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Retrospective Analysis of an Outbreak of B Virus Infection in a Colony of DeBrazza's Monkeys (*Cercopithecus neglectus*)

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Abstract | In 1981, an outbreak of herpetic disease developed in a colony of DeBrazza's monkeys (*Cercopithecus neglectus*). In seven of eight infected animals, clinical signs of infection included vesicular and ulcerative lesions on the lips, tongue, and/or palate. Histologic examination of lesions revealed intranuclear inclusion bodies, and electron microscopy revealed nucleocapsids and virions with typical herpesvirus morphology. Although a virus was isolated that appeared similar to monkey B virus, techniques available at the time did not allow precise identification of the virus. Analysis of serum from one surviving monkey collected 12 years after the outbreak revealed a pattern of reactivity characteristic of B virus-positive serum on the basis of results of ELISA and western immunoblot analysis. Polymerase chain reaction analysis of archived paraffin-embedded tissue specimens and molecular analysis of the one viral isolate obtained from a DeBrazza's monkey indicated that the virus responsible for the outbreak was a new genotype of B virus. Testing of sera from lion-tailed macaques (*Macaca silenus*) housed in an adjacent cage at the same zoo indicated that these animals harbored this virus and, thus, were the likely source of the virus that infected the DeBrazza's monkeys. This study documents usefulness of archiving samples from disease outbreaks for later analysis. In addition, this incident underscores the importance of considering herpes B virus infection when outbreaks of disease having characteristics of herpetic infections develop in nonhuman primates kept at institutions that also house macaques.

The herpesvirus family consists of three subfamilies: α-herpesviruses (simplex viruses), β-herpesviruses (cytomegaloviruses) and γ-herpesviruses (lymphotropic herpesviruses). The human herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and Cercopithecine herpesviruses 1 (*Herpesvirus simiae*; monkey B virus), 2 (simian agent 8; SA8) and 16 (*H. papio* 2; HVP-2) are members of the α-herpesvirus subfamily. Monkey B virus is indigenous in macaques (*Macaca* spp.), SA8 in African green monkeys (*Cercopithecus aethiops*), and HVP-2 in baboons (*Papio* spp.). In all of these monkeys, the natural history of their associated α-herpesviruses is similar to that of HSV in humans (1–4). Infections are usually asymptomatic or mild, with lesions largely being limited to oral and/or genital mucosal epithelial surfaces. During primary infection, α-herpesviruses establish latent infections in the host, usually in sensory ganglia. Reactivation of latent virus in response to diverse stimuli may result in recurrent lesions or asymptomatic shedding of virus in body fluids.

Although these viruses generally cause mild or inapparent infections in their natural host, several have been associated with severe infections when transmitted to other species. HSV-1 has been associated with a fatal infection in gibbon apes (2), and a related virus of squirrel monkeys, *Herpesvirus saimiri* 1, has caused fatal infections in marmosets and owl monkeys (3). Similarly, B virus has been found to cause severe and frequently fatal infections in colobus monkeys as well as humans (4–6).

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We describe an outbreak of a herpesvirus infection in a colony of DeBrazza's monkeys, and characterize the causative agent. Molecular analysis of the virus indicated that it represents a fourth genotype of monkey B virus, and was most likely transmitted indirectly to the DeBrazza's monkeys from a group of lion-tailed macaques.

Materials and Methods

Animals: A long-time colony of eight DeBrazza's monkeys (*C. neglectus*) was housed as a social group at the Woodland Park Zoo in Seattle, WA. Seven of the eight animals were born in the zoo and one (WD-16) was purchased from a commercial vendor in 1966 (15 years before the outbreak). Age ranged from 3 months to 20 years (Table 1). The colony was housed together in 3 interconnecting cages in the Primate House. The two adjacent cages on either side of the DeBrazza's monkey cage, one of which housed a group of ruff lemurs (*Lemur variegatus*) and the other a group of lion-tailed macaques (*Macaca silenus*), were separated by a solid concrete wall with no possibility for direct contact between the species. The DeBrazza's monkeys were fed Purina Monkey Chow supplemented with fruits and vegetables. The outbreak began February 12, 1981. On day 8 of the episode, all DeBrazza's monkeys were removed from the primate house, and animals with clinical signs of infection were housed individually. During the outbreak, all monkeys received antibiotic treatment (chloramphenicol, ampicillin and nystatin), ascorbic acid, and empiric treatment for clinical signs of disease.

Postmortem examination: The three monkeys that died were necropsied the day they died. Tissues were fixed in phosphate-buffered 10% formalin, embedded in paraffin, sectioned

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Table 1. Clinical signs of infection and progression of the herpesvirus outbreak

Monkey No.	Age (y)	Sex	Day of outbreak		Duration	Clinical signs				
			Onset of illness	Recovery or death	of signs (d)	Oral ulcers	Salivation	Anorexia	Skin lesions	Ocular lesions
WD-20	3 weeks	M	1[a]	6*	6	+	-	+	+	-
WD-1	20	F	3	10*	8	+	+	-	-	-
WD-19	1	F	8	38	21	+	+	-	+	+
WD-12	6	M	8	17	11	+	+	+	-	-
WD-13	5	M	10	17	9	+	+	-	-	-
WD-18	2	M	14	18	6	+	+	-	+	-
WD-8	8	F	14	18*	5	+	+	-	-	-
WD-16	3	M	-	-	-	-	-	-	-	-

[a] = Lesions were first noted on animal WD-20 on 2/12/81. This was designated as day 1 of the outbreak.
* = Animals that died.

