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Original Research

Characterization of Baboon Cytomegalovirus Infection in Healthy Adult Baboons (*Papio anubis*)

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Cytomegalovirus (CMV) is a common chronic herpesvirus found in humans and numerous other mammalian species. In people, chronic viruses like CMV can alter overall health and immunity and pose a serious risk for those with an inadequate immune system. In addition, CMV plays an important role in animal health, and could affect the health of vulnerable populations, like endangered species. Previous studies found a high rate of CMV seropositivity among adult baboons (*Papio anubis*), and results from our laboratory revealed that baboon CMV (BaCMV) seropositivity was correlated with altered immune cell populations. In the current study, we further characterized BaCMV infection in normal, adult baboons. Analysis of blood samples from baboons (age, 6 to 26 y) revealed a low overall prevalence of detectable of BaCMV DNA, with a higher detection rate in aged baboons (older than 15 y). Furthermore, data suggest that individual baboons maintain similar rates of recurrence and levels of BaCMV shedding in saliva over time. Finally, we evaluated multiple commercially available assays for antihuman CMV IgG and IgM for use with baboon sera. Results of this study will improve our understanding of BaCMV and may be directly relevant to other closely related species.

Abbreviations: BaCMV; baboon cytomegalovirus; CIA, chemiluminescent immunoassay; CMV, cytomegalovirus; CPS, counts per second

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Cytomegalovirus (CMV) is a chronic betaherpesvirus. Once a host is infected with CMV, the virus can act in various tissues to cause a low-level, productive, chronic infection with continuous expression of viral proteins.[6] Primary human CMV infection typically occurs during childhood and usually is accompanied by no to mild and self-limiting symptoms in healthy persons. However, human CMV can cause significant complications in immunosuppressed people, including transplant recipients and persons with HIV.[26] In addition, congenital CMV infection is a major cause of hearing loss and impairment in vision, cognition, or motor function in children.[18] Furthermore, CMV is implicated in aging of the immune system, a process known as immunosenescence. It is thought that a lifetime of CMV recrudescence exposes the host immune system to viral antigenic challenge, which can eventually lead to reduced effectiveness of the immune system.[10]

In addition to human CMV, cytomegaloviruses are commonly found in numerous mammalian species, including many NHP.[1] Likewise, chronic viruses like CMV have implications regarding the health of animals, including in the conservation of endangered primates. For example, a study investigating an unusually high number of sudden deaths in multiple wild chimpanzee communities found evidence of bidirectional CMV transmission between chimpanzees and gorillas.[16] Moreover, the authors suggested that reactivation of or reinfection with CMV may exacerbate outbreaks of bacterial or viral diseases, creating a major threat to small, highly vulnerable populations.[16] Not only do captive NHP serve as important models for diseases and conditions that affect human populations, they also serve as important models for their wild counterparts.

Baboons are naturally infected with viruses homologous to many common viruses found in people, including baboon CMV (BaCMV), herpesvirus papio 1 (homologous to Epstein–Barr virus in humans), herpesvirus papio 2 (homologous to human herpes simplex virus), and simian varicella virus.[16,27] Baboon CMV is ubiquitous in both captive and wild baboon populations.[4] In the captive baboon (*Papio anubis*) colonies at the University of Oklahoma Health Sciences Center (OUHSC), the conventionally reared study colonies test positive for BaCMV by 3 y of age.[19,32] Baboon CMV was first isolated from the OUHSC baboon colony in 2001 and was found to be closely related to both human and rhesus CMV.[4] An additional outcome of that study was the development an inhouse ELISA to detect antiBaCMV IgG in OUHSC colony baboons, by using BaCMV isolates.[4]

In addition, our laboratory previously found that a high concentration of antiBaCMV IgG was negatively associated with populations of T-helper lymphocytes, supporting the thought that chronic infection with CMV may impair immune function over time.[31] However, our understanding of the key characteristics of BaCMV infection is incomplete. No studies have

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examined the detection rate of BaCMV in WBC, a key source of latent CMV in people,[1] or the rate and frequency of viral shedding in healthy, adult animals. Therefore, in the present study, we determined the rate of BaCMV DNA detection in an adult (age, 6 to 26 y) population of baboons. We also characterized BaCMV viral shedding in saliva samples obtained noninvasively from baboons over a 9-mo period. Furthermore, commercially available assays measuring antihuman CMV IgG were evaluated in comparison with the inhouse antiBaCMV IgG assay for their potential use with baboon sera.

