Original Research

Effects of Pregnancy, Outdoor Access, and Antifungal Medication on Hair Loss in Breeding-age Female Pigtailed Macaques (*Macaca nemestrina*)

Rose Kroeker,^{1,*} Lee Chichester,² Grace H Lee¹, and Julie M Worlien¹

Over 18 mo, adult female pigtailed macaques (*Macaca nemestrina*) housed at a breeding facility in Arizona were monitored every 6 mo for alopecia. The study period coincided with the movement of a majority of animals from primarily outdoor housing to continuous indoor housing and a corresponding decrease in available space. These changes were made due to the newly recognized prevalence of coccidioidomycosis at this site. The effects of pregnancy status, changes in outdoor access or space, and administration of fluconazole for the treatment of coccidioidomycosis on alopecia were explored. In this group of pigtailed macaques pregnancy did not appear to affect alopecia, in contrast to findings from a closely related species, rhesus macaques. Fluconazole administration increased alopecia in older animals but not in the youngest age group. Conversely, the effects of limited outdoor access or decreased space on increasing alopecia were greatest in the youngest group of animals.

DOI: 10.30802/AALAS-CM-18-000108

Alopecia has been recognized as a vexing problem for captive laboratory-housed NHP in recent years and is increasingly a focus of regulatory scrutiny.^{4,16,18} Alopecia can be a secondary symptom for numerous disorders ranging from dermatologic to systemic in nature, and these possibilities have been cataloged in veterinary texts and a recent review publication.^{21,25} Despite the numerous potential etiologic options, many cases of alopecia still lack an identifiable cause.^{17,29} A survey of 4 primate colonies revealed that alopecia affected 49% of laboratory-housed rhesus macaques.¹⁸ In our own colony of pigtailed macaques, we documented a prevalence between 63% and 74%, dependent on season.¹⁶ In some NHP species (including the rhesus) there is a well-documented seasonal component to hair loss that occurs in the wild as well as in captivity.³²

To better understand influences to alopecia in macaques, we here report haircoat condition ratings obtained during an 18-mo period for adult female pigtailed macaques (*Macaca nemestrina*) at the Washington National Primate Research Colony's Arizona Breeding Colony. The reporting period encompasses the outbreak of coccidioidomycosis in our colony and the subsequent treatment of a large proportion of the colony with the antifungal agent fluconazole. Our observations also encompass a transition in colony housing, in which about 1/3 of the colony was moved from housing that allowed outdoor access to continuous indoor housing to minimize the threat of further *Coccidioides* exposure. Because the transition to indoor housing was accomplished by barring access to previously available outdoor space,

the transition necessarily resulted in less total space available to animals. The occurrence of these events during our reporting period, along with ongoing pregnancies in our breeding colony allowed us to examine the effects of these factors (pregnancy, fluconazole treatment, and outdoor exposure with space changes) on hair growth or loss in pigtailed macaques.

Coccidioides is a fungus that causes coccidioidomycosis and is endemic to the southwestern United States.^{6,31} The fungus grows in the soil and is then spread through the air in dry, dusty conditions. Most species of mammals can develop coccidioidomycosis but the severity of the disease is somewhat species-dependent.⁷ In humans, coccidioidomycosis most often causes a flu-like illness from which most people recover without treatment. However, the disease can be more serious, especially in people with compromised immune systems.^{5,12,27} In contrast to the typical disease course in humans, pigtailed macaques in our colony are more seriously affected, particularly in regard to dissemination outside the lungs.

The first line of treatment for coccidioidomycosis is the antifungal agent, fluconazole, which was FDA-approved in 1990.^{11,24} Two early case reports of fluconazole use in NHP^{1,8} do not report increased alopecia, although whether the studies monitored hair loss is unclear. In addition, alopecia was not noted as a side effect in human clinical trials of fluconazole, but hair loss is included in the label information as a potential side effect. What appears to be the first report linking fluconazole and alopecia describes alopecia in 12.5% to 20% of patients who were treated with fluconazole for various fungal infections.²² Alopecia was reported to begin a median of 3 mo after the initiation of fluconazole and to resolve by 6 mo after discontinuation or reduction of the dose. Although the underlying disorder cannot be ruled out as a contributing factor to alopecia, the authors argue that

Received: 28 Sep 2018. Revision requested: 17 Nov 2018. Accepted: 12 Feb 2019.

¹Washington National Primate Research Center, Seattle Washington, and ²Animal Resources Center, University of Texas Medical Branch, Galveston, Texas

*Corresponding author. Email: rkroeker@comcast.net

the timing of onset and resolution of the alopecia indicated a primary role for fluconazole.²³ They suggest that alopecia might not have previously been reported as a side effect of fluconazole use because of the delayed onset of the alopecia and the fact that many patients with fungal infections are treated for durations shorter than 2 mo.²³ We predicted that we would see an increase in alopecia for pigtailed macaques that had received fluconazole for at least 2 mo prior to assessment.

