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REOVIRUS ASSOCIATED MENINGOENCEPHALOMYELITIS IN BABOONS

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Abstract

Baboon orthoreovirus (BRV) is associated with meningoencephalomyelitis (MEM) among captive baboons. Sporadic cases of suspected BRV induced MEM have been observed at Southwest National Primate Research Center for the past 20 years, but could not be confirmed due to lack of diagnostic assays. An immunohistochemistry (IHC) based assay using antibody against BRV Fusion Associated Small Transmembrane protein p15, and a conventional PCR (PCR) based assay using primers specific for BRV were developed to detect BRV in archived tissues. Sixty-eight cases of suspected BRV induced MEM from 1989 to 2010 were tested for BRV, Alphavirus, and Flavivirus by immunohistochemistry. Fifty-nine out of 68 cases (87%) were positive for BRV by immunohistochemistry; one tested positive for Flavivirus (but was negative for West Nile virus and St Louis encephalitis virus by real-time PCR (qRT-PCR), and one virus isolation (VI) positive control tested negative for BRV. Sixteen cases (nine BRV negative and seven BRV positive cases, by immunohistochemistry), along with VI positive and negative controls were tested by PCR for BRV. Three (out of nine) IHC-negative cases tested positive, and three (out of seven) IHC-positive cases tested negative by PCR for BRV. Both immunohistochemistry and PCR assays tested one VI positive control as negative (sensitivity: 75 %). This study shows that the majority of cases of viral MEM among baboons at SNPRC are associated with BRV infection and the BRV should be considered as a differential for non-suppurative MEM in baboons.

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Keywords

Papio; Nervous system; Orthoreovirus; Primates; Encephalitis; Myelitis

Introduction

Spontaneous viral encephalitis is a rare pathology in baboons⁵¹ and is seldom diagnosed clinically. So far, the only reported outbreak of viral meningoencephalomyelitis (MEM) among baboons occurred during 1993–94 at the Southwest National Primate Research Center in San Antonio, TX (SNPRC)⁵¹. During this outbreak, eight juvenile baboons developed neurological symptoms and showed histopathological lesions of acute non-suppurative MEM⁵¹. A novel double stranded RNA (dsRNA) virus with 10 genomic fragments and syncytium inducing capability was isolated from five affected baboons^{28,51}. The isolated virus was inoculated into two healthy baboons to reproduce similar lesions of MEM⁵¹. Based on the morphological and genomic characteristics, the viral isolate was classified as a novel species (*Baboon orthoreovirus*; BRV, isolate 10895) of the genus *Orthoreovirus*, family *Reoviridae*^{26,28,51}.

The family *Reoviridae* is the largest and the most diverse family of the non-enveloped double stranded RNA (dsRNA) virus families²⁴. The family *Reoviridae* has two recognized subfamilies, based on the presence (subfamily *Spinareovirinae*) or absence (subfamily *Sedoreovirinae*) of spikes or turret proteins at each of the icosahedron vertices of the viral capsid^{68,69}. The subfamily *Spinareovirinae* include nine genera (*Orthoreovirus*, *Aquareovirus*, *Oryzavirus*, *Fijivirus*, *Mycoreovirus*, *Cypovirus*, *Idnoreovirus*, *Dinovernavirus*, *Coltivirus*), and subfamily *Sedoreovirinae* include six genera (*Orbivirus*, *Rotavirus*, *Seadornavirus*, *Phytoreovirus*, *Cardoreovirus*, *Mimoreovirus*)^{68,69}.

The genus *Orthoreovirus* includes viruses affecting a wide variety of vertebrate hosts including fish, birds, reptiles, and mammals. Distinguishing features include the following: 1) a segmented genome consisting of three large (L1, L2, and L3), three medium (M1, M2, and M3), and four small (S1, S2, S3, and S4) linear dsRNA segments, which code for three λ , three μ , and four σ translation products^{27,69}, 2) transmission by respiratory or fecal-oral route⁶⁹, 3) isolated only from vertebrate hosts, and 4) all members of all species groups, except the *Mammalian orthoreovirus* (MRV), form syncytia^{24,26,27,69}. Further, depending upon syncytium formation and the phylogenetic analysis of the viral capsid proteins, two subgroups have been proposed under the genus *Orthoreovirus*, the fusogenic and the non-fusogenic subgroups. The non-fusogenic clade/subgroup consists of the prototypical MRV species and its member isolates, while the fusogenic subgroup consists of the *Baboon orthoreovirus* (BRV), *Nelson Bay virus* (NBV), *Avian orthoreovirus* (ARV), and *Reptilian orthoreovirus* (RRV)^{19,26}.