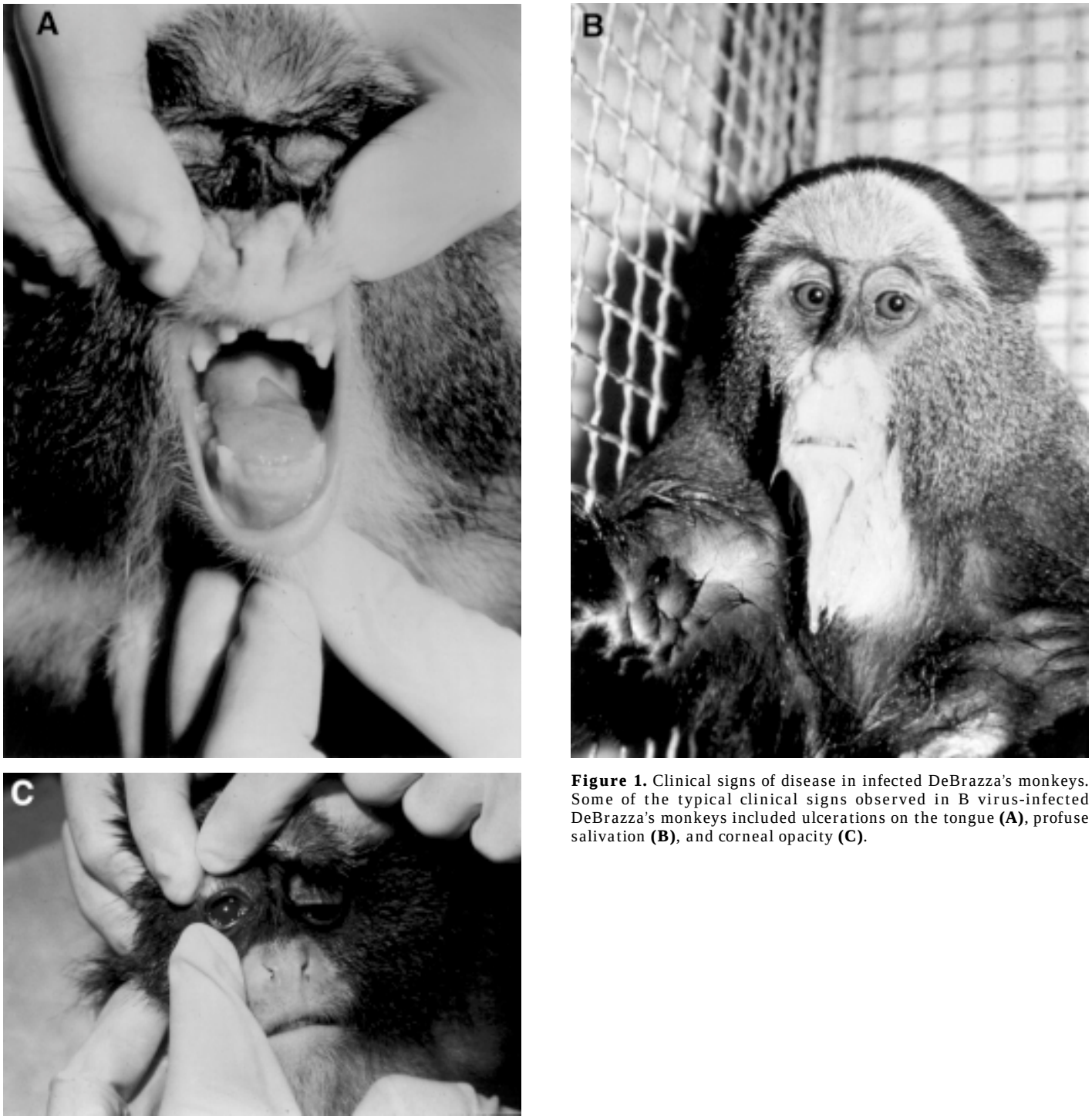

Figure 1. Clinical signs of disease in infected DeBrazza's monkeys. Some of the typical clinical signs observed in B virus-infected DeBrazza's monkeys included ulcerations on the tongue **(A)**, profuse salivation **(B)**, and corneal opacity **(C)**.

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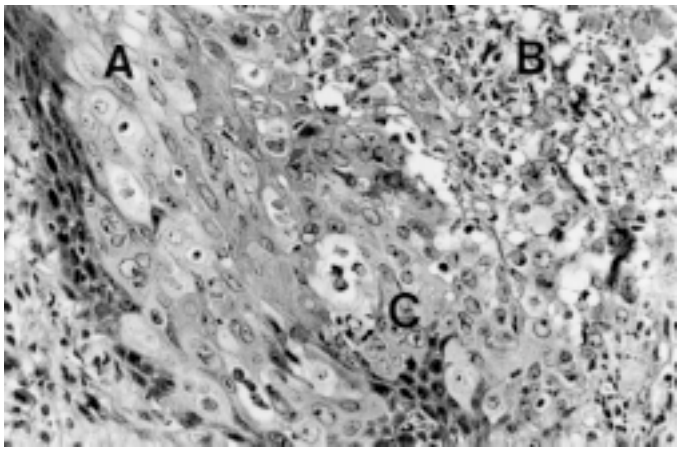

Figure 2. Histomicrograph illustrating the typical microscopic appearance of lesions observed in the oral and lingual mucosae. Notice mucosal epithelial cells have ballooning degeneration and peripheral margination of chromatin **(A)** surround a central region of necrotic cell debris **(B)**. Epithelial cells at the margin of the lesions are in various stages of degeneration and necrosis **(C)**. H & E stain. Magnification x40.

at 5-μm thickness, and stained with hematoxylin and eosin for microscopic examination. Tissue sections that contained intranuclear inclusions were stained with Fuelgen. For electron microscopy, 1-mm^3 sections were cut from paraffin blocks, deparaffinized in xylene, further fixed in osmium tetroxide, and embedded in plastic Epon. One-micron-thick sections were examined, and those selected for ultrathin sectioning were stained with uranyl acetate and lead citrate.

