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Epidemiology of Cercopithecine Herpesvirus 1 (B Virus) Infection and Shedding in a Large Breeding Cohort of Rhesus Macaques

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The epidemiology of B virus infection in a large (n = 157) cohort of rhesus macaques at the California Regional Primate Research Center was evaluated prospectively from September 1989 through January 1991 by serial physical examinations, a behavioral substudy (n = 51), and repeated diagnostic testing. Half were B virus antibody–positive at baseline; subsequently, incident cases of infection were documented through serology alone (42) or with virus isolation (5). Eight recurrent infections and a single symptomatic (primary) case were observed. Risk of B virus infection increased as monkeys aged, with few >3 years old remaining uninfected. Postpubertal monkeys and those entering sexual adolescence (2–3 years) were at greatest risk, although wounding by cagemates and breeding history (for females) were both significant predictors of time to infection. B virus was isolated from oral or conjunctival and genital tissues in equal proportions. Transmission occurred only during the breeding season, possibly coinciding with an elevation in social stressors in the population.

B virus (cercopithecine herpesvirus 1, or *Herpesvirus simiae*) is a naturally occurring alphaherpesvirus of the genus *Macaca* and a significant occupational health hazard for individuals working with macaques or their tissues. Morbidity associated with B virus in macaques is typically limited to vesicle and ulcer formation in the oral cavity [1, 2], but at least 25 well-documented cases of human B virus infection have occurred. Most of these cases have progressed to encephalomyelitis, resulting in at least 16 human deaths to date [3–8]. Additional human B virus cases have been reported but not confirmed with diagnostic tests [3], and asymptomatic infections may have occurred on several occasions [7–9]. The biology and epidemiology of B virus in macaques and humans has recently been reviewed [10].

Because of public health considerations and requirements for simian retrovirus research, the National Institutes of Health is funding efforts to establish B virus–free domestic breeding colonies of rhesus monkeys [11]. However, options available to assist these efforts have been limited by insufficient knowledge of the epidemiology of B virus in macaques. Observational studies are particularly useful to assess natural B virus transmission because experimental infectious work with this virus in monkeys is complicated by biosafety level 4 housing requirements [12]. Previous epidemiologic studies have predominantly been limited to serologic surveys of poorly defined populations without consideration of important variables such as monkey origin, age, sex, housing, and time in captivity, thus precluding complete epidemiologic interpretation [3].

Evidence suggests that the pathogenesis, immunology, and epidemiology of B virus in macaques closely resembles that reported for herpes simplex viruses in humans [3, 10]. Both oral-facial and genital sensory nerve tissue tropisms have been demonstrated [13–15]. Furthermore, extensive antigenic cross-reactivities exist between B virus and herpes simplex virus antibodies, historically complicating efforts to diagnose human B virus infections [16]. One recent descriptive study [17] concluded that most B virus transmission among macaques was venereal, but this possibility has not been specifically investigated. We undertook a prospective cohort study in an enclosed population of rhesus monkeys to improve epidemiologic understanding of B virus in that species. The study assessed the significance of venereal transmission of B virus relative to other forms of contact over a time period including two breeding seasons.

Materials and Methods

Study cohort. One of the California Regional Primate Research Center (CRPRC) outdoor breeding enclosures (north corral 4, NC4) was selected for this study since it contained the

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Monkeys were maintained in conditions compliant with standards of the National Institutes of Health (Guide for the Care and Use of Laboratory Animals, 1985, publication no. [NIH] 85-23).

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largest and most age-diverse population of three groups included in a recent cross-sectional survey for B virus antibodies [18]. Because the study objectives were to assess both incident cases and recurrent B virus infections for the entire enclosure, all monkeys initially present in NC4 or that subsequently entered the population were included in the cohort regardless of their B virus status or exposure history. The diverse age-sex distribution of CRPRC outdoor breeding populations allows for the formation of species-typical dominance hierarchies (social dominance ranks) for both males and females, largely analogous to the social structure of free-ranging macaques [19]. These are monitored by CRPRC behaviorists through ongoing observations of dyadic interactions within each cage. Infrequently, infants are cross-fostered between different enclosures to increase genetic diversity among otherwise isolated breeding groups. All infants born or fostered into NC4 were included in the study after their arrival. The breeding season for these populations usually extends from early September through March, with offspring born during February–August. First evidence of sexual cycling activity among females in CRPRC group-housed rhesus typically occurs during their second or third year of life.