The MRV is the only member of the non-fusogenic subgroup of the *Orthoreovirus* genus, and it affects a wide variety of hosts (including humans); however, it causes significant pathology in only a few species (most notably in mice). MRV usually causes mild self-limiting respiratory and gastrointestinal symptoms in children and infants^{59,60}. MRV isolates have been isolated from human cases of non-bacterial fatal pneumonia⁶⁴, hepatitis-

encephalitis⁴⁴, necrotizing encephalopathy⁵⁷, meningitis^{37,42,43}, and myocarditis⁶². Overall, it appears that MRV may at times cause serious/fatal infections in humans; however, in most cases it causes mild self-limiting disease. In contrast to humans, MRV isolates cause severe lesions in infant laboratory mice characterized by encephalitis, hepatitis, biliary atresia^{4,9,29}, pancreatitis, myocarditis, and bronchiolitis obliterans organizing pneumonia^{10,52,53}.

The fusogenic subgroup of *Orthoreovirus* consists of BRV, ARV, NBV, and RRV. The ARV mostly causes subclinical infections among birds and is associated with an important economic disease of young chickens, known as Viral Arthritis Syndrome (tenosynovitis)¹¹. The NBV was first isolated in 1968 from the blood of a grey headed flying fox (*Pteropus poliocephalus*, a fruit bat) from the Nelson Bay in New South Wales, Australia^{24,35}. So far, only one isolate of NBV is known. Recently, a number of *Orthoreovirus* isolates have been identified in human patients with a history of recent exposure to bats, in Malaysia, Hong Kong, and China^{17–19,16}. These isolates are genetically closely related and are strongly suspected to be of bat origin. It has been proposed that, based upon the uniformity of the carrier species (*Pteropine spp.*, bats), the NBV and the other aforementioned novel isolates be put together in one species by renaming the NBV as *Pteropine orthoreovirus* (PRV)¹⁹. It is very likely that these viruses are capable of species jumping from bats to humans¹⁹. The RRV was first isolated from a python (*Python regius*) with hemorrhagic kidney lesions^{2,24}, subsequently, a number of RRVs have been isolated from various types of snakes^{1,25,50,67}, lizards^{25,54}, tortoise^{25,54}, chameleon²⁵, and iguana^{13,25,36}. It has been suggested that in wild conditions, subclinical infections with RRVs are common²⁴. Experimental infection of snakes with RRV resulted in fatal proliferative interstitial pneumonia^{47,50}. At least three species are known among identified RRVs which are not species restricted, making it a distinct possibility that future isolates will be discovered^{13,27}.

As stated earlier, BRV was first isolated from an outbreak of meningitis, encephalitis, and myelitis (meningoencephalomyelitis, MEM) among baboons housed at SNPRC during 1993–94. There is limited antigenic similarity and sequence conservation between BRV and other members of genus *Orthoreovirus*. Phylogenetic analysis indicates that BRV evolved in parallel to ARV and NBV²⁸. BRV specific antibodies have been detected in serum samples of > 95% tested baboons (n > 100) from three captive bred populations from the US and Egypt, and in baboon sera preserved at the SNPRC from the early 1970s, suggesting that BRV may have co-evolved with baboons in nature and is not geographically restricted^{3,51}. During the previous outbreak at SNPRC, BRV antibodies were not detected in the serum samples of human caretakers who worked with infected baboons, or among other non-human primate species including macaques, langurs, and chimpanzees housed within the same facility³, indicating the species specificity of the virus.