Virus isolation and serologic testing: To culture for unknown but clinically suspected viruses, Vero cells were seeded in 24-well trays and allowed to grow to 95% confluency. Clinical specimens were inoculated onto the cell monolayers and allowed to adsorb for 30 minutes at 37°C in a humidified 5% CO_2 incubator. After adsorption, monolayers were fed with Eagle's minimal essential medium supplemented with 1.5% fetal bovine serum. Plates were incubated at 37°C in 5% CO_2 for 8 days, with daily microscopic observation to notice any cytopathologic changes. Viruses used as reference standards included HSV-1 strain KOS, HSV-2 strain 186, HVP-2 strain OU1-76, SA8 strain B264, and monkey B virus strains E2490 (rhesus genotype), E90-136 (cynomologous genotype), and Kumquat (pigtail genotype) (7). The DeBrazza's isolate designated 8100812 was kindly provided by Dr. R. Heberling of Virus Reference Laboratories, San Antonio, TX.

At the time of the outbreak, acute and convalescent serum samples were obtained from three monkeys (WD-12, WD-18, and WD-19) at 0 to 7 and 56 to 63 days after onset of oral lesions, respectively (2/25/81 and 4/21/81). Sera were initially tested by use of a neutralization test for antibodies to HSV-1, HSV-2, B virus and SA8 and subsequently by use of an ELISA. Samples that tested positive for antibody reactivity by ELISA were confirmed for virus specificity by use of a competition ELISA and western immunoblot analysis as described (8, 9). Serum from the sole surviving monkey (WD-19) obtained 11 years after the outbreak also was evaluated by use of western immunoblot analysis and ELISA. New animals were not added to the DeBrazza's monkey collection between the time of the outbreak and euthanasia of this animal. Serum samples from lion-tailed macaques were collected at various times between 1995 and 1998; samples from animals at the time of the DeBrazza's monkey outbreak were not available. Although details of the dynamics of the macaque colony over the 20 years after the outbreak were not available, it is known that new animals were not introduced into the group from an outside source. These sera were tested by use of ELISA and competition ELISA as described (7).

Polymerase chain reaction: Polymerase chain reaction (PCR) testing of DeBrazza's monkey tissue specimens for α-herpesvirus DNA sequences were performed basically as described (10). Fixed, paraffin-embedded tissue sections cut during the original investigation were used to isolate DNA for PCR testing to determine which viral agent was present in the samples. Briefly, tissue blocks were cut and tissue digested overnight at 37°C after addition of proteinase K followed by inactivation at 95°C. Samples were microcentrifuged for 30 seconds to pellet any remaining undigested material, and 20 μl of the supernatant was used in a standard PCR test as described (10). HSV-1, HSV-2, B virus and SA8 DNA were used as controls. Primers BV-1 and BV-2 located within the UL28 (ICP18.5) coding sequence amplify a 128-bp product from all four viruses. Virus identification was subsequently made on the basis of differential susceptibility of the product to cutting by various restriction enzymes as described (10).

Analysis of DNA sequence: Viral DNA was purified from infected cells on NaI gradients. Purified viral DNA was digested by use of various restriction enzymes, and fragments were separated on agarose gels as described (7). Sequences from two regions of the viral genome were amplified by use of PCR and were directly sequenced as described (7). Sequence data for the lion-tailed macaque monkey B virus isolated from the DeBrazza's monkey have been deposited in GenBank (Accession No. AF241218). Phylogenetic analysis of DNA sequences was performed with analogous sequences of various B virus isolates, using the MEGA program package as described (7, 11).

Results

Clinical aspects of the outbreak: In February of 1981, a three-week-old DeBrazza's monkey (WD-20) was examined because of its inability to cling to its mother (day 1 of the outbreak). On examination, it was found to have ulcerations on the tongue, hard and soft palate, and hind feet (Figure 1A). It later developed severe diarrhea and facial edema, and died on day 6 due to septicemia. On day 3, the dam of this infant (WD-1) had a serous nasal discharge that progressed to a mucopurulent nasal discharge. The dam also developed ulcerated lesions on the tongue and the hard and soft palate. It became septicemic and died on day 10 of the outbreak. The infant's older sister (WD-19) had oral lesions and profuse salivation on day 8 (Figure 1B). This animal subsequently developed conjunctivitis, epiphora, and bilateral corneal opacity that lasted through day 38 (Figure 1C). On days 8 and 10, oral ulcerative lesions were observed on monkeys WD-12 and WD-13, respectively. On day 14, monkeys WD-8 and WD-18 developed ulcerative lesions of the tongue and hard and soft palate. Monkey WD-18 recovered, but WD-8 died 4 days later due to systemic infection. Clinical aspects and timing of the disease outbreak observed in seven of the eight DeBrazza's monkeys at Woodland Park Zoo are summarized in Table 1.

At the time of death, all three monkeys that died had multifocal, individual to coalescing, 1- to 3-cm-diameter oral and lingual erosions. The erosions had rough borders, a few of which progressed to