Specimen collection and testing. Baseline evaluation of NC4 was done on 20 September 1989. Subsequently, the entire cohort was assessed eight more times for shedding of B virus, antibodies to B virus, and specific exposure variables described below. These assessments were done every other month for 16 months, concluding on 22 January 1991. During each assessment, cotton-tipped swabs (Baxter Scientific Products, McGaw Park, IL) were used to collect conjunctival (left and right), buccal or pharyngeal, and urogenital (glans or preputial region for males and vaginal or cervical for females) samples from all monkeys in the enclosure. Swab specimens were placed in virus transport medium immediately after collection, consisting of MEM with 2.0 m*M* L-glutamine, 2% fetal bovine serum, 4 μg/mL amphotericin B, 50 μg/mL gentamicin, and 200 units/mL penicillin-streptomycin (all from JR Scientific, Woodland, CA). Transport tubes were frozen at −70°C and shipped to the Southwest Foundation for Biomedical Research (SWFBR) for isolation of B virus using published methods [20]. Briefly, each specimen was plated on Vero cells and allowed to incubate for at least 9 days. If evidence of any herpesvirus-typical cytopathic effect was seen, the isolate was identified specifically by SDS-PAGE and autoradiography according to published methods [20].

Concurrent with the collection of tissue swabs, serum (2–3 mL) was obtained from all monkeys in NC4 to test for antibodies to B virus (also at SWFBR) by published methods [21, 22]. Briefly, specimens were evaluated via an ELISA [21] at four dilutions (1:5, 1:50, 1:500, and 1:5000) or via Western blot with B virus–infected whole cell (Vero) lysates as antigen [22] or both. Serum and swabs were also collected from 1 infant immediately before fostering it into the study enclosure to evaluate the possibility of B virus introduction via that individual. Daily health observations of the cohort allowed for monitoring of overt illnesses as they occurred.

Case definitions. B virus infection status was assessed from the results of combined serologic and virologic testing of collected specimens. To establish the earliest time point of B virus exposure, we classified sera as antibody-positive when they had B virus antibody titers ≥1:50 via ELISA or at least one B virus–specific band via Western blot. Monkeys both antibody-negative and virus-negative were considered at risk for subsequent B virus infection. Monkeys scored as antibody-positive were considered to be infected with B virus, at least latently, and capable of B virus reactivation and shedding for the duration of the study. Thus, B virus isolations from antibody-positive monkeys represented recurrent infections. Specimen testing also identified primary cases: first infection with B virus in monkeys lacking B virus antibodies in acute-phase serum. These definitions are analogous to those used for herpes simplex virus infections in humans [23, 24]. Primary cases represented initial B virus replication in host epithelial tissues, presumably before latent infections were established [10]. Incident cases were identified by primary infections and by seroconversions, that is, antibody-positive specimens from monkeys that tested antibody-negative on all previous occasions and showed no previous virologic or clinical evidence of B virus infection.

Exposure variable ascertainment. Monkey age, sex, social dominance rank, trauma lesions, and breeding histories (females only) were evaluated for possible association with B virus. Social dominance ranks were coded into three equal strata (per sex) representing low, medium, or high rank. Physical examinations were used to detect recent trauma via the presence or absence of penetrating skin lesions, presumed to result from direct aggression by cagemates; these observations were scored as dichotomous responses (trauma versus no trauma) for each preceding 2-month assessment interval. Infant birth records, uterine palpation per rectum, and vaginal inspection for semen plugs were used to determine the intervals during which breeding had occurred in female monkeys. Female monkeys ≥2 years old displaying genital sexual cycling activity were considered sexually receptive to males and copulation, regardless of the pregnancy outcome.