Alopecia seems to be more common in captive NHP than in their wild counterparts, although the underlying reasons are unclear.^{3,33} Numerous differences exist between wild and captive settings, and captive environments differ widely between each other. Despite the wide variation in captive environments, 2 obvious broad differences between captive and wild environments are access to outdoors and available space. Wild animals have few restrictions on outdoor access and space, whereas these features are restricted to various degrees for captive animals. Researchers have reported decreased alopecia in captive rhesus populations with either current outdoor access³⁰ or previous history of outdoor access.¹⁵ In addition, haircoat condition in rhesus macaques reportedly is affected by access to a substrate that more closely mimics a natural environment² and by the amount of space available to individual animals.³⁰ Whether decreased outdoor access similarly causes alopecia in pigtailed macaques is unknown. Further refinement of the role of outdoor access and space in haircoat quality can provide important husbandry information and potentially reveal specific mechanisms responsible for hair growth or loss. We predicted that restricted access to the outdoors would result in worsened alopecia in comparison to that of animals with increased outdoor access or space.

Postpartum hair loss in women has been documented since at least 1960.¹⁹ A recent cross-sectional study of pregnant and postpartum women documented significant differences in hair growth phases (telogen [resting phase] compared with anagen [growth phase]) between groups but noted the differences were quite small (18% telogen hairs in late pregnancy compared with 21% in postpartum).¹⁰ The authors note that this very modest increase in postpartum hair loss is unlikely to explain clinical cases of women seeking help for hair loss during the postpartum period and suggest that these cases likely have other underlying problems.¹⁰

Conversely, dramatic examples of hair loss associated with pregnancy have been reported in rhesus macaques.²¹ Because rhesus macaques are seasonal breeders and exhibit seasonal hair loss, it is difficult to determine the independent effect of pregnancy on hair loss. In addition, nonpregnant rhesus macaques can exhibit hair loss during seasonal molt, and not all pregnant animals lose hair.^{9,32} However, significant, independent effects of pregnancy on worsening alopecia have recently been documented by using sophisticated analyses with a relatively large sample.³ In that study, nonpregnant female rhesus macaques were 2.4 times more likely to have a better hair coat than were pregnant females that were of a similar age, social rank, and were housed under similar conditions. Furthermore, the study showed that hair condition was particularly affected during late pregnancy and in the immediate postpartum period.

Although closely related to rhesus macaques, pigtailed macaques are nonseasonal breeders. Anecdotal reports of dramatic hair loss concurrent with or after pregnancy are unavailable. To our knowledge, the effects of pregnancy on hair loss in pigtailed macaques have not been systematically studied. However, through a survey of alopecia among 3 macaque species at our Seattle colony, we found that relationships between alopecia

and other factors were unaffected by the addition or removal of a small sample of pregnant animals.¹⁶ This finding might indirectly indicate that pregnancy has little effect on hair loss in pigtailed macaques. In the current study, we had an opportunity to systematically study the potential effects of pregnancy on hair loss patterns in pigtailed macaques by using a larger sample of breeding-age females than previously. We predicted that females in the last 2 mo of pregnancy or at less than 1 mo postpartum would show increased alopecia in comparison to females at earlier stages of pregnancy and nonpregnant females.

Materials and Methods

Sample description. Animals were maintained in accordance with the *Guide for Care and Use of Laboratory Animals*,¹³ participated in the Washington National Primate Research Center Environmental Enhancement plan, and were fed a nutritionally balanced diet that was supplemented at least 3 times weekly with additional fruit and produce. The Washington National Primate Research Center is AAALAC-accredited, and all research was conducted under protocols approved by the University of Washington IACUC.

The sample comprised 144 female pigtailed macaques residing at the Arizona Breeding Center between September 2015 and March 2017. Specifically, female pigtailed macaques were included in the sample if they were at least 4 y old (breeding age) at the time of their semiannual health exam in Fall 2015 (period 1) and had alopecia scores from at least 2 of 3 subsequent health exams conducted in Spring 2016 (period 2; $n = 136$), Fall 2016 (period 3; $n = 133$), and Spring 2017 (period 4; $n = 132$). The sample included 8 animals initially at Arizona Breeding Center that were transferred to Seattle before the March 2017 assessment; alopecia scores collected in Seattle were used for the period 4 data for these animals.

