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Lanolin as a Treatment Option for Ringtail in Transgenic Rats

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Ringtail is a condition characterized by dry skin and annular constrictions that sometimes result in loss of portions of the tail. This condition most commonly affects preweanling rats, and low relative humidity is thought to be a principal cause. The use of transgenic rats in our facility has been increasing since 2002, and we recently diagnosed several litters from transgenic Fischer 344 rats (*Rattus norvegicus*) with ringtail. Treatment was necessary to maintain the health and integrity of the tails to allow genotyping. Lanolin ointment was chosen because it is a nontoxic, inexpensive, effective moisturizer used for treating human skin conditions. We examined 5 litters comprising 37 pups total, ranging in age from 7 to 17 days at the time of presentation. Animals in 3 litters were randomly assigned to a treatment or nontreatment group, and all animals in the remaining 2 litters were treated. Lanolin was applied to the tails of treatment groups once daily for 6 d. Treatment was tolerated well by pups and no animals were rejected by the dams. After treatment, tail condition was scored from 0 to 3, with 0 representing a tail normal in appearance, and 3 representing severe disease. Chi square testing showed marginal statistical significance, with a trend for a higher percentage of treated rats having healthier tails on day 7 compared to untreated pups. The Pearson correlation between treatment and tail condition scores was significant. Results indicate that lanolin was an efficacious treatment option for ringtail.

Abbreviations: : hPAP, human placental alkaline phosphatase; R26, ROSA26; RH, relative humidity

Ringtail is a pathologic condition of the tail of neonatal rats that is typically characterized by dry skin and the formation of annular constrictions**.** In severe cases in which the constrictions compromise blood supply, tissue distal to the constrictions can become painful and necrotic, and auto-amputation may result. Animals afflicted by severe disease might require euthanasia for humane reasons if the condition causes extreme discomfort or disfigurement of tail tissue. The condition has been reported most commonly in laboratory rats (*Rattus norvegicus*)[3,4,16,25] but has also been reported as a spontaneous condition in other rodent species, such as the white-tailed rat (*Mystromys albicaudatus*)[22] and the pouched mouse (*Saccostomus campestris*).[5] We have observed the spontaneous condition infrequently in laboratory mice (*Mus musculus*) in our facility, and conditions resembling ringtail in laboratory mice with targeted genetic mutations have been reported in the literature.[18,20] Previous studies report that the disease develops in laboratory rats between 2 and 19 d of age[3,4,25] and therefore seems to arise principally during the preweaning period.

The exact etiology of spontaneous ringtail is unknown, but environmental relative humidity (RH) less than 40% is most commonly implicated as a causative factor.[1,6,17] Consistent with other reports,[3,4,25] cases of ringtail in our facility have historically presented in rats during the preweaning period, with the highest incidence during winter and early spring, when the RH level in our animal housing facilities tends to be the lowest and fluctuates on an almost daily basis. Environmental RH levels as low as 16% were measured during the period when cases of ringtail described in this report first presented (Figure 1). This level is below the RH range of 30% to 70% recommended for rodents in the *Guide for the Care and Use of Laboratory Animals*.[15] Other factors such as ambient room and cage temperature, caging type, genetic predisposition, and nutritional status also may play a role in the incidence of the disease.[2,10,17,25] One study of ringtail affecting Munich Wistar Fromter rats reported that epidermal hyperplasia represented the principal microscopic lesion of the disease.[3] However, because epidermal hyperplasia is a histologic lesion common to many chronic dermatopathies, this finding does not elucidate the true etiology of the disease.

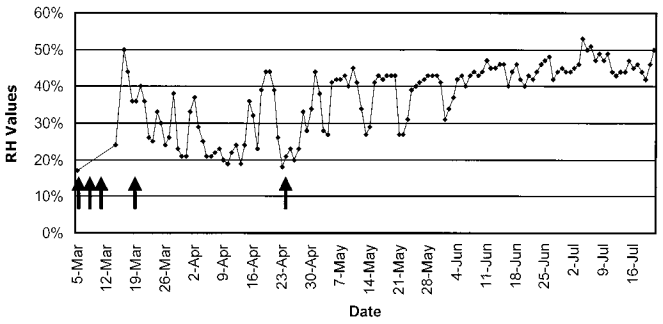

Figure 1. Average daily relative humidity (RH) values in the room housing the animals in our study. The values are those recorded by the environmental monitoring system for the room. Each diamond represents the average RH value for a single day. Each arrow represents one litter and the date when it was diagnosed with ringtail and included in the study. Due to a data storage malfunction, RH values for March 6 through March 13 are absent.