Behavioral substudy. A behavioral substudy (n = 51) was done concurrent with the cohort study to enable direct assessment of the risk of B virus infection relative to specific activities of select monkeys in the population. Only monkeys ≤3 years old were included, since previous survey information [18] indicated that most B virus infections had already been acquired by that age. Six monkeys (3 male, 3 female) were selected for observation from each of the four birth groups of interest (1987, 1988, 1989, and 1990) by simple random sampling. All 2- to 3-year-old monkeys in the enclosure were included during the breeding season (n = 14 for 1989, n = 25 for 1990) to aid in evaluation of venereal transmission. Behavioral activities of interest were those that involved physical contact between monkeys and could therefore potentially result in direct B virus transmission: play, grooming, passive aggression (study monkey was recipient), and sexual mounts. Behavioral data were collected by trained observers (one per session) using the focal animal technique, described elsewhere [25], with one-zero sampling [26]. Tallies of these behavioral displays were converted into seasonal activity rates per observed monkey (September–December 1989, January–August 1990, September–December 1991), permitting assessment of seasonal changes in specific behaviors relative to B virus incidence.

Statistical analyses. Age-stratified incidence rates and risk estimates [27] across specified time intervals were determined

Table 1. Demographics of rhesus macaques in north corral 4 at the California Regional Primate Research Center from 20 September 1989 to 22 January 1991.

Birth group	No. males	No. females	Total
1990	16*	12	28
1989	10	15	25
1988	10	15	25
1987	4	10	14
1986	5	7	12
1985	5	4	9
1984	4	8	12
1983	3	1	4
1981	1	0	1
1980	1	22	23
1979	2	0	2
1976	2	0	2
Total	63	94	157

* Includes one infant monkey cross-fostered into cohort.

using monkey-months of observation as the denominator for each stratum. Cumulative incidence proportions, another measure of average risk [27], were calculated for each 2-month testing interval by dividing the corresponding incident cases by the number of monkeys considered to be at risk immediately after the previous set of diagnostic test results. Cox proportional hazards regression models [28] were used to explore the relative importance of the exposure variables for B virus transmission. Monkey-months of age to infection was the dependent variable for these models, and the age of each monkey at risk provided the baseline time variable at study initiation. These models do not require specification of the baseline hazard function and can be used to describe the relationship between incidence rates of disease and a set of predictor (exposure) covariables, retain censored data (e.g., losses to follow-up) in the analysis, and can incorporate changes in the value of covariables over time (i.e., time-dependent covariates). Proportional hazards models were constructed using the program 2L [29] in a forward-and-backward stepwise procedure (F-to-enter, $P = 0.10$, F-to-remove, $P = 0.15$), forcing in possible confounding variables detected during each analysis. The likelihood ratio statistic was used to test the significance of individual exposure variables, while adjusting for other variables in the model, at an α level of .05. Trauma lesions, breeding history, and seasonal activity rates for play, grooming, aggression, and sexual mounts were modeled as time-dependent covariates. Comparisons between the behavioral activity profiles of monkeys from different birth groups over seasons were made by multivariate analysis of variance [30]. This analysis represented a one-within (activity represented by the four behaviors) and one-between (birth group) design with repeated measures (seasonal activity rate). Logistic regression [29] was used to explore possible associations between sex, social dominance rank, birth group, and B virus recurrent infections detected among antibody-positive monkeys.

Results

Demographic characteristics of the study cohort are shown in table 1. A total of 129 monkeys were present in the enclosure at study initiation. Thirty-three infants subsequently entered the population by birth; 1 other entered through cross-fostering. Fifteen monkeys left during the study period: 6 were culled, 4 were found dead, 3 were fostered to other enclosures, and 2 were removed for research protocols. No monkeys were removed or found dead for reasons attributable to their B virus status. Thus, of the 163 monkeys ever present, only 157 were in the population long enough to be sampled and contribute to the analyses.