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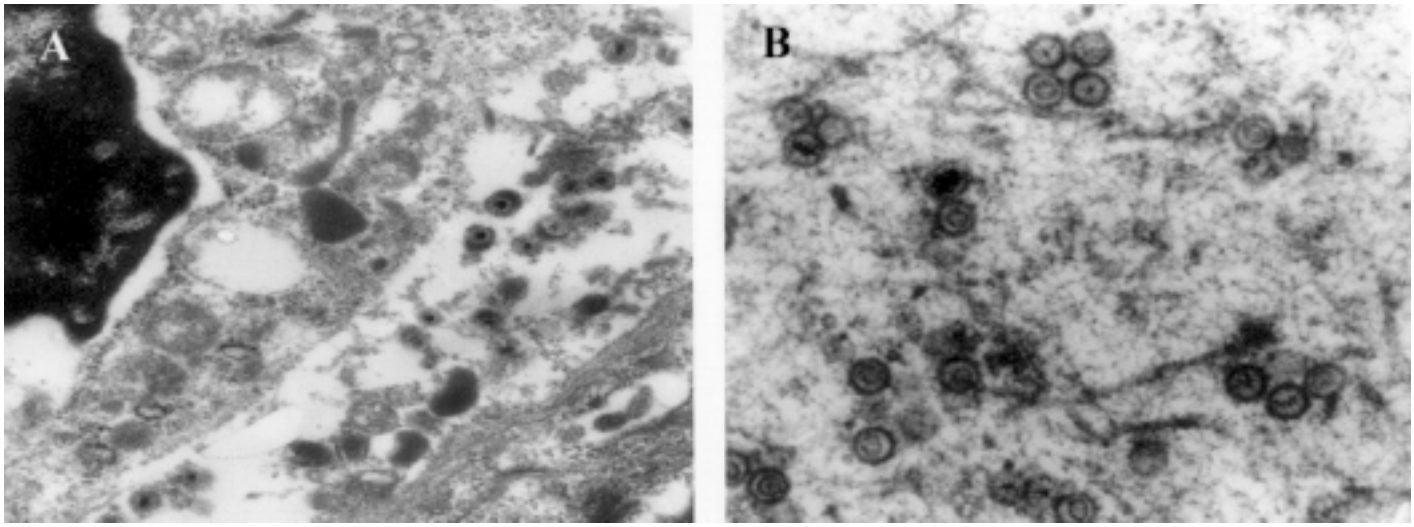

Figure 3. Electron micrograph showing intranuclear viral nucleocapsids **(A)** and cytoplasmic enveloped virions **(B)** present in a tongue lesion from a necropsy specimen collected during the course of the outbreak.

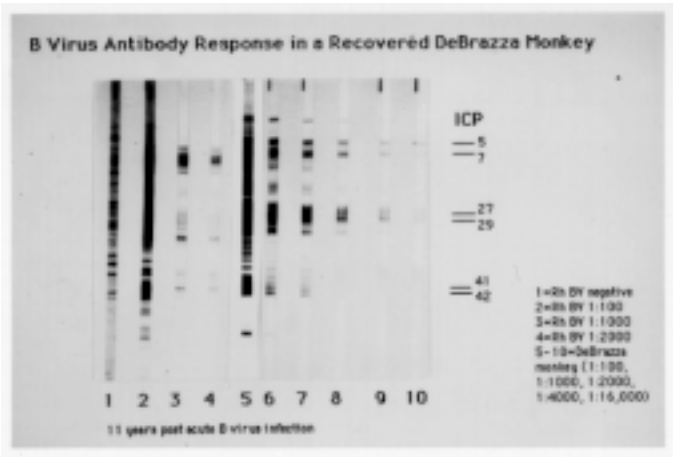

Figure 4. Western blot of serum from monkey WD-19 tested against B virus-infected cell polypeptides. Sera from a B virus-negative rhesus macaque, a B virus-positive rhesus macaque, and DeBrazza's monkey WD-19 were tested by immunoblot analysis against B virus-infected cell antigen. The B virus-infected cell polypeptides recognized by the positive sera are indicated at right.

Table 2. Virus neutralization by DeBrazza's monkey sera

Animal No.	Serum sample	Neutralization titer to: B Virus	HSV	SA8	Isolate
WD-12	Acute	<1:6	<1:6	ND	ND
	Convalescent	<1:6	1:12	<1:6	1:3
WD-18	Acute	1:6	1:6	ND	ND
	Convalescent	<1:6	1:12	1:6	1:3
WD-19	Acute	<1:6	1:24	ND	ND
	Convalescent	1:12	1:24	1:6	1:3

ND = Not done.

regions of central ulceration that were overlaid by a fibrinopurulent exudate. The histopathologic characteristics of the mucosal ulcers from necropsy and biopsy specimens were similar. Centrally, ulcers were filled with neutrophils, macrophages, lymphocytes, fibrin, and bacterial and necrotic debris (Figure 2). Nuclear swelling, margination of chromatin, and ballooning degeneration of mucosal epithelial cells were evident. Nuclei were filled with an amphoteric staining material, and multinucleated syncytia also were seen. Epithelial cells located at the ulcer edges were in various stages of degeneration and necrosis. There was a marked neutrophilic and lymphohistiocytic infiltrate within the submucosa. The two fatal adult cases (WD-1 and WD-8) had multifocal areas of adrenocortical necrosis. Inclusion bodies or syncytial cells were not observed in these lesions. Important lesions were not seen in the other tissues examined, including the CNS, brain, heart, testes, salivary glands, or any of the major blood vessels.

Identification of the viral agent: Electron microscopy of the ulcerative epithelial lesions revealed nuclear swelling, margination of chromatin, and viral nucleocapsids within nuclei of cells (Figure 3). These nucleocapsids were 95 to 103 nm in diameter and were either empty or contained electron-dense material. Nucleocapsids were usually solitary, but occasionally were seen in clusters. The cytoplasm and extracellular spaces contained large numbers of enveloped virions 120 to 180 nm in diameter that appeared as nucleocapsids with an additional electron-dense coating bounded by a unit membrane. The size, location within cells, and morphologic features were consistent with identification of the virus as a member of the herpesvirus family. Virus was isolated from swab and biopsy specimens of oral lesions from three monkeys (WD-8, WD-18, and WD-19). Growth characteristics of the viruses were consistent with those of α-herpesviruses, developing over a short time course, with rounding of infected cells and syncytia formation. Sera from these same three animals were tested by neutralization for antibodies against HSV-1, B virus and SA8, but results were equivocal (Table 2). None of the three monkeys had a substantial increase in titer to any of the viruses. One monkey (WD-19) had a twofold increase in titer to B virus, and the other two monkeys (WD-12 and WD-18) had a twofold increase in titer to HSV-1. Due to the extensive antigenic cross-reactivity among these viruses (3), it was not possible to determine the specific identity of the causative virus.