Colony housing. Animals were primarily housed in social groups of 10 to 15 adult animals and young infants. Social groups were either breeding groups, comprising one adult male and multiple adult females, or all-female groups, comprising adult females and their juvenile offspring. Smaller numbers of animals ($n = 8, 4, 4$, and 13 respectively for the 4 periods) were housed in pairs, individually, or individually with their infant. The number of noncompound-housed animals is higher for period 4 due to the inclusion of the 8 animals transferred to Seattle. As noted earlier, 5 compounds were transitioned from outdoor to indoor housing in June 2016 to minimize exposure to *Coccidioides* spp. After the indoor transition, macaques in these compounds were continuously indoors, with the exception of approximately 1 h each day when the compounds were cleaned. The space available to groups with indoor-only access averaged 219 ft², which is slightly more than 1/4 of the space available to groups in the same compounds when allowed both indoor and outdoor access. Indoor transitioning of these compounds is reflected in an increase in the percentage of our total sample that was housed indoors throughout the study (25% housed indoors during period 1 compared with 38% for period 4).

Alopecia scoring. All animals at the facility receive a hands-on physical exam under sedation every 6 mo. As part of the physical exam, they are assigned a haircoat score of 0 to 4, with 0 representing no alopecia and 4 indicating the most severe presentation. The scoring methodology has previously been reported in detail.⁴ In brief, the body is visualized as separated into 11 sections (head, left arm, right arm, chest, abdomen, upper back, lower back, left upper leg, left lower leg, right upper leg, and right lower leg), each comprising 9% of the body surface. The tail comprises the final 1%. The number of affected

body sections is then added to determine an overall alopecia score (Figure 1). All personnel involved in scoring are trained initially to a minimum reliability of 0.85 (κ) with a master scorer and complete annual reliability refresher training.

Coccidioidomycosis and treatment. The study period coincided with the increased identification and treatment of coccidioidomycosis in the center. Due to increasing identification of affected macaques, proactive screening for coccidiomycosis in animals under any suspicion of illness was instituted by late 2015. This process resulted in screening every 6 mo for most animals. Macaques with positive titers were treated with fluconazole. Those on treatment were regularly monitored for changes in blood chemistry and body condition because fluconazole can induce changes in liver enzymes, and loss of body condition can occur in chronic disease states.^{11,28} The subjects included in this study were judged to be clinically normal by the veterinarians attending to their care.

During the study period, the delivery of fluconazole changed. Beginning in February 2016, a feed containing fluconazole became available, thus making it more likely that animals could remain in their original social groups during treatment. Before introduction of the feed, it was more often necessary to move animals to smaller areas with fewer social companions, to facilitate medication delivery.

Data analysis. The effects of animal age, time period, pregnancy status, fluconazole administration, and access to the outdoors or increased space on the probability of increasing or decreasing alopecia scores was examined by using ordered logistic regression. All predictor variables were treated as categorical variables, as described following.

Age. Animals were categorized by their age at the time of the period 1 assessment into those younger than 6 y ($n = 46$), 6 to 8 y ($n = 38$), and older than 8 y ($n = 60$).

Time period. Sedation for semiannual health exams and alopecia scoring takes place in the spring and fall. Data for the current report included alopecia ratings obtained between 31 August and 13 October 2015 (period 1); 7 and 29 March 2016 (period 2); 10 August and 24 October 2016 (period 3); and 6 March and 3 April 2017 (period 4).

Pregnancy status. An animal was coded as pregnant when she was in the last 2 mo of pregnancy or had given birth less than 1 mo prior to assessment. This categorization was made in light of results from an earlier report regarding pregnancy effects in rhesus macaques.³

Fluconazole. Animals were coded as positive for fluconazole treatment when they had begun receiving fluconazole at least 2 mo prior to their alopecia assessment and were either continuing fluconazole at the time of their assessment or had been discontinued less than 2 wk before assessment. Macaques were categorized as negative for fluconazole use when they had no history of fluconazole administration, when fluconazole had been discontinued for more than 2 wk, or when fluconazole treatment had begun less than 2 mo prior to their assessment. The 2-mo criterion was chosen in light of previous research implicating this duration as the minimum needed to observe drug effects on hair loss.²²

Changes in outdoor access or space available. The number of days animals were housed with access to the outdoors in the 6 mo prior to their alopecia assessment was computed. Animals that spent at least 80% of those days in housing with access to the outdoors were categorized as positive for outdoor access/increased space. Animals with less than 80% of days with access to the outdoors were categorized as negative. The decision was made to treat this variable as binary rather than continuous due

Score	No. of body regions with alopecia	% of body surface affected
0	0	0
1	1 or 2	1–19
2	3 to 5	20–46
3	6 to 8	47–73
4	9 or more	74 and greater

Figure 1. Alopecia score assignment

to the bimodal distribution of 'days outside' at all time periods (that is, most animals either had access to the outside all the time or were housed inside all the time). The criterion was set at 80% to maintain roughly equal 'positive' and 'negative' groups.