Repeated assessments of the study cohort provided 1260 sera and 5036 epithelial tissue swab specimens. Sixty-five monkeys (50% of the baseline population) were already B virus antibody–positive at study initiation. Combined serologic and virologic testing determined that 92 monkeys were uninfected (at risk) sometime during the study period and that 47 (51%) incident cases of infection had occurred (21 males, 26 females). Forty-two of these were identified by seroconversions and the remaining 5 by primary virus isolations (4 of which subsequently seroconverted). These incident cases were used to evaluate exposure variables for B virus transmission. B virus was isolated on 14 occasions (table 2), representing 5 primary and 8 recurrent infections (one case unidentified). The latter group, occurring among 112 monkeys classified as antibody-positive during the study overall, was used to explore factors possibly related to B virus

Table 2. Monkey characteristics associated with 14 isolations of B virus from study cohort of rhesus macaques at the California Regional Primate Research Center from 20 September 1989 to 22 January 1991.

Type of infection, monkey no.	Age (years)	Sex	Month/year	Tissue site(s)
Primary				
23726	2	F	9/89	Gen/Conj
25007*	0.3	F	9/89	Gen
24227	1	M	11/89	Gen
24727†	1	F	1/90	Oral
24762	1	F	9/90	Oral
Recurrent				
21782	5	F	9/89	Conj
23035	3	F	9/89	Conj
22423	4	M	9/89	Conj
23037	3	M	9/89	Conj
19504	8	M	9/89	Gen
20620	9	F	1/90	Gen
23726‡	3	F	9/90	Gen
20575	10	F	1/91	Gen
Unknown§	—	—	9/89	—

NOTE. F = female; M = male; Gen = genital; Conj = conjunctival. Primary, first infection with B virus; recurrent, evidence of previous B virus infection.

* No antibodies to B virus detectable for subsequent 16 months of study.

† B virus isolated during symptomatic episode of disease.

‡ Primary infection detected during 9/89 testing.

§ Identification label lost in transit.

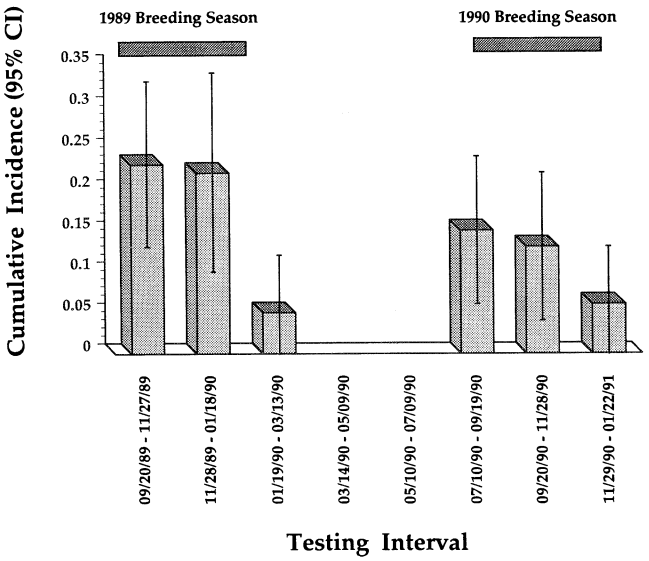

Figure 1. Cumulative incidence (risk) per 2-month testing interval for 47 cases of B virus infection in California Regional Primate Research Center north corral 4 rhesus macaques from 20 September 1989 to 22 January 1991. CI = confidence interval.

recurrence. Each infant tested (n = 28) was positive for B virus antibody, with a titer equal to that of its mother, which disappeared by an average of 5.5 months of age (95% confidence interval = 3–8 months). Censoring occurred on 45 occasions: 1 monkey died midstudy (bacterial septicemia), and 44 monkeys had no evidence of B virus infection at study termination.