Several cases of ringtail recently presented in litters from transgenic Fischer 344 dams housed in our facilities between the months of February and April. All cases reported here developed in animals that were part of a single colony housed in one room. Approximately 1 litter out of every 10 (10%) was diagnosed with ringtail during this time. The transgene construct

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Table 1. Summary of the characteristics of litters included in this study

Litter	Total no. of pups in litter	No. of pups treated	Age at time of treatment (days)	No. of transgene-positive pups in litter
1	10	5	8	8
2	11	6	9	11
3	6	6	7	Data unavailable
4	7	4	13	4
5	3	3	17	Data unavailable

carried by the dams in this colony was human placental alkaline phosphatase (hPAP) driven by the ROSA26 (R26) promoter[13] intended to be expressed ubiquitously in tissues. The transgene serves as a marker for tracking the fate of transplanted cells. In order to identify rat pups carrying the transgene described, laboratory personnel routinely followed an institutional animal care and use committee (IACUC)-approved protocol for biopsying distal portions of the tails for genotyping. The presence of severe ringtail occasionally made it difficult to obtain sufficient tail tissue for genotyping because of devitalization of the distal tail tissue and loss of tail tissue resulting from auto-amputation. In addition, tails occasionally became edematous and inflamed as tissue was constricted. During this stage, the disease appeared to be painful to the animal judging by their vocalization during tail manipulation,[24] making effective treatment important to the health and welfare of the animal.

Our primary goal was to find a treatment that maintained the integrity of the tail tissue, allowing genotyping as necessary, while promoting the general health and welfare of the animals. No discussions of ringtail treatment were found in the literature, so we empirically chose to use a commercially available, medical-grade lanolin ointment. We hypothesized that lanolin would serve as an effective treatment for ringtail because affected tails are often dry and scaly in appearance, and lanolin is an emollient that has been used effectively in the treatment of some dry skin conditions in humans[21,23] and for breastfeeding women.[12] We further hypothesized that it would not harm the animals if ingested, nor would its use result in dams neglecting or cannibalizing treated pups.

The University of Michigan Transgenic Core Facility has generated approximately 75 transgenic rat founders carrying 8 different transgenes,[19] and the expectation is that transgenic rat models will continue to gain in popularity as they hold the potential to be excellent models for many types of genomic studies.[8] We show here that lanolin can be an efficacious treatment option for ringtail that will enable researchers to consistently perform routine laboratory procedures such as tail biopsy for genotyping and will promote the health and welfare of affected animals.

Materials and Methods

Animals. We examined 5 litters comprising a total of 37 pups which ranged in age from 7 to 17 d old at the time of presentation. All animals were included in an IACUC-approved research protocol. The characteristics of the animals used in this study are summarized in Table 1. The number of animals in the study was relatively low because the incidence of this spontaneously occurring disease was sporadic throughout the colony. Laboratory personnel also needed to euthanize some affected pups for various experimental purposes during the course of our study, further reducing the number of animals completing this study.

All animals were part of 1 specific pathogen-free (SPF) breeding colony housed in a single room in which environmental variables such as temperature, relative humidity, and airflow were monitored using a Watchdog System (Edstrom Industries, Waterford, WI). Room temperature was maintained between 73 and 74 °F (22.8 to 23.3 °C), and RH ranged from 16% to 50% (Figure 1). Semiannual surveillance reports for these animals indicated that they were free from infection with sialodacryoadenitis virus, Kilham rat virus, Toolan's H-1 virus, rat parvovirus, Sendai virus, pneumonia virus of mice, *Mycoplamsa pulmonis*, Theiler's murine encephalomyelitis virus, lymphocytic choriomeningitis virus, murine adenovirus, cilia associated respiratory bacillus, and pinworms. The colony consisted of transgenic animals used for tissue transplant studies. The transgene was ubiquitously expressed hPAP driven from a R26 promoter that was designed to permit the tracking of transplanted cells. Dams and their litters were housed in standard plastic rat boxes (143 sq. in. of floor space) containing ¼-in corncob bedding (Bed-o'cobs, The Andersons, Maumee, OH) on ventilated racks (Allentown Caging Equipment Company, Allentown, NJ) calibrated to 60 air exchanges per h. The animals in this colony were offered Labdiet 5001 rodent diet (PMI Nutrition International, Richmond, IN) and water from an automatic watering system ad libitum.