Relationships among predictor variables were examined by using χ^2 analysis in SPSS version 19 (IBM, Armonk, NY). The full model that was tested initially included these 5 factors (time period, age, pregnancy status, fluconazole, and outdoor access or increased space) plus the interactions of period, pregnancy, fluconazole, and outdoor access with age. For the ordered regression, data were analyzed in R,²⁶ by using the ordinal package with the clmm command (cumulative link function for ordered logistic regression). Because the data contained only one 4 rating for alopecia, this value was pooled with the 3 scores for analysis. Terms were tested for their significance in the model by evaluating the change in the log likelihood scores for models with and without the predictor of interest by using ANOVA.

Results

Sample description. The pigtailed macaques in this study ranged in age from 4.0 to 17.8 y (mean, 8.2 y) at the time of their initial assessment. The percentage of the sample that was coded positive for pregnancy (that is, in the last 2 mo of pregnancy or the first month after delivery) was at its highest rate (25%) in period 3 before falling to the lowest level (10%) in period 4. The percentage of animals receiving fluconazole rose continuously throughout the study period, from 9% in period 1 to 42% by period 4. Of the 118 animals with all 4 assessments, 57 (48%) never received fluconazole during the study period. The percentage of animals outside for most (that is, at least 80%) of their time during the 6 mo preceding their assessments ranged from 40% to 65% and was highest in period 3. Trends for all of these variables across the study period are plotted in Figure 2.

Relations among predictor variables. Animals in the youngest group (that is, younger than 6 y) were more likely to be pregnant than animals in the older groups (that is, 6 to 8 y old or older than 8 y) in period 3 ($\chi^2 = 11.09$, $P = 0.004$); pregnancy and age were unrelated in all other periods (period 1: $\chi^2 = 0.76$, $P = 0.69$; period 2: $\chi^2 = 3.08$, $P = 0.21$; period 4: $\chi^2 = 2.65$, $P = 0.26$). In addition, for all 4 periods, age was unrelated to the likelihood of having outside access (period 1: $\chi^2 = 2.85$, $P = 0.24$; period 2: $\chi^2 = 0.36$, $P = 0.83$; period 3: $\chi^2 = 1.96$, $P = 0.38$; period 4: $\chi^2 = 1.77$, $P = 0.41$) and fluconazole administration (period 1: $\chi^2 = 2.59$, $P = 0.27$; period 2: $\chi^2 = 0.36$, $P = 0.83$; period 3: $\chi^2 = 0.86$, $P = 0.65$; period 4: $\chi^2 = 1.83$, $P = 0.40$).

Pregnancy status was unrelated to outdoor access or increased space in period 1 ($\chi^2 = 0.57$, $P = 0.30$). However, during periods 2 through 4, pregnant macaques were significantly more likely to have outdoor access and increased space (period 2: $\chi^2 = 5.76$, $P = 0.013$; period 3: $\chi^2 = 19.32$, $P < 0.001$; period 4: $\chi^2 = 4.51$, $P = 0.04$). In contrast, pregnancy status was unrelated to the likelihood of receiving fluconazole during any time period (period 1: $\chi^2 = 0.54$, $P = 0.46$; period 2: $\chi^2 = 0.94$, $P = 0.33$; period 3: $\chi^2 = 0.44$, $P = 0.51$; period 4: $\chi^2 = 0.35$, $P = 0.55$).

Figure 2. Rates (mean $\pm$ 2 SEM) for outdoor access, pregnancy, and fluconazole administration over the study period. The sample comprised 144 adult female pigtailed macaques. Outdoor access or increased space was defined as outdoor access available for at least 80% of the days during the 6 mo previous to assessment. Pregnant animals were those assessed fewer than 60 d before parturition to 30 d after birth. Fluconazole subjects were treated continuously from at least 2 mo to 2 wk prior to assessment.

Animals categorized as positive for fluconazole were less likely to have outdoor access and increased space during the first 2 periods (period 1: $\chi^2 = 6.6$, $P = 0.01$; period 2: $\chi^2 = 32.13$, $P < 0.001$), whereas fluconazole was unrelated to outdoor access or increased space in period 3 ($\chi^2 = 0.24$, $P = 0.63$) and period 4 ($\chi^2 = 1.61$, $P = 0.20$), which correspond to the time when animals were treated with fluconazole-containing feed and thus could be more easily treated in compounds than during the earlier periods.

Results of ordinal mixed regression. The final model included significant terms for time period, age category, outside access, and age $\times$ outside access, age $\times$ period, and age $\times$ fluconazole interactions. (Table 1). An animal's pregnancy status did not contribute significantly to the prediction of alopecia score. Alopecia scores for both older groups of animals were elevated compared with animals younger than 6 (6- to 8-y-olds, $P = 0.04$; older than 8 y, $P = 0.03$).

