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Fenbendazole Treatment and Litter Size in Rats

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Fenbendazole is commonly used in laboratory animal medicine as an anthelmintic for elimination of pinworms. It is generally regarded as a safe drug with minimal side effects. In our facility, 2 breeding colonies of rats were treated with fenbendazole to eliminate pinworms. Analysis of the breeding records revealed that feeding Sprague-Dawley rats a diet containing fenbendazole on a continuous basis for 7 consecutive weeks was associated with a significant reduction in litter size. Although the mechanism underlying this effect is unknown, the finding prompts caution when using fenbendazole to treat valuable breeding colonies or strains that are poor breeders.

Abbreviations: GEPR9, Genetically Epilepsy-prone Rats, substrain 9; KRV, Kilham rat virus; SD, Sprague-Dawley

Benzimidazoles, the class of drugs that includes fenbendazole, are versatile anthelmintics due to their wide range of effectiveness against gastrointestinal nematodes without reliance on systemic drug concentrations. In addition, benzimidazoles' adulticidal, larvicidal, and ovicidal properties make them unique among the various anthelmintic pharmaceuticals available.[11,19-21] Benzimidazoles are considered to act via inhibition of microtubule polymerization via binding to β-tubulin in susceptible parasites.[11,20,21]

Fenbendazole is a benzimidazole anthelmintic commonly used in both clinical and laboratory animal veterinary medicine. It is generally regarded as a safe drug with a high therapeutic index. Toxic side effects are reported at doses greater than 60-fold above the therapeutic dose.[19,20] In addition, hypersensitivity reactions secondary to antigen release from dying parasites can occur at therapeutic levels in some species.[19] Fenbendazole is only marginally absorbed after oral administration and is metabolized into the active compound oxfendazole (a sulfoxide) and a sulfone.[7,11,19,25] Feces contain about 44% to 50% of the administered dose, with less than 1% in the urine.[19] Fenbendazole is generally viewed as safe for use in pregnant domestic animals, with few reported complications.[19]

Fenbendazole is often used to treat oxyurid (pinworm) infections in laboratory rodents. Infection can be diagnosed by perianal cellophane tape test, fecal floatation, or direct examination of colonic or cecal contents, with the appropriate test depending on the species of pinworm.[2,3,7,13,20] Pinworm ova are persistent in the environment and pose a continual threat for both contamination of uninfected animals and recontamination in treated colonies. Although clinical problems are mild to unapparent, pinworm infections can have a profound effect on data collected from infected animals. Animals may demonstrate decreased weight gain and diminished growth rates, increased caloric demands for basal metabolism, and compromised overall nutritional status.[1-3,7,13,16,20,23,29] Parasite load can be influenced by animal age, weight, and sex.[4,5,8,9,17,20,29]

Rederivation is an effective method for elimination of parasites from rodent colonies, but this approach is expensive and time-consuming.[20] The most common pharmacologic treatments for rodent pinworms include thiabendazole,[16,27,28] avermectins such as ivermectin,[3,13,20] and fenbendazole. Although fenbendazole is not labeled for use in rodents, it has been proven to be effective against the mouse pinworms *Syphacia obvelata* and *Aspiculuris tetraptera*,[1,20] the rat pinworm *S. muris*,[7,13,20] the Mongolian gerbil pinworm *Dentostomella translucida*,[20,26,32] and *S. mesocricetus*, the pinworm of the Syrian hamster.[20,30,31]

Fenbendazole is often used for treatment of rodent colonies because of its wide margin of safety, ease of administration, and literature documenting efficacy. Few nonspecific effects on host physiology and behavior have been documented. For example, rat pups born to mothers fed fenbendazole during pregnancy had subtle alterations in righting reflexes, locomotor activity, and Morris water maze performance.[3] However, fenbendazole administration does not markedly alter a number of specific behavioral measures in rats or the onset of diabetes in mice.[14,24] This drug can be administered orally in specially compounded diets, treated water sources, or topically over feed. Fenbendazole treatment can eradicate *S. muris* and *A. tetraptera* from mice and rat colonies, assuming that appropriate quarantine and husbandry protocols are in place.[7,12,13,20] Feeding fenbendazole continuously for 7 d and alternating with traditional diet fed for 7 d for at least 3 cycles can eliminate all developmental stages of pinworms.[20]

We used fenbendazole administered in the feed to treat pinworms in 2 breeding colonies of rats maintained in our facility. After treatment was complete, we retrospectively examined the colony breeding records while animals were on and off fenbendazole treatment. In contrast to another report,[3] our data indicate that fenbendazole treatment may impair breeding performance in rats.