To identify the virus, PCR analysis was performed on specimens obtained at necropsy of the long-term survivor, WD-19. Three epithelial specimens, two adrenal sections, one kidney section, and one liver section were tested. Polymerase chain reaction products of the correct size (128 bp) were obtained from one adrenal and one epithelial tissue specimen (not shown). All other specimens were negative for herpesvirus DNA by use of PCR analysis. Both PCR products were cleaved by *Sac*1 into two

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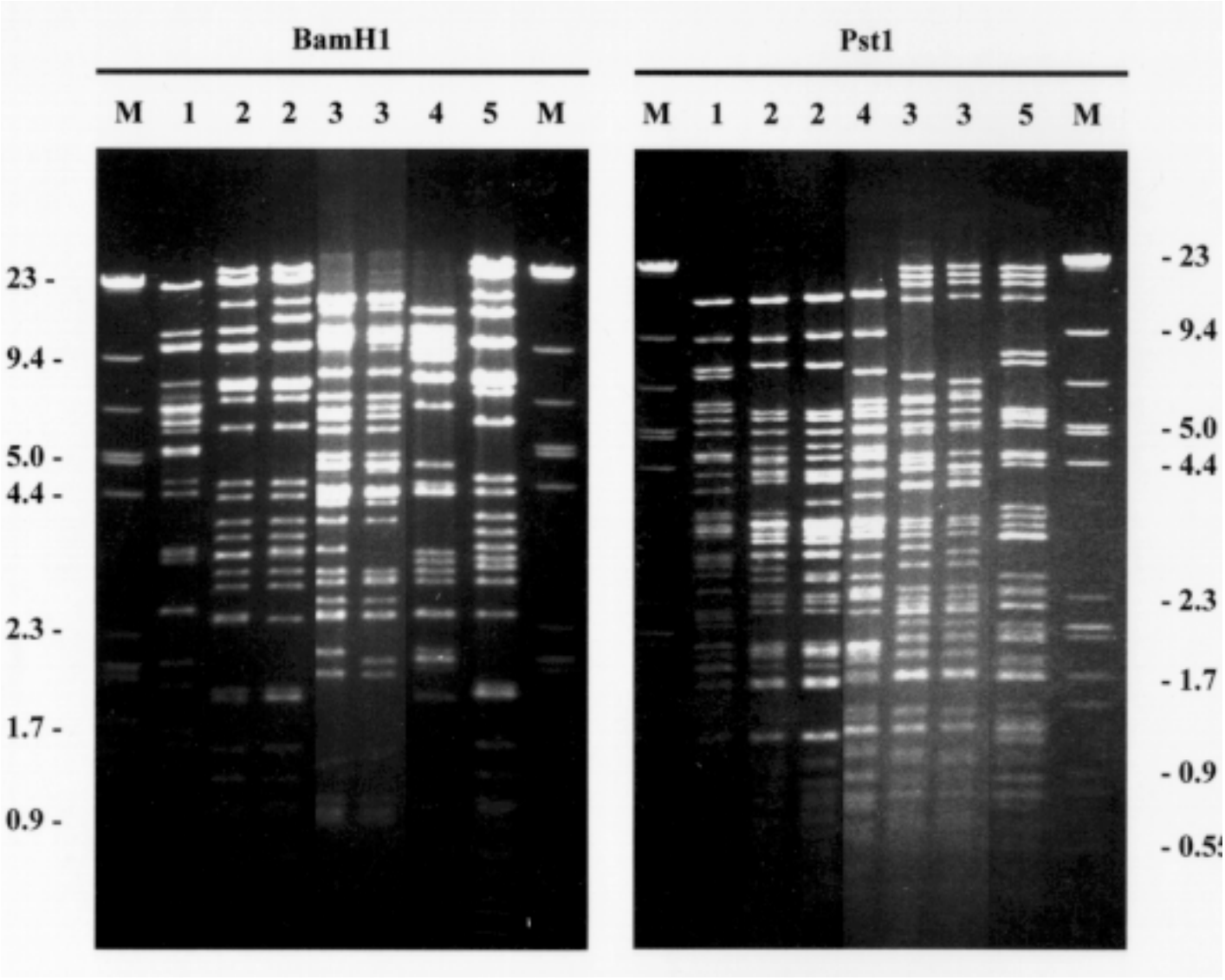

Figure 5. Restriction enzyme analysis of viral DNA. Viral DNA was purified from cells infected with B virus isolates from cynomologous (lane 1), rhesus (lane 2), pigtail (lane 3), or Japanese (lane 4) macaques or with the DeBrazza's monkey isolate 8100812 (lane 5). The DNA was cut with *Bam*H1 or *Pst*1 and was electrophoresed on agarose gels. Size markers (M) are indicated at right in Kbp.

fragments of 56 and 72 bp. This *Sac*1 cleavage pattern is diagnostic for B virus versus HSV-1, HSV-2 or SA8 (10), indicating that this animal was infected with B virus.

Retrospective analysis of serum from the one surviving monkey (WD-19) 12 years after the outbreak revealed a titer of 1:5,000 to B virus by use of ELISA. Using western immunoblot analysis, the specificity of this serum at a 1:100 dilution for B virus proteins was nearly identical to that of the control B virus-positive macaque serum (Figure 4). Further dilution of the serum indicated high amounts of antibody directed against B virus-infected cell polypeptides ICP5, 7, 27, 29, 41, and 42 (12). Amounts of antibodies directed against the major B virus glycoproteins (ICP7, 27, and 29) were particularly high in this serum, compared with those in the positive-control serum.

