and Shelties appear to have a reputation for not handling illness well. For example, 1 oncologist stated in an online discussion that 'Shelties are indeed "wimps" and they tend to join Collies in this category.'^c Another oncologist commented that 'Collies sometimes do not participate in their own recovery.'^c Indeed, the observation that led to the study reported here involved what appeared to be an RAI-like phenomenon in an MDR1 mutant Collie that had undergone a prolonged, but relatively benign, surgical procedure.

From this study, it appears that P-gp is an important component of the HPA axis in dogs. Dogs lacking P-gp (i.e., MDR1 mutant dogs) appear to have a chronically suppressed HPA axis compared with normal dogs. Further studies are necessary to determine the relationship between MDR1 genotype and RAI. However, it might be prudent for veterinarians to consider testing for RAI (ACTH stimulation) in critically ill canine patients of the herding breed group, since the MDR1 mutation has been identified in Collies, Shelties, Old English Sheepdogs, Australian Shepherds, and others.^{23,24} If these dogs have clinical signs consistent with RAI, then 'physiologic' doses of corticosteroids may be considered while awaiting test results. Treatment can be gradually discontinued in those dogs that appear to respond to treatment and have normal results in response to ACTH stimulation and can be continued in those dogs that fail to respond appropriately. Because

MDR1 genotyping is commercially available,^a many owners of Collies and other herding breeds know their dog's genotype. It is important to obtain this information and consider the implications of the MDR1 mutant genotype when treating these patients.

Acknowledgement

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Footnotes

- ^a Veterinary Information Network; www.vin.com/members/search.
- ^b Veterinary Clinical Pharmacology Laboratory, College of Veterinary Medicine, Washington State University, Pullman, WA; www.vetmed.wsu.edu/VCPL/.
- ^c Endocrine Diagnostic Service, Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL.
- ^d Excel 2002, Microsoft Corporation, Redmond, WA.
- ^e NCSS Number Cruncher Statistical Systems, Kaysville, UT; NCSS.com.
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