Materials and Methods

The animal facility of the Southern Illinois University School of Medicine (Springfield, IL) accommodates multiple species and users and maintains full accreditation from the Association for the Assessment and Accreditation of Laboratory Animal Care, International. Animal room temperatures for rats are maintained at 22.2 ± 0.5 °C with a humidity range of 30% to 70%. All rats described in this study were maintained on a 12:12-h light:dark schedule. Rats described in this report belonged to 2 distinct breeding colonies that were both maintained under conventional housing conditions (that is, autoclaved caging and filter tops were not used, research personnel could enter

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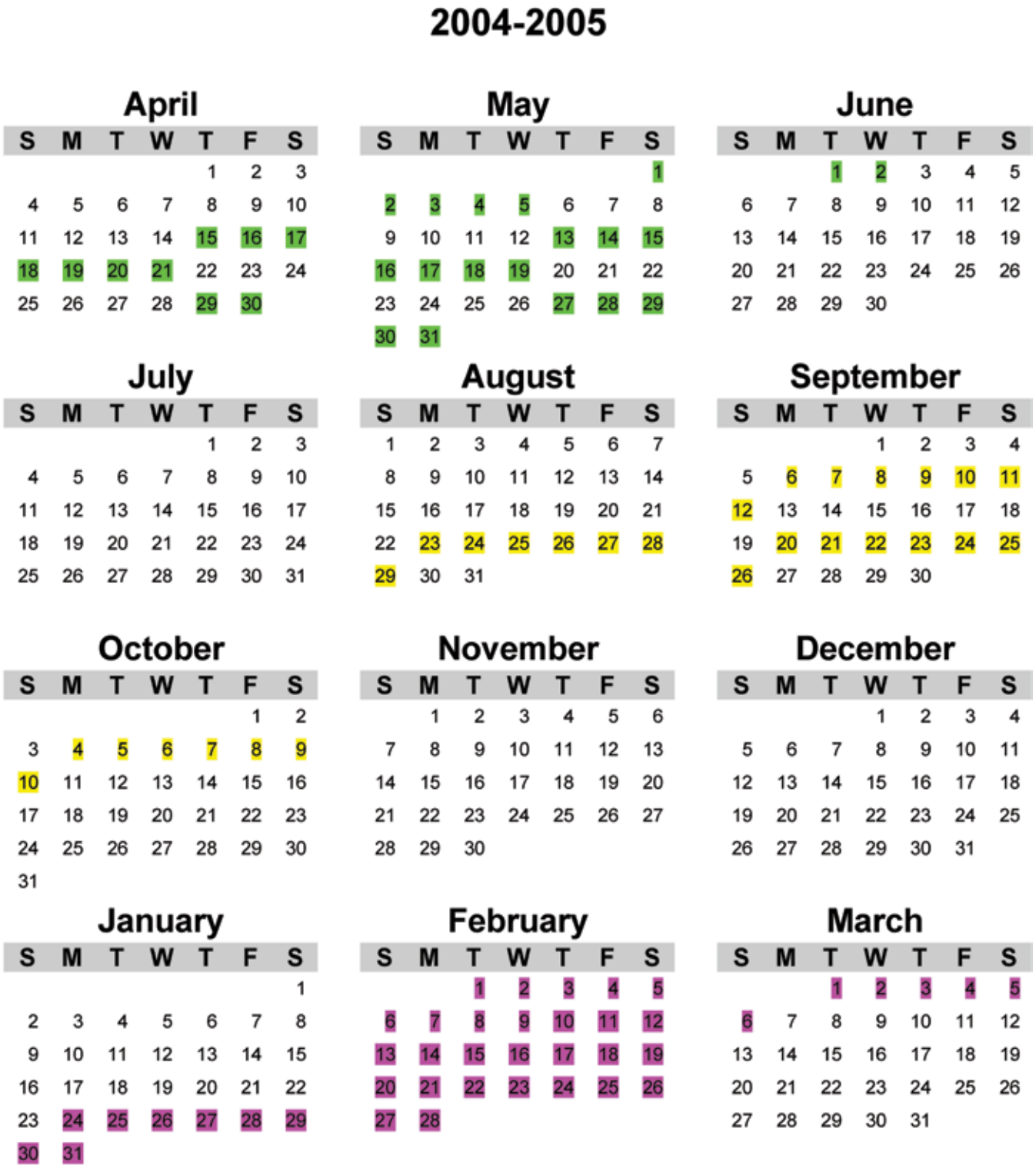