Although the significant coefficients for later periods suggest that alopecia scores are elevated in all subsequent time periods in comparison to period 1, inspection of the plotted mean scores (Figure 3) shows that period effects cannot be interpreted separately from significant interaction with animal age. All age groups showed worsening alopecia in period 2. However, in periods 3 and 4, both older age groups showed improvement in alopecia scores in comparison with period 1. Only the animals in the youngest age groups continued to show slight worsening of alopecia in comparison to period 1 (Table 1, Figure 3).

Animals that had outdoor access or increased space for more than 80% of the time had lower ($P = 0.0001$) alopecia scores than did macaques who had less outdoor access or space. Again, this main effect was qualified by an interaction with age. Compared with the oldest group, animals in the youngest group derived the most ($P = 0.0004$) benefit from outside access (Figure 4).

Finally, fluconazole administration had a negligible effect on alopecia for the youngest animals but was associated with increased alopecia scores for macaques in the 2 older age groups ($P = 0.02$ for both comparisons; Figure 5).

Discussion

In this study, we retrospectively investigated semiannual alopecia assessments in breeding-age female pigtailed macaques in

relation to outdoor housing access, pregnancy, and fluconazole treatment. We also explored the relationship of alopecia with age within our adult sample and changes in alopecia related to seasonal variation. Age significantly affected alopecia status: older animals were more likely to have higher alopecia scores regardless of other factors. In addition, a macaque's age disproportionately affected the alopecia scores of the oldest animals receiving fluconazole and magnified the beneficial effects of outdoor access or increased space for the youngest animals.

Alopecia scores for all age groups were significantly elevated in period 2 compared with period 1. Scores remained elevated for only the youngest animals in periods 3 and 4. The reasons for this variation remain unclear. Seasonal variation may provide a partial explanation, because the scores for both of the springtime periods (periods 2 and 4) were higher than for the fall periods preceding them. In many mammals, haircoats are thicker in the fall months than in the spring.^{30,32} However, other clear differences between periods 2 and 4 are not explained by seasonal variation. Alopecia scores for the colony were the worst in period 2, but this worsening pattern was not repeated to the same extent the following spring (period 4). The exceptionally low humidity experienced in February and March 2016 in the Mesa Arizona area (40 d with humidity below 30% compared with 14 d for the same period in the previous year)²⁰ may have contributed to the decreased scores during period 2. Humidity has previously been reported to be related to coat condition in rhesus macaques.³⁰

We consistently found no effect of pregnancy status on hair loss in pigtailed macaques. Despite the sometimes dramatic hair loss seen in the closely related species of rhesus macaques, pigtailed macaques do not appear to experience hair loss during pregnancy. This difference highlights the importance of gathering species-specific data concerning alopecia etiologies because generalizing—even from closely related species—may provide misleading guidance in our attempts to understand and ameliorate alopecia in colony NHP. It is still possible that pigtailed macaques experience more subtle increased shedding at the end of pregnancy, similar to that documented in women. Investigating more subtle changes in the hair growth cycle related to pregnancy requires some analysis of individual hairs.¹⁰ Regardless of whether subtle differences exist, we argue that these results give us confidence to include pregnant animals in future population studies of hair loss factors, without a concern that their inclusion will invalidate the results.

Fluconazole disproportionately affected the haircoats of older animals yet had minimal to no effect on animals in the youngest age group. For both older age groups, receiving fluconazole more than doubled the probability of receiving an increased alopecia score. To our knowledge, this study is the first to document potential effects of fluconazole use on hair loss in NHP. In addition, we are unaware of other research involving either human or animal subjects that implicates age as a potential mediating variable of fluconazole effects on alopecia. Consideration of subject age in future research may help to reveal fluconazole effects that have previously been obscured. Information regarding fluconazole effects is especially important given that pigtailed macaques are an important HIV model, and fluconazole is commonly used prophylactically for immunosuppressed study animals.

It is crucial to recognize that the incidence of coccidioidomycosis and fluconazole treatment are unavoidably confounded in our data set. Despite this drawback, the increase in alopecia noted with fluconazole animals seems unlikely to be primarily attributed to coccidioidomycosis. Because the macaques at the

Table 1. Results of logistic regression examining effects of assessment period, age, fluconazole, pregnancy status, and outdoor access or increased space on alopecia in pigtailed macaques