Materials and Methods

Animals and sample collection. Adult baboons (*P. anubis*) were housed at the National Baboon Research Resource (Department of Comparative Medicine, OUHSC) and were under the care of 3 clinical veterinarians. Samples for the studies presented herein were collected at the National Baboon Research Resource between 2013 and 2016. All conventionally reared baboons are positive for BaCMV in this facility. To determine the frequency of BaCMV DNA in WBC in healthy adult (that is, older than 5 y) baboons, we evaluated a breeding troop. Male and female baboons were group-housed in large hierarchical groups of approximately 60 to 80 animals, which were designed to mimic breeding baboon troops found in the wild. Housing consisted of outdoor corrals with attached indoor group cages that included enrichment items for climbing, exercise, and play. The adult animals enrolled in the study included 34 female and 7 male baboons ranging in age from 6 to 26 y (mean age, 13 y), corresponding to a human age range of approximately 18 to 78 y.[22,24] The study population included 24 baboons younger than 15 y o and 17 baboons 15 y and older (the average lifespan of captive baboons is approximately 21 y[5]). Sex and social status (dominant, intermediate, or subordinate) were recorded for each baboon.

Venous blood samples were collected under anesthesia (ketamine, 10 mg/kg IM) from all adult baboons during routine semiannual tuberculosis testing and health checks, to avoid additional procedures. Whole blood was collected into vacuum phlebotomy tubes containing EDTA as an anticoagulant, and WBC were harvested for later isolation of DNA. Serum was collected from a clotted sample after centrifugation at 500 × *g* for 15 min. Serum and WBC were transported to the laboratory on ice and frozen at −80 °C until analysis. Baboons housed in small groups in an indoor facility (n = 2 or 3 animals per group) were used for the validation of antiCMV IgM assays in serum samples obtained from animals newly infected with BaCMV (n = 3) and for the longitudinal CMV viral shedding study (n = 5). The small-group animals were not housed in the outdoor, large-group facilities due to behavioral or health concerns. Small-group housing included enrichment that promoted natural behaviors of climbing, exercise, and play. For the validation of antiCMV IgM assays, former SPF baboons known to be negative for BaCMV (that is, 3 adult males; mean age, 10 y) were used. Serum samples were collected, as described earlier, before, 2 to 3 wk after, and 3 mo after primary infection with BaCMV infection. In addition, antiBaCMV IgG levels were measured before and after BaCMV infection, to confirm seroconversion to BaCMV. For the CMV shedding study, samples of serum and EDTA-treated blood were collected from adult, conventionally reared, CMV-positive baboons (that is, 1 adult female, 4 adult males; mean age, 12 y). Blood samples were collected at study initiation and study end, and collections were coordinated with the baboons' routine semiannual tuberculosis testing and health checks. In return for food rewards, buccal swabs (Toothette Oral Swab, Sage Products, Cary, IL) were collected noninvasively approximately every 3 mo for a total of 9 mo (from June through March of the following year) during enrichment training sessions. The diet for all study baboons consisted of a commercial monkey chow, fresh fruits, and vegetables. Water was provided without restriction. The research adhered to the provisions of the Animal Welfare Act and Animal Welfare Regulations[28] and the principles of the *Guide for the Care and Use of Laboratory Animals*.[14] All animal procedures were approved by the University of Oklahoma's IACUC.

Detection of BaCMV DNA in blood and saliva. DNA was extracted from WBC by using QIAamp DNA Blood Mini Kits (Qiagen, Hilden, Germany) according to the protocol provided by the manufacturer. DNAzol (Invitrogen, Carlsbad, CA) was used to extract of salivary DNA from buccal swabs, according to the manufacturer's protocol. The Qubit dsDNA BR assay kit and fluorometer (Life Technologies, Carlsbad, CA) was then used to determine DNA concentrations. Baboon CMV copy number in saliva was determined by *Taq*Man real-time PCR analysis (Applied Biosystems, ThermoFisher, Waltham, MA) by using a standard curve method. To generate the standard curve, a 524-bp fragment of BaCMV was cloned into the TOPO TA vector (Invitrogen) according to the manufacturer's protocol. Serial dilutions of the plasmid DNA template ranging from 300,000 to 30 copies were used to generate the calibration curves for the *Taq*Man PCR assays. The BaCMV-specific primer pair and *Taq*Man probe sequence were: forward, 5′ GTT TAG GGA ACC GCC ATT CTG 3′; reverse, 5′ GTA TCC GCG TTC CAA TGC A 3′; and *Taq*Man probe, 5′ 6FAM–TCC AGC CTC CAT AGC CGG GAA GG–TAMRA 3′. The *Taq*Man assay was validated and optimized by using baboon salivary DNA samples; optimal concentrations were 200 nM of probe, 900 nM of each primer, and 40 ng of starting DNA template. All PCR reactions were performed by using a model 7500 Real-time PCR System (Applied Biosystems, Foster City, CA), and a positive control was included in each run. To confirm the identities of the PCR product and cloned fragment, samples were separated by gel electrophoresis, gel-purified, and sequenced. Sequences were confirmed as BaCMV by using the BLAST database.