Identification of affected animals and treatment with lanolin. Husbandry and veterinary technicians identified litters of pups exhibiting tails with the dry, flaky skin or annular constrictions typically described in cases of ringtail. After litters were identified, we examined the animals and included them in the study if a diagnosis of ringtail was confirmed. In all instances, whole litters appeared to be affected with the condition at the time of diagnosis, but, as noted earlier, some animals were euthanized by laboratory personnel prior to completion of the study. Physical examinations of affected animals were otherwise unremarkable, as animals appeared to be in good general health and body condition.

Animals were identified using permanent marker on the rump, or holes were punched in the ear pinnae by using a commercially available device (National Band and Tag Company, Newport, KY), depending on the age of the animals. Marker was reapplied as necessary. Animals in 3 litters then were randomly assigned to either treatment (n = 24) or nontreatment (control; n = 13) groups. Because of the success in using lanolin to ameliorate ringtail early in our study, all animals from 2 litters (n = 9) were treated for humane reasons. All pups in the treatment groups were restrained individually by holding the scruff area, and pure lanolin ointment (Lansinoh Laboratories, Alexandria, VA) was applied in a thin layer to the entire tail. Treatment took only a few seconds for each pup, after which pups were returned immediately to their cages with their mothers. Dams readily accepted treated pups upon return to their cage, and no pups were rejected or injured by dams.

Treatment was administered once daily for 6 d. On day 7, an unbiased, experienced animal technician blinded to treatment groups assigned a tail condition score between 0 and 3 to each animal. A score of 0 represented normal tail condition (Figure 2A), 1 represented a tail exhibiting some scaling (Figure 2B), 2 represented a tail with evidence of annular constrictions and

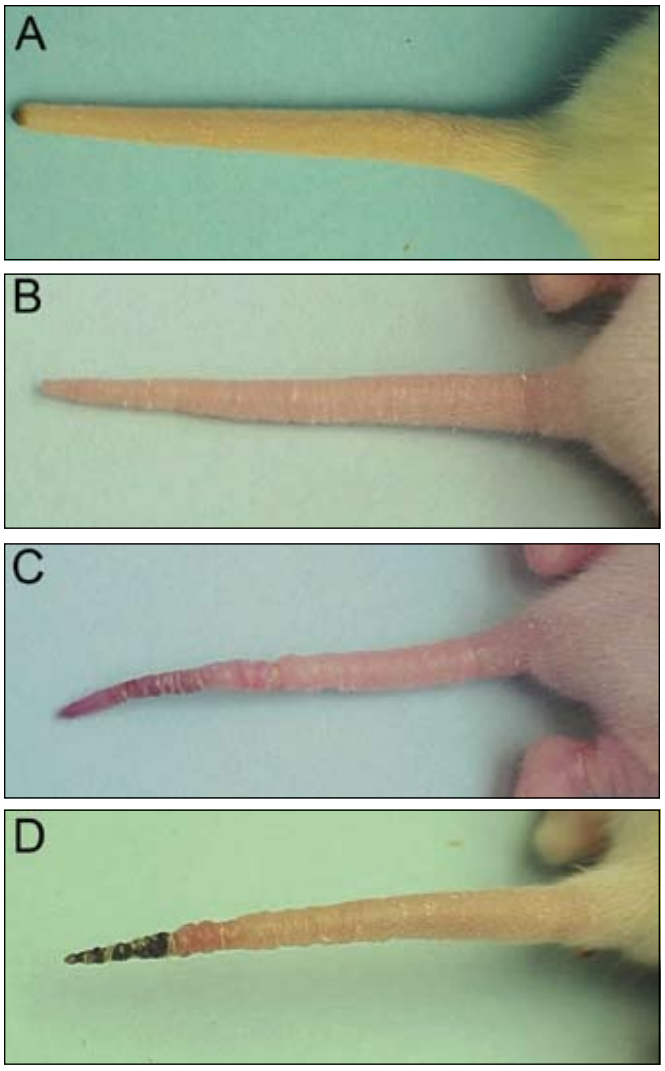

Figure 2. Representative clinical cases of ringtail used in our study. (A) Normal, healthy rat pup tail. This tail was given a condition score of 0. Note that the distal portion of the tail has been biopsied for genotyping. (B) Rat pup tail showing some flaking of the skin with mild constrictions. This tail was given a condition score of 1. (C) Rat pup tail clearly exhibiting annular constrictions and some malformation of tail tissue. This tail was given a condition score of 2. (D) Rat pup tail exhibiting annular constrictions with some malformation of tissue, and the tail tip appears necrotic. This tail was given a condition score of 3.