	Effect size (odds ratio)	β coefficient	SEM	P
period 2	2.403197	0.8768	0.2796	0.002
period 3	1.963444	0.6747	0.3025	0.026
period 4	2.165822	0.7728	0.3027	0.011
Age 2 (6 to 8 y)	2.129953	0.7561	0.3650	0.038
Age 3 (older than 8 y)	2.037045	0.7115	0.3288	0.03
Fluconazole (yes)	0.694683	-0.3643	0.2892	not significant
Outdoor (yes)	0.441225	-0.8182	0.2142	0.0001
period2×age2	0.69385	-0.3655	0.3915	not significant
period3×age2	0.301978	-1.1974	0.4328	0.006
period4×age2	0.285475	-1.2536	0.4305	0.004
period2×age3	0.855046	-0.1566	0.3480	not significant
period3×age3	0.282437	-1.2643	0.3808	0.0009
period4×age3	0.271797	-1.3027	0.3842	0.0007
Age2×outdoor	1.17539	0.1616	0.3147	not significant
Age3×outdoor	2.686932	0.9884	0.2798	0.0004
Age2×fluconazole	2.821306	1.0372	0.4527	0.02
Age3×fluconazole	2.350669	0.8547	0.3698	0.02

Figure 3. Interaction between age and time period. The youngest animals showed worsening alopecia at subsequent assessment periods relative to the initial assessment. Both groups of older animals showed worsening alopecia during the second assessment followed by slight improvement during the third and fourth periods. Animals were classified according to their age at the first assessment period.

breeding were closely monitored for this disease during the study period, the majority of the animals that were targeted to receive fluconazole did not have apparent symptoms of the disease even though they had elevated titers. Furthermore, hair loss is not listed as a symptom of coccidioidomycosis in clinical literature.²⁴ It is certainly likely that animals with advanced illness exhibit widespread symptoms of system failure that could include alopecia, and indeed alopecia was noted by veterinary staff for at least some macaques with advanced illness in our colony. However, our study population included very few macaques with advanced illness, due to the initiation of intensive screening that coincided with the beginning of our study and to our inclusion requirement that animals had to be assessed during multiple sessions of alopecia scoring.

In this study, macaques that had access to the outdoors at least 80% of the time during the 6 mo prior to alopecia assessment had significantly improved alopecia scores when compared with animals with less outdoor access and decreased space. Moreover, animals in the youngest age group benefited

Figure 4. Interaction between age and outdoor access or space. Unlike the oldest animals, the youngest macaques showed distinct improvement in alopecia scores when given outside access or increased space.

the most (in terms of improved alopecia scores) from outdoor access and increased space or—conversely stated—suffered the most from a lack of outdoor access and decreased space. Indeed, restricting outdoor access and space for the youngest age group resulted in alopecia scores comparable to those of older animals, essentially erasing any benefit of age. The effect of outdoor access or space is somewhat remarkable given the considerable change in the classification of animals' indoor–outdoor status from period to period. In our sample, only 21 animals (14%) were consistently classified as 'outdoor' or 'indoor' for all 4 periods. In fact, animals classified as 'outdoor' for period 2 were significantly more likely to be "indoor" in the following period.

A limitation of this study is the confounding of outdoor access with space available. Therefore, despite our finding of dramatic effects with the move to indoor space, we cannot say whether this difference is an effect of outdoor restriction, of limited space, or a combination of both factors. Decreasing available space to an established NHP colony has the potential to disrupt the existing hierarchical group structure and thus trigger behavioral disturbance and increased stress for individual animals.¹⁴

Figure 5. Interaction between age and fluconazole administration. Older animals given fluconazole showed a significant change in alopecia score; animals in the youngest age group did not show this effect.

Increased stress might have been a factor in the worsened haircoat condition in some of our animals. However, comparison of behavioral records tracking maladaptive behaviors for the same locations in 2016 (during outdoor access) and 2017 (after indoor transition) actually showed a slight decrease in 2017. In addition, it is notable that hair pulling, one of the maladaptive behaviors that was tracked and that is known to be related to hair loss, was not observed in any of the transitional compounds during either the 2016 or 2017 comparison periods and therefore appears to have no explanatory role in the observed haircoat changes coinciding with transitions to indoor space. Although it is possible that more subtle behavioral changes were present in the groups but were not captured in our monthly group scans, it does not appear that large behavioral changes accompanied indoor transition.

In addition, inferences from this study are limited by our lack of available histologic data for alopecia cases in this retrospective study. It is important to note that mild to moderate hair loss is commonly seen in primate colonies and typically has defied diagnosis.^{17,29} A retrospective review of veterinary records for the most severely affected animals in our study revealed no obvious medical conditions (skin conditions, nutritional deficiencies) that were specifically related to alopecia. No skin biopsies were obtained from study animals during the study period, in response to veterinary concerns. General health probably affected ratings in a minority of cases. Although having histologic data could have been valuable in further delineating causes of alopecia, we don't believe the lack of this information invalidates our basic findings of relations between alopecia ratings and colony management practices.

In this study, we found that age affected hair coat quality both directly and in interaction with environmental variables. In our adult population, the youngest animals had the best hair coats but also were the most affected by the environmental factors of outdoor access and space changes. Older adults' hair coat quality was affected by fluconazole status, which had no discernable effect on young adults. Contrary to our hypothesis, pregnancy had no apparent effect on hair coat in pigtail macaques, unlike the effects documented in rhesus macaques.