The cumulative incidence for each 2-month testing interval is shown in figure 1. All incident cases occurred during the two breeding seasons encompassed by this study, with 28 (60%) of 47 occurring during the first breeding season. Repeated testing allowed examination of the extent to which B virus antibody titers fluctuated over time among seropositive individuals; ELISA antibody titers were consistently high (≥1:500) for all monkeys meeting the incident case definition and did not fluctuate detectably for the 8 recurrent B virus episodes observed. Seasonality was also apparent for 14 B virus isolations resulting from primary or recurrent infections (table 2). No B virus was recovered during the nonbreeding season of these monkeys. One overt case of B virus disease was observed in a 9-month-old female undergoing a primary infection, which manifested as unilateral conjunctivitis with a small (0.5 cm) ulcer on her upper lip. Four of the 5 with primary cases seroconverted during the 2 months before the next testing date. However, B virus was isolated from genital tissues of a 4-month-old female that remained persistently antibody-negative for the subsequent 16 months. Interim specimens were available for the single overt case observed; B virus antibodies were first evident via Western blot 1 week after disease, but no attempts to reisolate the agent were successful. No herpetic-type genital lesions were noted on any monkey, despite thorough physical examinations and 7 of 14 B virus isolations from genital tissues. Neither birth group, sex, nor social dominance rank was significantly associated with B virus recurrence and shedding.

Age-stratified incidence rates and associated risk of B virus infection are shown in table 3. Only 7 monkeys >3 years old were found to be at risk of B virus infection studywide; all 7 seroconverted during the first breeding season, resulting in the highest risk estimate for any age group overall. The next greatest risks were found in monkeys entering the 2- to 3-year-old stage of sexual adolescence (i.e., those born in 1987 or 1988) during the first or second breeding seasons encompassed by this study, respectively. However, 23 (49%) of 47 incident cases occurred in younger age strata (<2 years old) not known to be sexually active.

The behavioral substudy included 489 observation sessions. These data indicated a significant multivariate main effect for season (Hotelling's T^2 = 139.2, $P < .001$) and significant univariate effects for grooming (T^2 = 14.6, $P < .01$), play (T^2 = 14.9, $P < .01$), and aggression activity rates (T^2 = 71.8, $P < .001$). Thus, overall activity profiles of monkeys changed seasonally, explained by differences in the individual rates for grooming, play, and aggression. The multivariate interaction term of season with birth group was also significant (T^2 = 91.0, $P < .01$). However, the activity profiles of monkeys 1 year apart in age were not significantly

Table 3. Incidence rates and risk of B virus infection for 92 rhesus macaques at risk in the study cohort by birth group, stratified by time intervals overlapping the successive breeding seasons.

Time interval, birth group	No. at risk	No. incident cases/ total months observed	Incidence rate	Risk of infection*
9/20/89–5/9/90				
1986 or before	7	7/16	.44	.97
1987	11	9/44	.20	.80
1988	21	10/124	.08	.47
1989	25	2/190	.01	.08
1990	21	0/29	0	0
5/10/90–1/22/91				
1986 or before	0	0/0	0	0
1987	2	1/16	.06	.38
1988	11	7/80	.09	.51
1989	23	11/145	.08	.47
1990	28†	0/234	0	0
Overall				
1986 or before	7	7/16	.44	.97
1987	11	10/60	.17	.88
1988	21	17/204	.08	.74
1989	25	13/335	.04	.51
1990	28	0/263	0	0

* Average risk across time interval for each stratum separately or combined into overall estimates [27].

† Includes 7 monkeys born after 5/10/90.

Table 4. Best-fitting Cox proportional hazards models of exposures influencing time to B virus infection in study cohort of rhesus macaques at the California Regional Primate Research Center.

Model number, exposure variable	Hazard rate ratio	95% CI	*P* value of LRS
1, Trauma lesions	4.1*	2.3–7.6	<.0001
2, Trauma lesions	4.7*	1.9–11.5	<.001
Breeding history	3.9†	1.1–14.4	.03
3, Trauma lesions	2.7*	0.6–13.3	.17
Play‡	1.1	1.0–1.2	.04
Aggression‡	1.1	0.9–1.4	.50
4, Trauma lesions	3.0*	0.2–46.8	.44
Play‡	1.9	1.2–2.9	<.001
Grooming‡	0.9	0.7–1.0	<.01

NOTE. Model 1 includes all monkeys at risk during study period (n = 92); model 2, all female monkeys at risk during study period (n = 66); model 3, all monkeys at risk for which behavioral observation data were available (n = 28); model 4, all female monkeys at risk for which behavioral observation data were available (n = 17). All exposure variables included in resulting models were time-dependent covariates. CI = confidence interval; LRS = likelihood ratio statistic.