In addition, BaCMV DNA in WBC was assessed by using a nested PCR method. The assay contained 100 ng of starting DNA template, and the conditions for the first round of PCR were 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 50 °C for 45 s, and 72 °C for 1 min. A presence–absence assay using TaqMan real-time PCR analysis was then completed on the first-round PCR product. The assay was validated by using positive-control samples from primary BaCMV-infected animals.

Measurement of antiCMV IgG and IgM. Three commercial assays for measuring human antiCMV IgG were assessed for use in baboons and compared with our previously validated inhouse assay for measurement of antiBaCMV IgG:[4,19] CMV IgG ELISA (DRG International, Springfield, NJ; IgG ELISA 1), CMV IgG ELISA (Gold Standard Diagnostics, Davis, CA; IgG ELISA 2) and CMV IgG Chemiluminescent Immunoassay (Immulite 1000 CMV IgG, Siemens, Los Angeles, CA; IgG CIA). Assays were performed as suggested by manufacturers, with modifications: for comparison with our inhouse antiBaCMV IgG assay, where outcome is expressed in OD units,[4,19,31] ELISA values were not converted to the arbitrary units of the commercial assay standard curves but instead reported as OD units. We also assessed 2 commercial assays for measuring human anti-CMV IgM—CMV IgM ELISA (Gold Standard Diagnostics; IgM ELISA) and Immulite 1000 CMV IgM (Siemens; IgM CIA)—for use in baboons. Commercial assays for antihuman CMV IgM

were performed according to the manufacturers' directions. Assay validation of human ELISA for use in baboon serum was determined through parallelism between diluted pooled baboon samples and a standard curve (extended to 6 or 7 standards), percentage recovery, and linearity of serial diluted baboons samples spiked with known concentrations. Appropriate human Immulite assays were validated through percentage recovery and linearity of serially diluted baboons samples spiked with known concentrations of antiCMV IgG and IgM. Banked serum samples from SPF baboons, negative for BaCMV,[32] were used as negative controls (n = 18). For all assays, the inter- and intraassay coefficients of variance were below 15% and 10%, respectively.

Statistical analyses. Data were tested for normality and homogeneity of variance and were transformed when needed. Repeated-measures ANOVA and the Tukey HSD test for multiple comparisons were used for longitudinal data. CMV IgG assays were compared by using Pearson correlation coefficients and a Bland–Altman difference plot was used to determine agreement between assays. For validated assays, baboon samples were considered positive for antiCMV IgG or IgM when values were more than 2 or 3 SD above the raw-count mean (OD units or counts per second) of known BaCMV-negative baboon serum samples (n = 18 BaCMV-negative samples). Statistical significance was defined as a P value less than 0.05. JMP 7 Statistical Discovery (SAS Institute, Cary, NC) was used for all statistical analyses.

Results

Frequency of BaCMV DNA detection in WBC and characterization of BaCMV shedding. In the total population of 41 adult baboons, 4 animals (9.8%) tested positive for BaCMV DNA in blood (Table 1). BaCMV DNA was detected in only 1 (4.2%) of the 24 baboons younger than 15 y. However, aged animals (15 y or older) in the population exhibited a higher proportion of BaCMV detection in WBC (3 of 17 animals; 17.6%). Of the 4 baboons with detectable BaCMV DNA in blood, 2 were dominant males (8.0 and 17.9 y old), and 2 were subordinate females (15.6 and 26.4 y old; Table 1).

In the longitudinal BaCMV shedding study (Table 2), BaCMV DNA was detected in 17 of 20 (85.0%) saliva samples obtained during the 4 noninvasive buccal swab collections from small-group-housed, BaCMV-positive baboons (n = 5). Although there was some variation in CMV viral load over time within animals, 3 of the 5 baboons that shed BaCMV at the initial collection time point shed virus at all other periods examined. Mean BaCMV viral load did not differ significantly between baboons, but the 2 animals with the lowest mean viral load did not shed virus consistently. In addition, the inconsistent shedders exhibited the lowest mean levels of serum antiCMV IgG; however, one inconsistent shedder (animal 5) did not have a significantly different antiCMV IgG concentration compared with the other 3 baboons (Table 2). No BaCMV DNA was detectable in WBC from the pre- or poststudy blood samples from any baboon included in the viral shedding study, and no effect of season was observed in this study group (data not shown).