We believe this study has broad implications, both practical and theoretical. Practically, the results provide increased understanding of how husbandry-related (compared with experimental) variables might influence alopecia in animals participating in research experiments. Theoretically, it is interesting that 2 of the 3 factors we investigated showed significant interactions with age. In both cases, the youngest age group was affected

differently than the older 2 age groups. Although the youngest macaques had better haircoats overall, restricted outdoor access had a more deleterious effect on younger animals than older ones. In contrast, fluconazole treatment, which might be considered a challenge to the organism, had significant detrimental effects for older animals but had no discernable effect on alopecia for the youngest animals. Given that all animals in this study were of adult age, the differences reported here highlight the importance of continuing to consider age, even in adult animals.

The current study demonstrates that a pigtailed macaque's haircoat quality is determined by naturally occurring processes (aging, seasonal variation) in combination with factors that may relate to wellbeing, such as health status or exposure to environmental stressors. Although haircoat condition certainly provides some reflection on primate wellbeing, it is increasingly clear that the assumption of a direct relationship is overly simplistic. Recognition of naturally occurring processes in hair loss will be essential in developing an increasingly nuanced understanding of the role that wellbeing plays in haircoat quality.

Acknowledgments

We thank Rita Bellanca and Caroline Mead for providing expertise and assistance with alopecia scoring, and for assistance with data extraction. This research was supported by NIH ORIP grant P51 OD010425.

References

1. Barrie MT, Stadler CK. 1995. Successful treatment of *Cryptococcus neoformans* infection in an Allen swamp monkey (*Allenopithecus nigroviridis*) using fluconazole and flucytosine. *J Zoo Wildl Med* 26:109–114.
2. Beisner BA, Isbell LA. 2008. Ground substrate affects activity budgets and hair loss in outdoor captive groups of rhesus macaques (*Macaca mulatta*). *Am J Primatol* 70:1160–1168. <https://doi.org/10.1002/ajp.20615>.
3. Beisner BA, Isbell LA. 2009. Factors influencing hair loss among female captive rhesus macaques (*Macaca mulatta*). *Appl Anim Behav Sci* 119:91–100. <https://doi.org/10.1016/j.applanim.2009.03.016>.
4. Bellanca RU, Lee GH, Vogel K, Ahrens J, Kroeker R, Thom JP, Worlein JM. 2014. A simple alopecia scoring system for use in colony management of laboratory-housed primates. *J Med Primatol* 43:153–161. <https://doi.org/10.1111/jmp.12107>.
5. Centers for Disease Control. [Internet]. 2009. Biosafety in microbiological and biomedical laboratories, 5th ed. Section VIII-B. [Cited 14 May 2018]. Available at: <https://www.cdc.gov/labs/BMBL.html>.
6. Centers for Disease Control and Prevention. [Internet]. 2018. Valley fever awareness. [Cited 14 May 2018]. Available at: www.cdc.gov/features/valleyfever/index.html.
7. Clemons KV, Capilla J, Stevens DA. 2007. Experimental animal models of coccidioidomycosis. *Ann N Y Acad Sci* 1111:208–224. <https://doi.org/10.1196/annals.1406.029>.
8. Dannemiller SD, Watson JR, Rozmiarek H. 1995. Fluconazole therapy in a rhesus monkey (*Macaca mulatta*) with epidural *Trichosporon beigelii* in a cephalic recording cylinder. *Lab Anim Sci* 45:31–35.
9. Dettmer AM, Rosenberg K, Menard MT, El-Mallah SN, Woodward RA, Suomi SJ, Meyer JS. 2017. Differential relationships between chronic hormone profiles in pregnancy and maternal investment in rhesus monkey mothers with hair loss in the neonatal period. *Am J Primatol* 79:1–8. <https://doi.org/10.1002/ajp.22489>.
10. Gizlenti S, Ekmekci TR. 2013. The changes in the hair cycle during gestation and the postpartum period. *J Eur Acad Dermatol Venereol* 28:878–881. <https://doi.org/10.1111/jdv.12188>.
11. Graybill JR, Griffith L, Sun SH. 1990. Fluconazole therapy for coccidioidomycosis in Japanese macaques. *Rev Infect Dis* 12 Suppl 3:S286–S290. https://doi.org/10.1093/clinids/12.Supplement_3.S286.