Figure 1. Fenbendazole treatment timeline. This calendar indicates the 3 treatment periods for fenbendazole administrations. Dates highlighted in green comprise the 1st period of intermittent treatment (SD colony), those in yellow are the 2nd period of intermittent treatment (GEPR9 colony), and those in purple indicate the continuous treatment of all rats in the facility.

the room garbed in a clean labcoat over street clothes, and transportation of rats back and forth to the laboratory was permitted). Cages were polycarbonate (17.5 × 9.5 × 8 in.), contained wood chip bedding (aspen), and were changed twice a week. Rats described in this report were housed either as dams with pups or as breeding pairs. Under nontreatment conditions, rats were fed ad libitum Rodent Diet 5001 (LabDiet, PMI Nutrition international, Richmond, IN) and received city water via an automatic watering system.

One colony consisted of approximately 35 adult Sprague-Dawley (SD) rats (*Rattus norvegicus*; 30 breeding females and 5 breeding males). This colony was used to produce preweanling pups for experimental purposes. Adults were not used experimentally. The other colony consisted of 50 GEPR9 (Genetically Epilepsy-prone Rats, substrain 9) rats (40 breeding females and 10 breeding males). The GEPR9 strain, which was derived

from SD rats,[22] demonstrates spontaneous audiogenic seizures and is used to study seizure mechanisms.[10] The breeding stock described in this report was not used experimentally. For both colonies, female breeders were typically between 2.5 and 12 mo of age. Replacement breeders were added on an as-needed basis by using rats weaned from the colonies.

In both rooms, sentinel rats housed in open caging on the lowest shelf of each rack were tested monthly for rodent microbial pathogens via serology and for parasites via fecal flotation, perianal tape test, and pelt evaluation. Both colonies were free of external parasites. Based on sentinel serology results, the SD colony was free of infection with Sendai virus, coronavirus, pneumonia virus of mice, rat parvoviruses, *Mycoplasma pulmonis*, Theiler encephalomyelitis virus, reovirus 3, and lymphocytic choriomeningitis virus (RADIL, Columbia, MO). The GEPR9 colony was seropositive for Kilham rat virus (KRV) but was negative for antibodies against the other agents listed.

The history of pinworm detection and fenbendazole treatment in the facility is summarized in Figure 1. Monthly colony surveillance revealed pinworms in the SD colony in April 2004. The pinworms were probably *S. muris*, because adult worms were located in the cecum, and eggs were recovered using anal tape impressions. All rats in the affected room received fenbendazole-medicated feed (Teklad Global 18% Protein Rodent Diet, Sterilizable, with 150 ppm Fenbendazole; Harlan Teklad, Madison, WI) on alternating weeks during April and June 2004 for a total of 4 treatment cycles. In August 2004, pinworms were discovered in 2 previously negative rat rooms, including the GEPR9 colony. These rats were treated with fenbendazole feed in the same manner as previously described. Rats treated in this manner are hereafter referred to as the intermittent treatment groups. Pinworms were detected once again in sentinel rats in January 2005. At this time, all rats in the facility (6 rooms) were treated for 7 consecutive weeks with fenbendazole feed in an attempt to eliminate pinworms from the entire facility. Rats treated in this manner are hereafter referred to as the continuous treatment groups. Colony composition (for example, age and parity of breeders) was not consistent across time, as breeders were periodically added and retired.