discoloration (Figure 2C), and a score of 3 indicated the most severe disease, where the tail exhibited distinct constrictions and evidence of devitalized tissue (Figure 2D). Tail condition for animals in 2 of the 5 litters (n = 10) was scored both before and after treatment to monitor progression of the disease and determine whether the condition of tails prior to treatment appeared to correlate with treatment success. Concurrent with treatment, RH levels in the rooms measured by the environmental monitoring system were recorded. In addition, a hand-held, combination digital thermometer–hygrometer (VWR International, West Chester, PA) was used periodically to measure temperature and RH inside representative cages containing affected litters. Measurements were taken for 10 min.

Statistical analysis. Chi-square statistics were calculated to compare tail condition scores in treated and control animals. Pearson correlation coefficients were calculated to determine whether treatment group, age at time of treatment, and litter identification were correlated with tail condition scores. All statistical analysis was performed using statistical software (SPSS, Chicago, IL).

Table 2. The number of animals in the control and treatment groups receiving each tail condition score

Group	Score	Frequency	%
Control	0	4	30.8
	1	4	30.8
	2	2	15.4
	3	3	23.1
		Total 13	100
Lanolin-treated	0	14	58.3
	1	5	20.8
	2	5	20.8
	3	0	0
		Total 24	100

Values shown are the total number of animals within each group receiving the indicated score (frequency) and the percentage of animals within each group receiving the indicated score.

Results

A summary of the number of animals in each group and their tail condition scores is given in Table 2. All results include the 2 nonrandomized litters because statistical analysis indicated that neither age at the time treatment was initiated nor litter identification appeared to correlate with tail condition scores ($r = -0.052$, $P = 0.761$). Including these 2 litters increased sample size and statistical power. Results showed that treatment with lanolin maintained affected tails in better condition compared with nontreated tails. Animals treated with lanolin had lower average tail condition scores (that is, healthier tails) than those in the control group. Of the animals in the treatment group, 58% (14 of 24) received a condition score of 0, compared with 31% (4 of 13) in the control group (Table 2). In addition, 23% (3 of 13) of the animals in the control group were given a score of 3 (worst tail condition), whereas no animals in the lanolin-treated received this score (Table 2). The difference in the percentage of animals in each score category clearly shows a trend toward healthier tails with treatment, but the difference was not statistically significant ($\chi^2 = 7.3$, $P = 0.062$). Relatively low incidence of spontaneous disease and limited sample size resulted in reduced statistical power, precluding further definitive interpretation of these results.

The correlation between treatment and tail condition scores was statistically significant ($r = –0.33$, $P = 0.047$); that is, the presence of treatment correlates with lower tail condition scores. There was no significant correlation ($r = -0.052$, $P = .761$) between age of pups at the time treatment was initiated and condition scores, indicating that the age of the affected pup did not influence treatment success. For the 2 litters (4 and 5) that were assigned scores before and after the 6-d treatment period, 2 treated animals improved (from score 1 to 0 and from 2 to 1; Figure 3A, B), and 4 treated animals received the same score before and after treatment. The condition of only 1 treated pup worsened (from a score of 1 to 2), but the tail tissue still appeared viable. In the control group, 1 animal received the same score at the beginning and end of treatment, whereas 2 control animals were worse on day 7 compared with the day treatment was initiated (from score 1 to 2 or 3). These results suggest that lanolin treatment can halt the progression of ringtail in animals