Molecular characterization of the virus: One viral isolate designated 8100812 was further characterized to establish with certainty its identity and relationship to other primate α-herpesviruses. Restriction enzyme cleavage of viral DNA from HSV-1, HVP-2, B virus and isolate 8100812 was performed. Using *Bam*-H1, the restriction cleavage pattern of 8100812 was considerably different from that of HSV-1, HSV-2, SA8, and HVP2, and from the reference B virus genotypes isolated from rhesus, cynomologous, and pigtail macaques as well (Figure 5). Cleavage of viral DNA with *Pst*1 indicated that, although minor differences were apparent, isolate 8100812 appeared similar to the pigtail macaque B virus genotype. Analysis of infected cell proteins and glycoproteins by use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed similar differences and similarities between isolate 8100812 and various B virus genotypes (Figure 6). Thus, although similar to other B virus isolates in many respects, the 8100812 isolate did not appear identical to any of the three known B virus genotypes.

To better determine the relationship of the DeBrazza's monkey virus isolate to other B virus isolates, a region from the UL27

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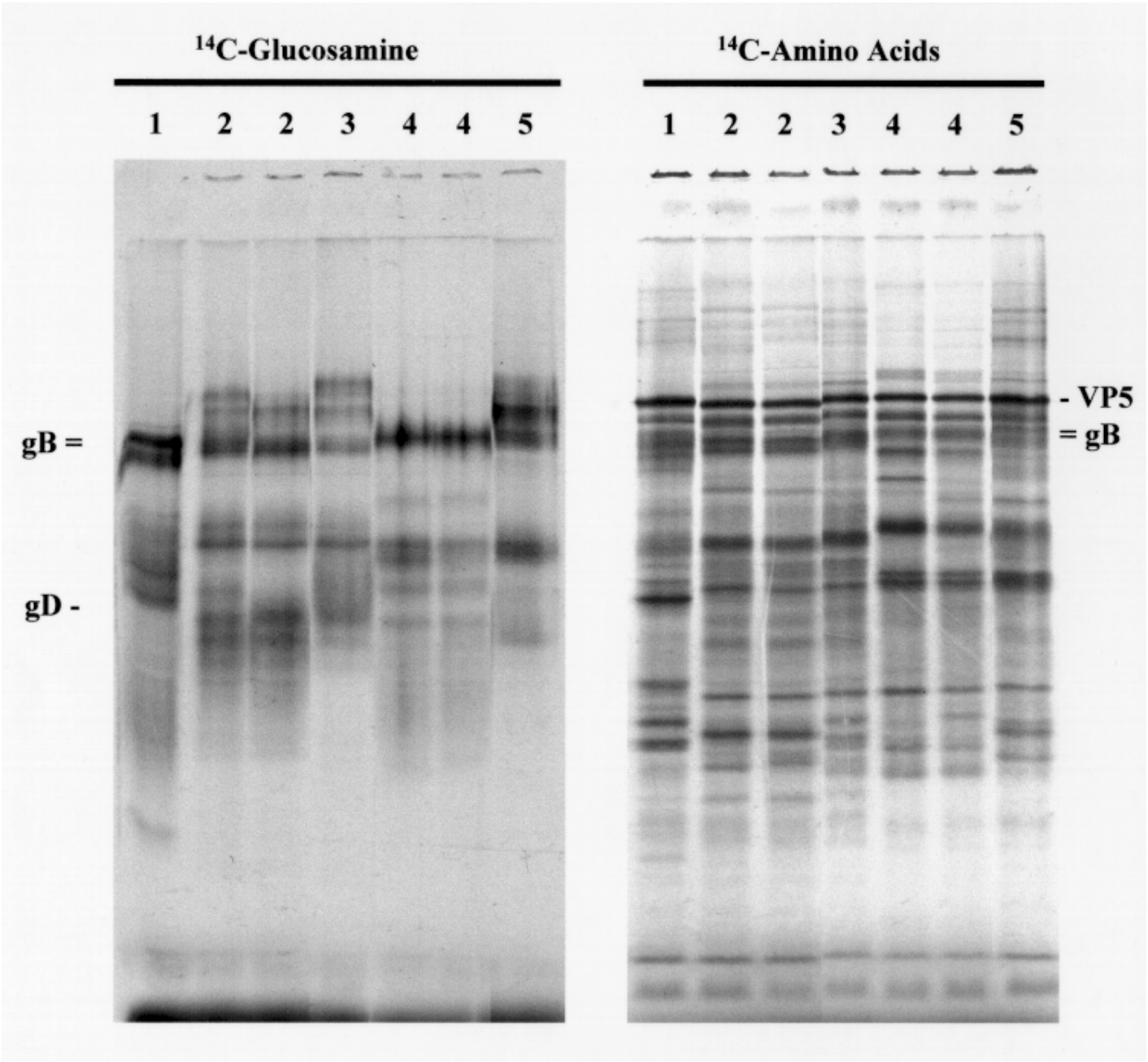

Figure 6. Comparison of viral infected cell proteins. Vero cells infected with the baboon virus HVP-2 (lane 1), B virus isolates from rhesus (lane 2), cynomologous (lane 3), pigtail (lane 4), or Japanese (lane 5) macaques, or the DeBrazza's isolate 8100812 (lane 6) and radiolabeled with ^{14}C-glucosamine or ^{14}C-amino acids from 4 to 24 hours after infection. Infected cell proteins were then separated by electrophoresis on 8% sodium dodecyl sulfate-polyacrylamide gels. Major viral proteins are indicated.

(glycoprotein gB) gene was amplified by PCR analysis and was sequenced. Although variable among different primate herpesviruses, this region of the UL27 gene is conserved within strains of a given virus (7). The sequence of this PCR product from isolate 8100812 had only minor variation from that of other B virus sequences. Phylogenetic analysis using homologous sequences from other primate α-herpesviruses confirmed the identification of isolate 8100812 as a strain of B virus (Figure 7A).