Measurement of antiCMV IgG and IgM. The IgG CIA was not suitable for use with baboon sera. However, IgG ELISA 1 was validated for use in baboons and was compared with our inhouse antiBaCMV IgG ELISA. The mean OD reading of BaCMV negative-control samples was 0.092 ± 0.050 unit (n = 18), and a cutoff value of 0.192 OD unit (negative control mean + 2 SD) was used for CMV IgG-positive samples. The Pearson correlation coefficient indicated that results from the 2 assays were strongly correlated (Figure 1 A; n = 41; r = 0.7; $P < 0.0001$). In addition, IgG ELISA 2 was validated for use with baboon sera. The mean OD reading of the BaCMV negative-control samples was 0.038 ± 0.023 unit (n = 18), and a cutoff value of 0.108 OD unit (negative control mean + 3 SD) was used to determine CMV IgG-positive samples. In contrast to IgG ELISA 1, IgG ELISA 2 showed poor correlation with the BaCMV assay (Figure 1 B; n = 41; r = 0.3; $P < 0.0001$).

Bland–Altman plot analysis was used to assess agreement between the inhouse antiBaCMV and commercial antihuman CMV IgG assays. Comparison of the BaCMV assay with IgG ELISA 1 revealed a bias of –0.04, with –0.32 as the lower limit of agreement and 0.24 as the upper limit of agreement (Figure 2 A; n = 41). The slight negative bias appeared to due to measurements exceeding 0.5 OD unit (Figure 2 A). The magnitude of the systematic difference was estimated as a –0.086 to 0.008 95% CI of the mean difference, whereas 95% CI of agreement limits, as an estimate of the size of potential sampling error, were –0.040 to –0.0241 for the lower limit of agreement and 0.163 to 0.325 for the upper limit. In addition, all samples positive for antiBaCMV IgG according to the inhouse ELISA were more than 2 SD above the mean OD reading of the negative controls in IgG ELISA 1.

The bias for the comparison of the BaCMV with IgG ELISA 2 was –0.15, with –0.54 as the lower limit of agreement and 0.23 as the upper limit of agreement; the moderate negative bias was likely due to measurements exceeding 0.35 OD unit (Figure 2 B; n = 41). The 95% CI of the mean difference was –0.215 to 0.090, and 95% CI of agreement limits were –0.644 to –0.428 and 0.122 to 0.339 for the lower and upper limits of agreement, respectively. All samples in the dataset that were positive for antiBaCMV IgG were more than 3 SD above the mean OD value of the negative controls in IgG ELISA 2.

Previously there were no validated assays for measurement of antiCMV IgM in baboon sera; therefore, we examined the feasibility of commercial antihuman CMV IgM assays for use with baboons. The commercial IgM ELISA was validated for baboon sera (Table 3). For human sera, the index value for positive antiCMV IgM antibodies is 1.1 units or greater; and our expected positive CMV IgM samples collected at 2 to 3 wk after primary BaCMV infection (n = 3) all tested positive for IgM antibodies according to the cutoff values for normal human serum (Table 3). Both preinfection samples and the 3-mo postBaCMV infection samples tested negative for antiCMV IgM. The mean calculated value of known BaCMV-negative samples was 0.429 ± 0.118 U (n = 18).

When the IgM CIA was assessed, all baboon sera tested as nonreactive for antiCMV IgM according to the cutoff values for normal human serum samples. However, this assay was suitable for use with baboon sera according to a negative cutoff value set 2 SD above the raw-count (in counts per second; cps) mean of known BaCMV-negative baboon serum samples (n = 18). The baseline mean of the 18 known BaCMV negative samples was 228,561 cps (Table 4); values for 1 SD and 2 SD were 36,139 and 72,278 cps, respectively, with the negative cutoff for antiCMV IgM in baboons as 300,838 cps (and below). According to this calculated cutoff value, samples collected at 2 to 3 wk after primary BaCMV infection (n = 3) all tested positive for antiCMV IgM antibodies, whereas the preinfection and 3-mo postinfection samples tested negative for antiCMV IgM (Table 4).