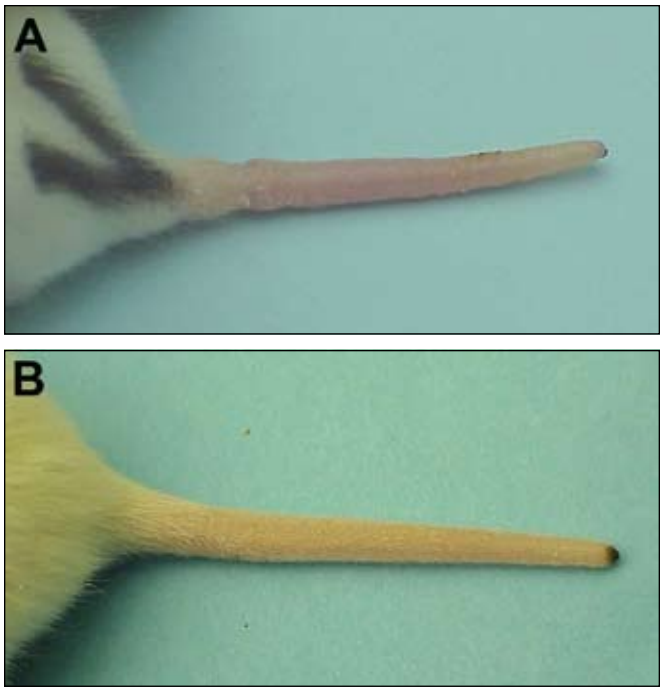

Figure 3. Treatment with lanolin resulted in marked improvement in tail condition. (A) Tail of animal from litter number 4 prior to treatment (score, 1). (B) The same tail after 6 d of treatment with lanolin (score, 0).

affected with the disease and can maintain tail viability.

Room temperature ranged from 73 to 74 °F (22.8 to 23.3 °C), and intracage temperatures ranged from 74.3 to 76.8 °F (23.5 to 24.9 °C). Room RH levels ranged from 16% to 50% during the course of our study, while within-cage RH levels were consistently 6% to 10% higher than room RH levels. Throughout the course of our study, the average room RH was approximately 29% and the mean intracage RH was approximately 36%. The differences between room and within-cage temperature and RH values that we observed were consistent with what has been reported for mouse cages in other studies.[11,14] Room RH levels lower than those recommended in the *Guide* (minimum of 30%) occurred periodically during the course of this study. This deficiency is a general concern in our vivaria during dry months and reflects the inability of building systems to completely restore humidity in some rooms. In our experience, periods of extremely low RH levels are often sporadic and of short duration and generally don't appear to affect the health of our animal colonies. If very low RH levels are noted for an extended period of time, additional measures are taken to increase RH in the rooms housing animals to ensure animal health and welfare. It should be noted that our environmental monitoring system experienced a data storage malfunction for a period of 8 d (March 6 through March 13) early in the course of this study (Figure 1).

Discussion

We evaluated the effectiveness of lanolin as a treatment for ringtail in pups from transgenic Fischer 344 rats. Our primary objectives were to maintain the general health and welfare of affected animals and keep tail tissue viable so that tail tips could be biopsied for genotyping. Application of lanolin to tails was consistently effective, as no treated animals developed severe disease with devitalized tissue in the distal tail.

Ringtail most commonly affects rats prior to the time of weaning. It has been reported to develop as early as 2 d of age[25] and as late as 19 d of age,[3] with most cases presenting at about 1 wk of age.[4,6,16] In our study, ringtail was diagnosed in pups between 7 and 17 d of age with a mean age at presentation of 10.8 d. This might suggest a peak in susceptibility to the disease at around 1 wk of age; however, the condition can develop almost anytime during the preweaning period. Interestingly, our study showed that the age of pups at the time of disease onset and treatment initiation did not appear to influence the response to treatment. This finding suggests that the pathology and the progression of the disease are similar regardless of the age of onset.

The true etiology of ringtail is unknown, but there are likely several factors playing a role in its development. Low ambient RH levels traditionally have been cited as a primary cause of the disease.[6,16,17,25] Given the dry and scaly appearance of tails affected by the condition, it is reasonable to hypothesize that lesions occur due to a dry ambient environment. Most reported cases of ringtail have occurred during the winter months, and a seasonality to the disease has been noted.[3,4,22,25] All ringtail cases in our facility occurred during the winter months when RH levels were generally low and fluctuated considerably compared with those later in the year; however, our findings do not indicate that low RH levels alone are causative. One case in our study developed when room RH levels were near 40%. Two other studies[3,4] also reported cases of ringtail during times when RH levels were considerably above 40%. Furthermore, we found that intracage RH levels were 6% to 10% higher than the room RH. Another study reported that within-cage RH levels for ventilated cages were 20% higher than room RH levels.[9] If low RH levels alone were causative, it is unlikely that ringtail would have developed during these periods when the RH was within the normal range suggested by the *Guide for the Care and Use of Laboratory Animals.* Furthermore, we would expect to see an annual occurrence of the condition during winter and early spring months when RH levels are low, which has not been our experience at this institution.