* Compared with monkeys lacking trauma lesions.

† Compared with female monkeys lacking evidence of recent breeding.

‡ Represented as seasonal behavioral activity rate per monkey at risk.

different when rates from the first and second breeding seasons were compared. Thus, the 1989 breeding season activity rates for monkeys born in 1987 were not different from the 1990 breeding season rates for monkeys born in 1988, and so forth, thereby demonstrating consistency within these data. Furthermore, the behavioral profiles of each birth group changed significantly over breeding seasons as monkeys aged (data not shown). Only male and female monkeys ≥2 years old were seen to copulate, and 75 (95%) of 79 observed copulations occurred during the typical breeding season. Five of 11 female monkeys 2–3 years old had genital sexual cycling activity and were observed being mounted (one pregnancy resulted), while no mounts or pregnancies were recorded among 14 noncycling females of the same age. Only 13 penetrating bites were documented through the behavioral substudy, 10 of which occurred in monkeys uninfected before the bite; seroconversion to B virus was observed in bite recipients during the subsequent 2-month testing interval for 5 of these 10.

Four Cox proportional hazards models were constructed to test all possible combinations of exposure variables (sex, social dominance rank, trauma lesions, breeding history, and seasonal behavioral activity rates for play, grooming, aggression, and sexual mounts) possibly related to B virus infection, since breeding histories were available only for female monkeys and seasonal activity rates were determined only for a subset of the NC4 population. Thus, none of the resultant models are directly comparable, each representing a comprehensive analysis of available information for separately defined groups. Table 4 lists the exposure variables best fitting the data for each model using this approach. Both trauma lesions and breeding histories were represented by dichotomous variables, so the hazard rate ratio (HRR) associated with each is directly interpretable as a relative risk estimate, with the positive coefficient sign implying a greater hazard with increasing levels of the exposure. Behavioral activity rates were represented by continuous variables, so the associated HRRs correspond to the exposure variable assuming a value of 1. The trauma variable was forced into models 3 and 4 because of its independent association with infection in these analyses and because it confounded the relationship of play and aggression rate variables with age to B virus infection. The breeding history variable was not significantly associated with B virus infection in model 4, although 10 of 13 incident cases had the exposure compared with no evidence of breeding recorded for those female monkeys remaining uninfected. This result was likely due to small sample size, as suggested by the large SE (0.616) for the breeding history variable tested alone.

Discussion

A prospective cohort study of B virus in rhesus macaques enabled proper temporal assessment of exposure factors relative to B virus infection. A behavioral substudy provided exposure data in a manner novel to epidemiologic research involving monkeys and was warranted because of the high social complexity of the species. Our results indicated that the risk of B virus infection increased substantially as monkeys aged, with few uninfected monkeys remaining so beyond the 2- to 3-year-old stage, during which sexual activity was observed to begin within the enclosure. Additional evidence from the present study strengthening the role for hypothesized venereal transmission [15, 17] included the breeding seasonality for both incident cases and B virus recurrences, our success in isolating the agent from genital tissues in 3 of 5 primary cases of B virus infection, and the HRR associated with breeding history in model 2. However, the significance of nongenital modes of B virus transmission was documented by our observation of 49% of incident cases occurring in monkeys <2 years old, none of which had any evidence of sexual exposures. Furthermore, Cox regression analysis indicated that the trauma variable's effect superseded that of all other exposures tested. In agreement with previous results [18], neither gender nor social dominance rank were significantly associated with B virus infection in any analysis.