Discussion

Although human CMV is generally thought of as a benign chronic virus, it has major implications in immunosuppressed

Table 1. Frequency of detection of baboon CMV (BaCMV) DNA in WBC from adult baboons (age, 6 to 26 y)

		BaCMV-positive WBC		Details regarding animals with BaCMV-positive WBC		
Age (y)	No. of animals	No. of animals	Proportion of population with BaCMV+ WBC	Sex	Age	Social status
< 15	24	1	0.042	Male	8 y	Dominant
≥ 15	17	3	0.176	1 male 2 females	17.9 y 15.6 and 26.4 y	Dominant Both subordinate
Total	41	4	0.098			

In the study population, male and female baboons were group-housed in large hierarchical groups (approximately 60 to 80 animals), which were designed to mimic breeding baboon troops found in the wild. Housing consisted of outdoor corrals with attached indoor group cages that included enrichment items. The study population comprised 34 females and 7 males ranging in age from 6 to 26 y (mean age, 13 y), corresponding to a human age range of approximately 18 to 78 y.

Table 2. Characterization of baboon CMV (BaCMV) shedding over time

			No. of BaCMV DNA (copies /µL)					Mean antiCMV IgG level
Animal	Age (y)	Sex	Sample 1	Sample 2	Sample 3	Sample 4	Mean ± SEM	(OD unit)
1	12	Male	667.2	ND	ND	ND	667.21	0.1455[a]
2	11	Male	15728.3	8717.2	579.6	480.1	6376.3 ± 3666.4	0.6560[b]
3	8	Male	7035.3	7790.7	2270.0	435.4	5087.1 ± 1795.7	0.7285[b]
4	12	Male	1685.4	20934.0	14564.5	13167.7	12587.9 ± 4008.0	0.6500[b]
5	18	Female	3181.1	1076.9	ND	ND	2129.0	0.5445[b]

ND, not detected
Baboons were housed in small groups in an indoor facility that included enrichment and promoted natural behaviors. The small-group animals were not housed in the large-group facilities due to behavioral or health concerns. Buccal swabs were collected noninvasively during enrichment training sessions in return for a food reward at approximately every 3 mo for a total of 9 mo (that is, 4 collections from each of 5 baboons)
Different lowercase letters indicate significant differences ($P < 0.05$) between groups.