After cessation of fenbendazole treatment, we received reports from technicians in 2 laboratories regarding a decline in breeding performance that occurred when the rats were maintained on the medicated feed. Both laboratories maintained breeding records that identified breeding pairs, the interlitter intervals, and litter sizes before, during, and after fenbendazole administration. Several GEPR9 rats (n = 7) received both intermittent and continuous fenbendazole treatment and were included in both the intermittent and continuous groups. Rats in either colony that produced no offspring on either diet were eliminated from the data analysis. Records for both colonies and all treatment periods were compiled for statistical analysis (paired *t* test; SAS statistical software package, Cary, NC).

Results

SD rats maintained on fenbendazole-medicated feed on an intermittent basis produced a mean of 6.8 pups per litter (range, 0 to 19), as compared with an average of 10.0 pups per litter (range, 4 to 18) when given the nonmedicated diet ($P = 0.14$). SD rats given fenbendazole-medicated feed continuously had mean litter sizes of 8.2 pups (range, 0 to 18) as compared with 12.3 pups per litter (range, 8 to 16) when the same rats were maintained on unmedicated feed ($P = 0.04$, paired *t* test; Table 1).

The GEPR9 colony is highly inbred and produces small litters even under normal conditions. Litter size was not affected in GEPR9 rats that received fenbendazole on an intermittent basis ($P = 0.74$; Table 2). When maintained on a continuous fenbendazole diet, GEPR9 rats produced a mean litter size of 2.6 pups (range, 0 to 6) as compared with a mean of 3.7 pups per litter (range, 0 to 4) on the unmedicated diet ($P = 0.21$).

Table 1. Litter sizes in Sprague-Dawley rats maintained on fenbendazole-medicated and standard diets

	None	Fenbendazole	*P*[a]
Intermittent treatment			
Total no. of female rats[b]	18	17	
Age range (mo)	1.5–16.5	2–13.5	
Mean age (d)[c]	208.4 ± 53.3	227.3 ± 113.3	0.604
Median age (d)	211.5	206.0	
No. of litters	77	21	
Mean litter size (no. of pups)[c]	10.0 ± 3.5	6.8 ± 6.5	0.14
Median litter size (no. of pups)	12	8	
Continuous treatment (49 d)			
Total no. of female rats[b]	11	11	
Age range of female rats (mo)	2–9	4.5–11.5	
Mean age (d)[c]	142.2 ± 29.4	292.4 ± 60.9	0.0001
Median age (d)	146.5	312.0	
No. of litters	26	9	
Mean litter size (no. of pups)[c]	12.3 ± 2.2	8.2 ± 7.5	0.04
Median litter size (no. of pups)	13	4	

[a]By paired *t* test.
[b]Only female rats that had litters were included in this analysis. Given the low numbers of female rats and litters during fenbendazole treatment, the number of pups born per week was too low to provide meaningful information. The number of pups weaned per female rat per week was not recorded. Data were not available for analysis during the post-treatment period.
[c]± standard error of the mean.

A retrospective assessment of husbandry conditions (for example, changes in bedding, lighting, caging) and general environmental factors (for example, room relocation, construction) during the course of this study did not reveal any obvious factors that might have adversely influenced normal patterns of breeding. The colonies have remained free of pinworms since the colony-wide treatment regimen was completed in March 2005.

Discussion

Despite the wide acceptance of fenbendazole as a safe drug, the data presented here indicate that fenbendazole treatment may reduce productivity in rat breeding colonies. A previous anecdotal report suggested an adverse effect of fenbendazole on breeding performance, but this observation was not substantiated by data.[13] Our data indicate that SD rats receiving a continuous diet of fenbendazole-medicated feed have reduced litter sizes compared with those of rats fed an unmedicated diet.