To determine the relative relationship of the DeBrazza's monkey isolate to various B virus genotypes, PCR amplification and DNA sequencing of a 1.3-Kbp section from the unique short region of the 8100812 viral genome extending from the 3' end of the US4 (gG glycoprotein) open reading frame (ORF) through the US5 (gJ glycoprotein) ORF and the 5' third of the US6 (gD glycoprotein) ORF was performed. The DNA sequence of 8100812 in coding and non-coding regions varied considerably from that of the three B virus genotypes previously reported (7). Phylogenetic analysis placed isolate 8100812 firmly within the B virus clade, being more closely related to B virus isolates from pigtail macaques than to isolates from rhesus, Japanese, or cynomol-

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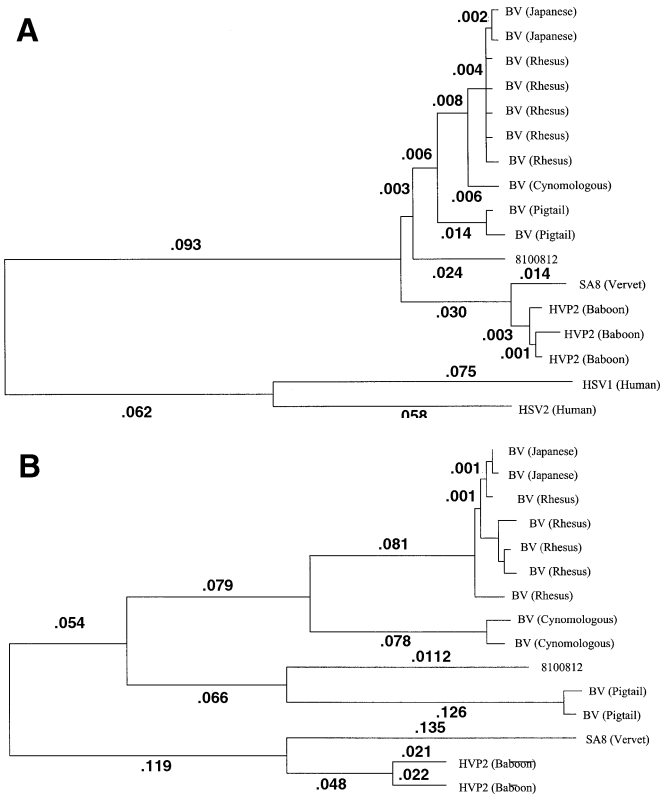

Figure 7. Phylogenetic analysis of primate α-herpesvirus DNA sequences. Approximately 430 bp of sequence from the UL27 (gB) gene **(A)** and 1.3 Kbp of sequence from the Us region of the genome spanning the US4-6 (gG, gJ, and gD) genes **(B)** were aligned, and phylogenetic analysis was performed, using Tamura-Nei distance and complete gap deletion. The Neighbor-Joining method was used to construct the trees shown.

gus macaques (Figure 7B). However, 8100812 was sufficiently distant from pigtail macaque B virus isolates that it represents a fourth genotype of B virus. This same tree topology was obtained when coding, non-coding, or total sequence data were analyzed, and when different algorithms were used to compute distances and/or construct trees.

Identification of the source of the virus. Since the infections in the DeBrazza's monkeys were so severe and since B virus is indigenous in Asiatic macaques rather than African cercopithecine monkeys, it was suspected that the virus may have been introduced into the DeBrazza's monkeys from a macaque species. Sera from a group of lion-tailed macaques housed in a cage adjacent to the Debrazza's monkey unit were, therefore, tested for B virus antibodies. Of 16 animals tested, 4 were strongly seropositive, raising the possibility that these animals could have been the source of the B virus that infected the DeBrazza's monkey colony.

The molecular analyses indicated that the DeBrazza's monkey isolate represented a new genotype of B virus. Although antigenically similar, different B virus genotypes have some genotype-specific antigenic determinants that can be used to identify them (7). Two of the positive lion-tailed macaque sera were, therefore, tested by cELISA to determine whether these animals were infected with this particular B virus genotype. As indicated (Figures 8A and 8B), the reactivity of lion-tailed macaque sera with either pigtail or rhesus B virus antigen was competed equally well with all three soluble B virus antigens. In contrast, the reactivity of lion-tailed macaque serum with DeBrazza's B virus antigen was more efficiently competed with soluble DeBrazza's B virus antigen than with either soluble pigtail or rhesus B virus antigens (Figure 8C). These results are consistent with the lion-tailed macaques being infected with a B virus genotype antigenically identical to the DeBrazza's monkey B virus isolate 8100812, and suggests that this isolate represents a B virus genotype indigenous to lion-tailed macaques.

Discussion

Evidence from each facet of the investigation indicated that the identity of the virus associated with the disease outbreak in the DeBrazza's monkeys was herpes B virus. Herpesvirus virions were visualized by electron microscopy, B virus DNA was detected in monkey tissues by PCR, and testing of serum for antibodies indicated B virus infection of the DeBrazza's monkeys. The fact that DNA was detected in adrenal and epithelial specimens from one monkey some eleven years after the disease outbreak suggests that B virus had remained latent in this animal, as there was no evidence that this was a primary infection. This is most interesting since the DeBrazza's monkey is a foreign host for this herpesvirus.

The source of B virus in this outbreak was not conclusively identified. Although the seven monkeys born and reared at the zoo had no known exposure to macaques, possible indirect contact with lion-tailed macaques in an adjacent cage cannot be excluded. Although clinical signs of herpetic disease were not observed in the lion-tailed macaques at the time of the outbreak, several of these animals had high titer of B virus antibodies when tested in 1998. Furthermore, evidence presented here suggests that these macaques harbor a genotype of B virus identical to that isolated from the DeBrazza's monkeys. Although direct contact between the lion-tailed macaques and the DeBrazza's monkeys does not appear to have been possible, indirect contact between the species cannot be ruled out. In unrelated studies we have found connections between infected animals visiting the same hospital location, as well as with handler-associated materials from contaminated areas (unpublished observations). Whether such transmission occurred in this zoo facility remains open to speculation.