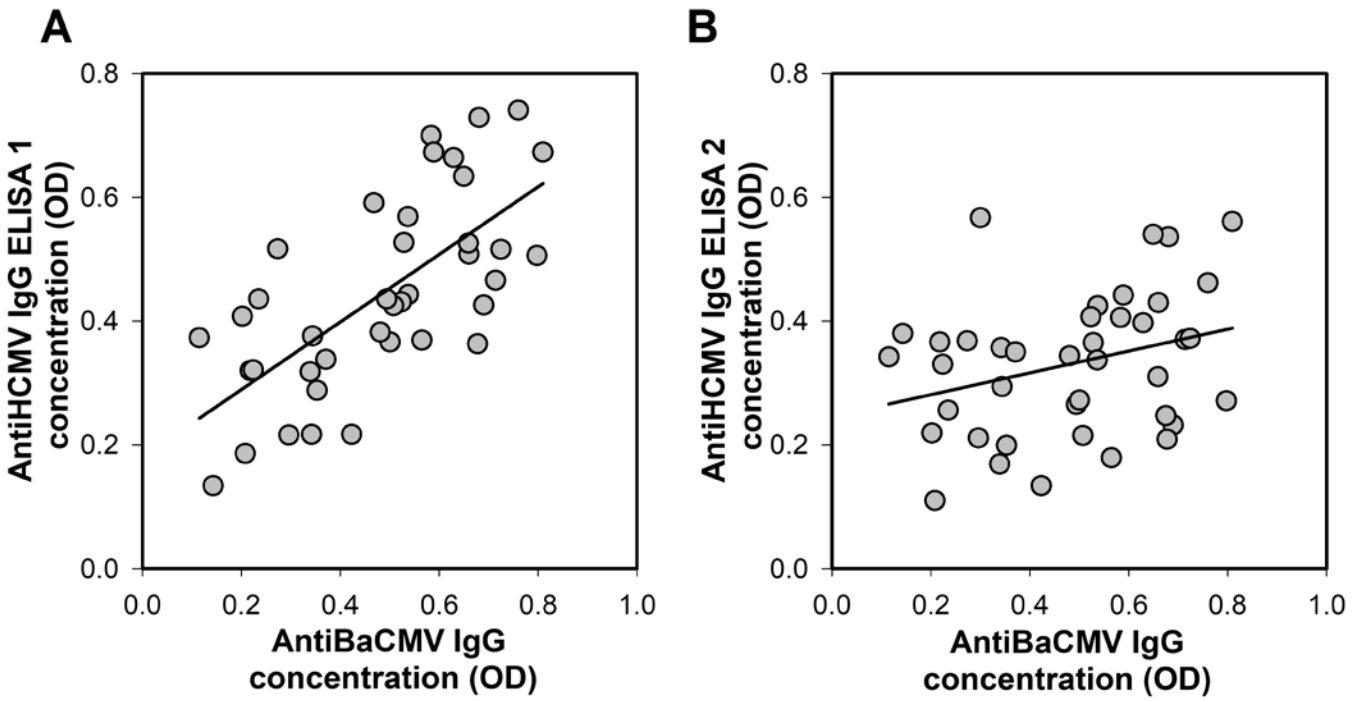

Figure 1. The relationship between the antiBaCMV IgG ELISA and commercially available antihuman CMV IgG ELISA ($n = 41$). (A) The correlation between the antiBaCMV IgG assay and IgG ELISA 1 (r = 0.7; $P < 0.0001$). (B) The correlation of the antiBaCMV IgG assay with IgG ELISA 2 (r = 0.3; $P < 0.0001$). HCMV, human CMV.

patients, in neonatal infants, and during the aging process. In addition, chronic viruses have important roles in the health of various animals, including NHP and endangered species. Characterizing pathogens with which endangered species are infected, including chronic viruses like CMV, is important for assessing the risks that may affect endangered populations and their conservation.[16,21] To date, the majority of research on NHP CMV has been done in rhesus macaques, due to the close