12. **Hohl TM.** 2014. Overview of vertebrate animal models of fungal infection. *J Immunol Methods* **410**:100–112. <https://doi.org/10.1016/j.jim.2014.03.022>.
13. **Institute for Laboratory Animal Research.** 2010. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
14. **Judge PG, De Waal FBM.** 1997. Rhesus monkey behaviour under diverse population densities: coping with long-term crowding. *Anim Behav* **54**:643–662. <https://doi.org/10.1006/anbe.1997.0469>.
15. **Kramer J, Fahey M, Santos R, Carville A, Wachtman L, Mansfield K.** 2010. Alopecia in rhesus macaques correlates with immunophenotypic alterations in dermal inflammatory infiltrate consistent with hypersensitivity etiology. *J Med Primatol* **39**:112–122. <https://doi.org/10.1111/j.1600-0684.2010.00402.x>.
16. **Kroeker R, Bellanca RU, Lee GH, Thom JP, Worlein JM.** 2013. Alopecia in 3 macaque species housed in a laboratory environment. *Am J Primatol* **76**:325–334. <https://doi.org/10.1002/ajp.22236>.
17. **Luchins KR, Baker KC, Gilbert MH, Blanchard JL, Liu DX, Myers L, Bohm RP.** 2011. Application of the diagnostic evaluation for alopecia in traditional veterinary species to laboratory rhesus macaques (*Macaca mulatta*). *J Am Assoc Lab Anim Sci* **50**:926–938.
18. **Lutz CK, Coleman K, Worlein J, Novak MA.** 2013. Hair loss and hair-pulling in rhesus macaques (*Macaca mulatta*). *J Am Assoc Lab Anim Sci* **52**:454–457.
19. **Lynfield YL.** 1960. Effect of pregnancy on the human hair cycle. *J Invest Dermatol* **35**:323–327. <https://doi.org/10.1038/jid.1960.127>.
20. **National Oceanic and Atmospheric Administration (NOAA).** [Internet]. 2019. Climate data online: dataset discovery. [Cited 18 October 2017]. Available at: www.ncdc.noaa.gov/cdo-web/datasets
21. **Novak MA, Meyer JS.** 2009. Alopecia: possible causes and treatments, particularly in captive nonhuman primates. *Comp Med* **59**:18–26.
22. **Pappas PG, Kauffman CA, Perfect J, Johnson PC, McKinsey SD, Bamberger DM, Hamill R, Sharkey PK, Chapman SW, Sobel JD.** 1995. Alopecia associated with fluconazole therapy. *Ann Intern Med* **123**:354–357. <https://doi.org/10.7326/0003-4819-123-5-199509010-00006>.
23. **Pappas PG, Kauffman CA, Sobel JD.** 1996. Alopecia associated with fluconazole therapy—response. *Ann Intern Med* **125**:153–154. <https://doi.org/10.7326/0003-4819-125-2-199607150-00022>.
24. **Parish JM, Blair JE.** 2008. Coccidioidomycosis. *Mayo Clin Proc* **83**:343–348. <https://doi.org/10.4065/83.3.343>.
25. **Patel A, Forsythe P, Smith S.** 2008. Small animal dermatology. New York (NY): Elsevier/Saunders.
26. **R Core Team.** [Internet]. 2018. R: a language and environment for statistical computing. R foundation for statistical computing. [Cited 18 September 2018]. Available at: <https://www.R-project.org/>.
27. **Romani L.** 2011. Immunity to fungal infections. *Nat Rev Immunol* **11**:275–288. <https://doi.org/10.1038/nri2939>.
28. **Shubitz LF.** 2007. Comparative aspects of coccidioidomycosis in animals and humans. *Ann NY Acad Sci* **1111**:395–403. <https://doi.org/10.1196/annals.1406.007>.
29. **Steinmetz HW, Kaumanns W, Neimeier KA, Kaup FJ.** 2005. Dermatologic investigation of alopecia in rhesus macaques (*Macaca mulatta*). *J Zoo Wildl Med* **36**:229–238. <https://doi.org/10.1638/04-054.1>.
30. **Steinmetz HW, Kaumanns W, Dix I, Heistermann M, Fox M, Kaup FJ.** 2006. Coat condition, housing condition and measurement of faecal cortisol metabolites—a noninvasive study about alopecia in captive rhesus macaques (*Macaca mulatta*). *J Med Primatol* **35**:3–11. <https://doi.org/10.1111/j.1600-0684.2005.00141.x>.
31. **Tabaoda J.** [Internet]. 2014. Coccidioidomycosis. Merck Veterinary Manual. [Cited 14 May 2018]. Available at: <http://www.merckvetmanual.com/generalized-conditions/fungal-infections/coccidioidomycosis>.
32. **Vessey SH, Morrison JA.** 1970. Molt in free-ranging rhesus monkeys, *Macaca mulatta*. *J Mammal* **51**:89–93. <https://doi.org/10.2307/1378535>.
33. **Zhang P.** 2011. A noninvasive study of alopecia in Japanese macaques *Macaca fuscata*. *Curr Zool* **57**:26–35. <https://doi.org/10.1093/czoolo/57.1.26>.