Other variables such as ambient temperature, diet, and caging type might also influence the development of ringtail. Mild elevations in ambient room temperature seem to increase the incidence of ringtail, but whether it is a significant factor is unclear.[25] Room temperature in our facility is maintained within a narrow range and remained between 73 and 74 °F during the course of our study. In light of this small variance, we think it is unlikely that temperature influenced the incidence of the disease in this study. Experimental alterations in diet have induced the formation of lesions in rats resembling ringtail. After 5 to 6 wk on a vitamin B_6-deficient diet, rats were observed with lesions resembling ringtail,[10] and exclusion of fat from the diet also induced the formation of lesions resembling ringtail in the tails of rats;[2] however animals in that study also exhibited other skin lesions as well as loss of body condition. Rodents housed in our facility receive nutritionally balanced, commercially available diets, and it is therefore unlikely that any dietary deficiencies caused the described cases because the vast majority of these animals never exhibit ringtail. The effect of static versus ventilated caging type has not been analyzed experimentally; however, it has been shown that RH levels are higher in static cages than in ventilated.[9] Independent measurements of intracage humidity taken during and subsequent to the current study support other published findings. Because all animals in our study were housed in ventilated cages, as are the majority of rats in our facility, we cannot draw any conclusion regarding the effect of cage type.

It has also been suggested that there might be a genetic

predisposition for ringtail,[6,17] and the role that the transgene played in disease incidence in our study should therefore be considered. There is no evidence in the literature to suggest that the R26–hPAP transgene alters susceptibility to ringtail, and we suspect that it did not play a role in disease development. The R26-hPAP transgene is intended to be an innocuous, ubiquitous cell marker. Kisseberth and colleagues[7] developed 2 R26-hPAP transgene-positive lineages in rats that developed to adulthood normally and were fertile. The dams and sires of litters in our study were heterozygous for the transgene. It would be predicted that 75% of the offspring would carry a copy of the transgene. In some litters, 100% of the pups carried the transgene, as documented by genotyping performed by laboratory personnel. This distribution resulted in a relatively homogeneous population with respect to the transgene, thus reducing the likelihood that it influenced the development of ringtail. If this transgene increased the likelihood of an animal developing ringtail, we would have expected a higher prevalence of the disease within the colony. In his study of ringtail in Norway rats, Totton[25] observed that affected parents produced normal offspring and normal parents produced affected offspring, leading to the conclusion that there was no noteworthy genetic predisposition. Cases of ringtail have not presented in this transgenic rat colony in the 2 years since these cases developed, which further supports our conclusion that the transgene played little or no role in the development of ringtail in these animals. Future studies will be designed to examine the transgene as a variable, should spontaneous disease present again in this colony.

All existing data indicate that ringtail is a disease of preweanling rats and likely has multiple etiologies. Given the absence of information regarding treatment options for ringtail and the fact that it is recognized as a relatively common disease in rats, our current report should benefit the laboratory animal community. Our report shows that lanolin ointment can be an effective treatment option for ringtail. We hypothesize that lanolin's emollient properties effectively moisturize the tail tissue, similar to its effect in ameliorating dry skin conditions in humans.[21,23] We also believe that the mechanical effect of applying the ointment, which might aid in removing the dry skin and potentially constrictive rings, plays a role in its effectiveness. Treatment with lanolin should be initiated as soon as the earliest clinical signs of ringtail, such as flaky, scaly skin, are evident on the tail. Prophylactic treatment beginning at approximately 7 d of age for groups of animals with a history of the disease should be considered also. Our results indicate that 6 d of once-daily treatment is effective, but longer and more frequent application might be necessary in some circumstances. The transgenic laboratory rat has the potential to become a powerful model for the study and discovery of gene function; therefore, as the number and use of transgenic rats in research facilities increases, it will be imperative to have a safe and economical treatment option for ringtail lesions.

Acknowledgments

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