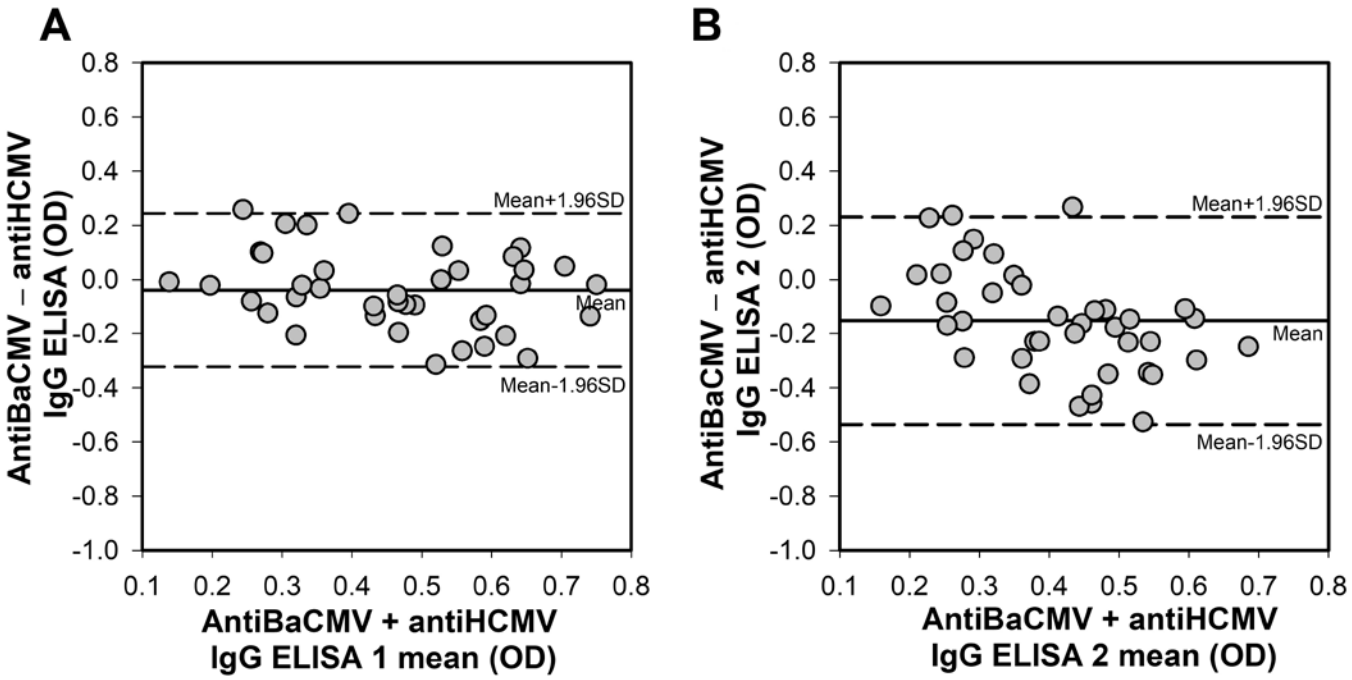

Figure 2. Agreement between the antiBaCMV IgG ELISA and commercially available antihuman CMV IgG ELISA (Bland–Altman plot analysis; n = 41). (A) The agreement of the antiBaCMV IgG assay with IgG ELISA 1. Assay comparison revealed a bias of –0.04, with -0.32 as the lower limit of agreement and 0.24 as the upper limit of agreement. The 95% CI of the mean difference was –0.086 to 0.008, and 95% CI of agreement limits were –0.040 to –0.024 and 0.163 to 0.325 for the lower and upper limits, respectively. (B) The agreement of the antiBaCMV IgG assay with IgG ELISA 2. Assay comparison revealed a bias of –0.15, with –0.54 as the lower limit of agreement and 0.23 as the upper limit of agreement. The 95% CI of the mean difference was –0.215 to 0.090, and 95% CI of agreement limits were –0.644 to –0.428 and 0.122 to 0.339 for the lower and upper limits, respectively. HCMV, human CMV.

similarity between rhesus CMV and human CMV and the accessibility of rhesus macaques as an animal model for people.[15] Moreover, captive baboons provide excellent models, for both other NHP and humans, in which to study chronic pathogens. Previous studies have reported some of the characteristics of BaCMV,[19,31,32] but further examination of CMV in baboons is needed. Therefore, the current study was designed to further characterize CMV DNA and viral shedding in baboons and to assess the feasibility of commercially available antihuman CMV Ig assays for use in baboons.

In humans and rhesus macaques, detectable CMV DNA is generally cleared from blood after primary infection.[17,34] Although monocytes are considered a source of CMV latency, CMV DNA is not frequently detected in blood of either healthy people or rhesus macaques.[2,3,11,20,27,30,33] Similarly, the present study found that BaCMV is detected at a rate of less than 10% in the WBC of adult baboons. However, the detection of BaCMV DNA in blood was increased in baboons 15 y of age and older compared with younger baboons (17.6% compared with 4.2%). The age-related increase in the detection rate of BaCMV DNA in WBC observed in the current study is consistent with studies of human CMV DNA detection in blood donors, wherein lower levels of CMV DNA detection have been found in young blood donors when compared with elderly donors (that is, 60 y of age and older).[11] Previously, baboon social status was found to affect antiBaCMV IgG concentration.[31] Of the 4 baboons that tested positive for BaCMV DNA in WBC, 2 were dominant males and 2 were subordinate females. It is possible that with the inclusion of more baboons, a difference between CMV detection rates might be observed between socially high-ranking and subordinate animals, but no difference was observed in the current study.

The transmission of CMV is typically through close contact with infected bodily fluids, such as saliva, blood, and urine.[7]

Table 3. Measurement of antiCMV IgM in baboons with primary BaCMV infection by using the commercial IgM ELISA

Animal	Time point	Calculated value	Relative to cutoff value
1	preinfection 1	0.226	negative
2	preinfection 1	0.159	negative
3	preinfection 1	0.265	negative
1	preinfection 2	0.351	negative
2	preinfection 2	0.382	negative
3	preinfection 2	0.313	negative
1	3 wk postinfection	1.709	positive
2	2 wk postinfection	1.110	positive
3	2 wk postinfection	1.761	positive
1	3 mo postinfection	0.465	negative
2	3 mo postinfection	0.598	negative
3	3 mo postinfection	0.545	negative
CMV IgM-negative samples (n = 18; mean ± SD)		0.429 ± 0.118	

Serum samples were collected before, at 2 or 3 wk after, and at 3 mo after primary BaCMV infection. Banked samples from SPF baboons (negative for BaCMV) were used as negative controls.

Salivary glands are a known site of CMV replication and latency, and it has been proposed that salivary glands are an independent site of CMV latency from mononuclear cells in other species.[9,23] In people, the percentage of adults who shed CMV in saliva varies between 9% and 23%,[7] with increased shedding

Table 4. Measurement of antiCMV IgM in baboons with primary BaCMV infection by using the commercial IgM chemiluminescent assay

Animal	Time point	cps	Relative to cutoff value
1	preinfection 1	215,147	negative
2	preinfection 1	228,839	negative
3	preinfection 1	204,223	negative
1	preinfection 2	254,376	negative
2	preinfection 2	204,404	negative
3	preinfection 2	194,774	negative
1	3 wk postinfection	1,380,763	positive
2	2 wk postinfection	377,845	positive
3	2 wk postinfection	1,247,871	positive
1	3 mo postinfection	224,812	negative
2	3 mo postinfection	288,174	negative
3	3 mo postinfection	168,658	negative
CMV IgM-negative samples (n = 18; mean)			228,561 cps
Baseline 1 SD			36,139 cps
Baseline 2 SD			72,278 cps
Baseline mean + 2 SD			300,838 cps

cps, counts per second
Serum samples were collected before, at 2 or 3 wk after, and at 3 mo after primary BaCMV infection. Banked samples from SPF baboons (negative for BaCMV) were used as negative controls.