Continuous and intermittent treatment regimens both tended to reduce litter size in both colonies of rats, although the effect only attained statistical significance in SD rats maintained continuously on fenbendazole. The GEPR9 colony, which comprises inbred rats, has small litters under normal conditions (3.7 pups per litter for GEPR9 versus 12.3 for SD), making detection of a significant reduction in litter size more difficult in GEPR9 as compared with SD rats. Thus, the lack of statistically significant

Table 2. Litter sizes in GEPR9 rats maintained on fenbendazole-medicated and standard diets

	None	Fenbendazole	*P*[a]
Intermittent treatment			
Total no. of female rats[b]	22	22	
Age range (mo)	2–14.5	2.5–13	
Mean age (d)[c]	187.0 ± 39.7	209.4 ± 92.8	0.32
Median age (d)	195.0	215.8	
No. of litters	80	38	
Mean litter size (no. of pups)[c]	4.6 ± 2.3	4.5 ± 4.1	0.74
Median litter size (no. of pups)	3	0	
Continuous treatment (49 d)			
Total no. of female rats[b]	15	15	
Age range of female rats (mo)	2–12	2.5–10	
Mean age (d)[c]	194.3 ± 48.0	229.1 ± 86.8	0.0071
Median age (d)	196.9	259.5	
No. of litters	29	23	
Mean litter size (no. of pups)[c]	3.7 ± 3.4	2.6 ± 3.7	0.21
Median litter size (no. of pups)	0	0	

[a]paired *t* test.
[b]Only female rats that had litters were included in this analysis. Given the low numbers of female rats and litters during fenbendazole treatment, the number of pups born per week was too low to provide meaningful information. The number of pups weaned per female rat per week was not recorded. Data were not available for analysis during the post-treatment period.
[c]± standard error of the mean.

decrease in the GEPR9 strain may be related to a floor effect, such that large numbers of subjects would be necessary for detection of a statistically significant reduction in litter size. Despite lack of statistical significance, the suggestion of a potential biologic effect could be important in management decisions relevant to colonies like GEPR-9 that are known to be poor breeders.

Although the reduction in litter size was statistically significant in the outbred SD rats, which normally have relatively large litters, several factors influence the interpretation of this finding. First, individual rats were used as breeders for 7 to 12 mo, yet treatment periods lasted for only 7 wk at a time. Therefore, most dams produced more litters while on the normal versus the medicated diet, and mean litter sizes calculated during the control intervals were more accurate and less variable than those calculated during the treatment periods. In addition, dams were somewhat older during the treatment period as compared with the pretreatment period, although age ranges were largely overlapping. Nonetheless, the somewhat greater age of the rats on medicated diet could have contributed to the reduced litter size we observed.

Another important factor influencing interpretation of these data is that the basic compositions of the medicated and nonmedicated diets were not identical. The fenbendazole-added diet contained 18.8% crude protein, 3.8% crude fiber, 57.3% carbohydrate, 41.2% starch, and 4.9% sugar. In contrast, the standard rodent diet used in our facility is comprised of 23.4% crude protein, 5.3% crude fiber, 59.8% carbohydrate, 31.9% starch, and 5.9% sugar. Most of the mineral, amino acid, and vitamin levels are also slightly different. However, both diets are formulated to provide balanced nutrition for all stages of life for rodents and are commonly used in laboratory animal facilities. The changes in breeding productivity that we observed seem unlikely to be due to these minor differences in the diet, although this possibility cannot be ruled out and could be tested in future experiments.

Finally, sentinel rats in the GEPR9 colony were seropositive for KRV, which is transmitted via urine, feces, blood, and milk and by the transplacental route. Developing rat fetuses and neonates are at greatest risk for contracting the virus and at greatest risk for severe illness or death. Infection with parvoviruses can cause infertility, fetal resorption, abortion, and reduced litter sizes in rodents.[6,15,18] The virus also can cause runting, ataxia, cerebellar hypoplasia, and jaundice in neonates and pups.[16,18] Other reported clinical problems include scrotal cyanosis, ascites, dehydration, symptoms of severe illness, and death.[16] None of these signs of disease have been noted in the GEPR9 colony, but it nonetheless displayed a tendency toward smaller litters when dams were maintained on fenbendazole-containing feed. Furthermore, the SD colony, which was negative for KRV, showed a significant reduction in litter size when maintained on medicated diet.

In summary, the data presented here demonstrate that SD rats fed a fenbendazole-medicated diet continuously for 7 wk show reduced litter size while on treatment. Additional studies are needed to elucidate the precise mechanisms by which the drug impairs reproduction. The data suggest that the potential effect of fenbendazole on fecundity should be considered when developing treatment plans for rats with pinworms.

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