The analyses in this report of B virus in DeBrazza's monkeys were not possible at the time of the outbreak; available technologies gave equivocal results despite strong suspicion of B virus infection. With the current availability of PCR, DNA sequencing, and more sensitive serologic techniques, it was possible to identify the agent responsible for this outbreak as a new genotype of B virus. This underscores the value of saving specimens from outbreaks where it is difficult to identify the pathogen involved.

The outbreak described here is similar to what was described in a colony of bonnet macaques in 1973 (13) and in 3 patas monkeys and a black and white colobus monkey in 1981 at another zoo (6). In those reports, the monkeys became ill over the course of 3 to 4 weeks, manifesting signs described as lethargy, conjunctivitis, respiratory dysfunction, diarrhea, and vomiting. In both of those reports, the virus was identified as a herpesvirus, but in the case of the patas monkeys and the black and white colobus, it was not possible to distinguish between HSV- 1, B virus, or a novel herpesvirus (6).

It should be noted that not all of the DeBrazza's monkeys that were infected with B virus in this outbreak succumbed to the infection. Since α-herpesviruses typically establish latent

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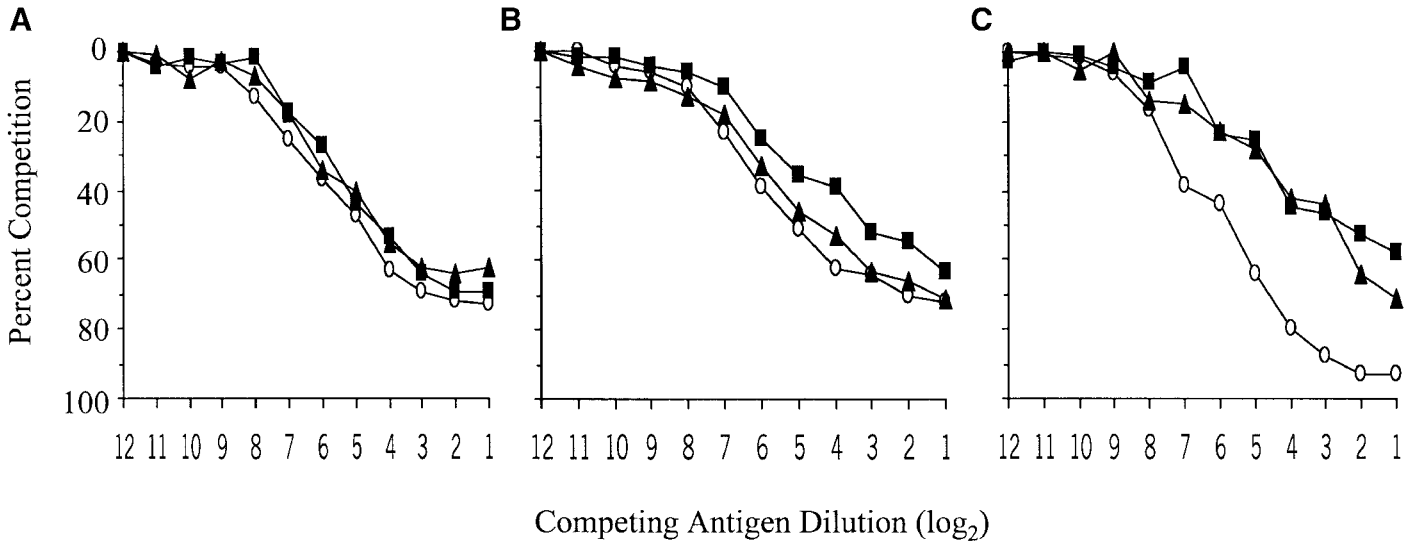

Figure 8. Competition ELISA of lion-tailed macaque sera. Soluble viral antigens were used to compete the reaction of serum from a B virus-positive lion-tailed macaque with viral antigens coated onto microtitration plate wells. Viral antigens coated onto the microtitration plates were **(A)** rhesus B virus; **(B)** pigtail B virus; and **(C)** DeBrazza's monkey isolate 8100812. Competing soluble viral antigens were rhesus B virus (△), pigtail B virus (□), or DeBrazza's monkey virus (❍).

infections in sensory ganglia after primary infection of mucosal surfaces, it is likely that surviving animals will remain infected with B virus and, thus, may shed infectious B virus intermittently, either asymptomatically or in association with clinically apparent recurrent lesions. Consistent with this, during routine sedation for a physical examination in 1993 (12 years after recovery), monkey WD-19 was observed to have oral ulcerations. The monkey was euthanized at that time, and tissues were tested by the B Virus Resource Laboratory at Southwest Foundation for Biomedical Research. Considering the lapse of time between the lesions and the original outbreak in the zoo animals (12 years), it appears that monkey WD-19 was latently infected and experienced reactivation of virus. Thus, any animals surviving B virus infections need to be handled with the same caution as macaques. Since such survivors represent potential sources of infectious B virus, serious consideration should be given to the practicality of maintaining such animals.

This outbreak of disease in DeBrazza's monkeys caused by B virus is important in several respects. Although not immediately recognized at the time, the infected animals manifested a classic clinical picture of a primary herpetic infection: ulcerating oral lesions, rapid onset, and some fatality. The high lethality associated with B virus in non-macaque species, including humans, reinforces the importance of recognizing potential α-herpesvirus infections in captive primates. Although B virus is indigenous to Asiatic macaque species, the appearance of herpes-like infections in other primate species in facilities where macaques are also housed should be considered as possible B virus infections and treated as such (14). Even though no human infections were associated with this outbreak, the potential exists for such infections to occur as a result of handling and treatment of diseased animals. Thus, it is important that personnel dealing with captive primates be aware of the signs of B virus infection and the potential zoonotic hazards associated with treatment of primates potentially infected with B virus.

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