in children (with probable primary infection) when compared with adults.[7,25] Studies in rhesus macaques have similarly found that juvenile animals shed rhesus CMV at a higher rate than do adult animals, but the percentage of adult macaques that shed virus in saliva (approximately 40% to 71%) is higher than what has typically been observed in people.[2,13] In addition, a study of viral shedding of human CMV in young children found that those shedding a high viral load were more likely to continue to shed virus consistently; in contrast, seropositive children who were inconsistent shedders were still capable of shedding CMV periodically but typically at a reduced viral load.[8] Therefore, we wanted to assess BaCMV shedding among healthy adult baboons to determine whether individual animals maintain a similar shedding frequency and amount over time, such that a single sample might be useful for identifying high shedders within the population. The current study revealed an extremely high rate of shedding among adult baboons. However, 2 of the 5 baboons shed virus inconsistently during the study period. Interestingly, the 2 baboons that shed BaCMV inconsistently had the lowest mean viral copy numbers and the lowest mean antiCMV IgG concentrations. Taken together, the results indicate that adult baboons appear to shed CMV in saliva at a high rate, similar to what has been found in rhesus macaques.[2,13] Furthermore, these data also indicate that the pattern of BaCMV shedding in saliva is similar to the pattern found in young children,[8] wherein baboons shedding a high viral load are more likely to consistently shed a high level of virus. Therefore, saliva should be considered to a major source of BaCMV transmission and reinfection in all baboons.

After primary infection with CMV, CMV IgG antibody is persistently secreted, whereas CMV IgM is secreted only during early CMV infection, and its detection is suggestive of active, acute, or recent infection.[16,29] The commercially available IgG ELISA 1 kit for antihuman CMV IgG showed strong correlation and good agreement, as assessed through Bland–Altman analysis, with our inhouse antiBaCMV IgG ELISA, and no discordant measurements were observed. Although the small bias (–0.04) appeared to be due to samples with measurements of 0.5 OD unit and above, the overall agreement and assessment indicate that measurements can be considered interchangeable between methods, with measurements expressed as OD units. Whereas the antihuman CMV IgG ELISA 2 was appropriate for use with baboon sera, it showed poor correlation to the antiBaCMV IgG ELISA. Furthermore, although the agreement between the 2 assays was adequate and there were no discordant measurements, we would not consider measurements from the inhouse baboon assay and IgG ELISA 2 to be interchangeable. Consequently, we suggest that baboon and potentially other NHP samples measured by using IgG ELISA 2 should be assessed relative to each other and not directly compared with measurements from the inhouse baboon assay or IgG ELISA 1.

Both commercial kits for measurement of antihuman CMV IgM examined were suitable for use in baboons. Although the 3 primary BaCMV-infected baboons all tested positive for anti-CMV IgM according to the cutoff value for normal human sera (determined as the mean + 3 SD), our data suggest that baboons may have a slightly lower cutoff of approximately 0.783 U, according to the mean + 3 SD of negative-control baboon samples (0.429 ± 0.118 U). Likewise, the IgM CIA was appropriate for baboon serum samples, with a negative cutoff value according to the mean of negative-control baboon sera + 2 SD. The commercially available assays for antihuman CMV IgG or IgM examined in the current study offer many benefits, including ease of use, short completion time, and full automation. Furthermore, the assays may be easily adaptable to apes and to other Old World species.

In summary, we found that, although the overall detection of CMV DNA in blood was low, healthy aged baboons have a higher prevalence of detection in blood, similar to what has been found in people. These data provide evidence that baboons shedding high levels of BaCMV are likely to be identified by using a single measurement. The results also indicate that baboon saliva can provide a source of viral transmission even during adulthood, long after natural primary infection with BaCMV (which occurs before 3 y of age).[19,32] It is possible that reinfection with different strains of BaCMV could influence shedding in saliva of adult animals. However, the small group-housed baboons used in the current study had few opportunities for direct interaction with different or new baboons with either an active infection or that were shedding BaCMV themselves, thus suggesting that the viral shedding of BaCMV in saliva we observed was persistent shedding years after primary infection. BaCMV is an informative model for human CMV and could also be used to examine how chronic viral infections affect the health of endangered NHP. The validation of several commercially available ELISA for antihuman CMV IgG or IgM for use in baboons will help provide other researchers and clinicians with easily accessible methods for characterizing antiCMV antibodies in baboons and potentially other NHP species.

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