The trauma variable was used to explore the importance of biting and scratching for B virus transmission, presumably via direct inoculation of virus-laden saliva. Potentially, this could be directly assessed by the aggression activity rate variable alone. Aggression was found to be a significant predictor in model 3, but its effect was greatly overshadowed by the simultaneous inclusion of the trauma variable. Limited be-

havioral data and small sample size hindered our ability to fully assess activity rates in the study population. A degree of misclassification bias [27] probably existed for the trauma data, as not all lesions were inflicted by cagemates and many lesions may have gone undetected. However, such bias would likely be nondirectional with respect to disease; that is, the likelihood of being bitten or scratched during a specified time interval would not change on account of a monkey's B virus infection status. Since similar arguments hold for breeding histories and behavioral activity rates, hazard rate ratios for individual variables presented in table 4 are probably conservative measures of the true risk of infection due to those exposures [27].

The significance of the play variable in models 2 and 3 implicates other interactions between monkeys for spreading B virus. Possibly, certain types of adolescent play escalated into bites and scratches by annoyed cagemates, promoting B virus transmission. Interestingly, the grooming variable remained (inverted) in model 4 after the inclusion of trauma, but severe confounding was evident. Notably, cell-free B virus is not expected to persist long in the environment [31, 32], and aerosol transmission of the agent between monkeys is not typically important [10, 17, 33].

Our results indicated that uninfected monkeys were primarily exposed to B virus via oral, conjunctival, or genital mucosal sites, depending on age, during the breeding season. Seasonality could have emerged by the appearance of stressors sufficient to cause the reactivation of latent infections in the population. Increases in stress-related behaviors, including aggression, have been described for at least two species of group-housed macaques during the breeding season [34, 35]. A variety of stressors have been associated with recurrences of related herpes simplex viruses in human beings [31], and stress has been described as an important factor preceding outbreaks of symptomatic B virus in macaques [36–38]. A fall-winter pattern in B virus lesions was observed by Keeble [2], who believed that seasonal monsoons increased susceptibility of monkeys to infection. More frequent sampling of the cohort may have increased our success in identifying B virus recurrences and primary infections, but the stresses imposed by excessive capture and handling may have predisposed infected monkeys to such recurrences. Future studies may benefit from use of highly sensitive molecular techniques such as the polymerase chain reaction, which has proven capable of detecting small amounts of herpesvirus DNA in clinical specimens [39–42] and does not require the presence of viable specimens.

B virus was a common infection of low pathogenicity in NC4 rhesus macaques during this study. We observed only 1 symptomatic case of B virus disease in 157 monkeys examined over a 16-month period; however, B virus lesions that occurred only inside the mouth or on the genitals between examinations could have been missed, as they have been shown to heal without scarring or other evidence of their existence [1, 2]. We isolated B virus on only 14 occasions despite serologic test results indicating high prevalence and incidence rates of infection. Other investigators [15, 17, 43–46] have reported difficulty in detecting asymptomatic B virus recurrences from antibody-positive macaques. This result could be due to collecting specimens at inappropriate times and to the low multiplicity of infectious virus present in asymptomatic recurrences. Birth group, sex, and social dominance rank failed to predict B virus recurrences among seropositive monkeys. However, the small number of recurrent cases observed limited our power to identify such associations, if they existed.

Our study provides information regarding the distribution and determinants of B virus infection in a well-defined population of rhesus macaques in hopes of contributing toward efforts to eradicate the virus from breeding colonies. Combined virologic and serologic testing of monkeys may be warranted considering our observation of a primary B virus infection in an infant that failed to seroconvert over a 16-month period; all other primary cases in this study occurred in monkeys that seroconverted within 2 months. More information is needed regarding the sensitivity and specificity of available serologic tests for B virus diagnosis. Our results indicate that monkeys <2 years old should be selected for B virus–free colonies. Repeated testing of seronegative monkeys housed singly or in pairs during a quarantine period lasting at least 4 months should be sufficient to identify most infected monkeys that have not yet seroconverted before their release into new B virus–free breeding groups. The seasonality of B virus infection and recurrences observed in this study suggests that the optimal time for selection of uninfected juvenile monkeys from infected enclosures may be during the nonbreeding months.

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