Over the past 20 years at SNPRC there have been sporadic cases of meningitis, encephalitis, and myelitis (meningoencephalomyelitis, MEM) among baboons. Some of the cases had confirmed bacterial etiology, but most cases were categorized as suspected viral/BRV etiology based on clinical history, gross, and histopathological lesions. Although BRV was always high on the list of suspected etiologies, it could not be confirmed due to lack of a proper diagnostic assay. Since the isolation and molecular characterization of BRV, several

recent reports have studied the BRV-Fusion Associated Small Transmembrane (FAST) family of proteins, specifically p15^{20–23,61,65,66} which is a nonstructural protein required for syncytium formation. Using polyclonal antibody specific for C terminus of BRV-p15 protein (residues 90–140)²³, and primers specific for S4 genome segment (which codes for BRV-p15 protein), we developed immunohistochemistry (IHC) and conventional PCR (PCR) based assays to detect the presence of BRV in archived formalin fixed tissue samples of baboons. This paper presents the findings from a retrospective study of 68 cases of suspected viral/BRV induced MEM at SNPRC from 1989–2010 which were tested by IHC and PCR for BRV, Alphavirus, and Flavivirus genera (which are known to be present in geographical vicinity of SNPRC).

Materials and Methods

Case selection

An internal pathology database (apath) at the SNPRC was searched for the terms “encephalitis”, “meningoencephalitis”, “encephalomyelitis”, “myelitis”, and “meningitis” as the morphologic diagnoses at the time of necropsy from the years 1989 to 2010. Cases with a determined bacterial or traumatic etiology were excluded. Only spontaneous neurological cases (n = 68) for which no specific etiology was determined were selected. The majority of these cases had histologic lesions of lymphocytic or non-suppurative meningitis, encephalitis, and myelitis, sometimes with hemorrhage and necrosis. Four cases from the previous outbreak, from which BRV was detected by virus isolation (VI), were used as positive controls. Confirmed cases of Eastern Equine Encephalitis (EEE) and West Nile virus (WNV) were used as positive controls for Alphavirus and Flavivirus respectively (gift from Dr Matti Kiupel, Michigan State University, Diagnostic Center for Population and Animal Health, Lansing, MI). Four healthy baboons that were sacrificed for routine colony management that had no clinical symptoms, and no gross or histological lesions of any neurological disease were selected as virus negative control animals. The original medical records, gross necropsy reports, and histopathology reports were retrieved and reviewed for confirmation or clarification, as needed.

Primary antibodies

Baboon orthoreovirus—Rabbit anti-BRV primary polyclonal antibody was a generous gift from Dr. Roy Duncan (Department of Microbiology and Immunology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada)²². The antibody is specific for the C terminus of the p15 protein (residues 90 to 140) which is a member of FAST protein family and helps in the dissemination of virus to the adjacent cells by syncytium formation²². There is no significant sequence identity between BRV FAST p15 protein and the FAST proteins identified in other members of genus *Orthoreovirus*²².

Alphavirus—Commercially available mouse anti-Alphavirus monoclonal antibody clone 3581 (Santa Cruz Biotechnology, Cat # SC-58088) raised against Alphavirus was used.

Flavivirus: Commercially available mouse anti-Flavivirus monoclonal antibody clone 3571 (Santa Cruz Biotechnology, Cat # SC-58128) raised against purified St. Louis Encephalitis virus (SLE) was used.

Immunohistochemistry (IHC)

Formalin fixed paraffin embedded nervous tissue blocks of the selected cases were obtained from the tissue archives and 3.5 µm sections were cut and placed on propylsilane coated glass slides. Tissue sections were paired with a primary antibody control (diluent only), virus positive controls (BRV, EEE, and WNV), and nervous tissue sections from four normal baboons as negative controls. Formalin fixed paraffin-embedded tissue sections were deparaffinized (Varistain Gemini ES, Thermo Scientific, Inc) and antigen unmasking was done by immersing in antigen retrieval solution (Dako, Cat # S1669) in a decloaking chamber (Biocare, Cat # DC2002) at 94 °C for 35 min followed by 90 °C for 10 min. For BRV, tissue sections were quenched in 0.3% hydrogen peroxide (in methanol) for 10 min, washed twice with distilled water, and blocked in non-fat dried milk (1%) for 30 min followed by another round of washing with washing buffer (Dako, Cat # S3006). Rabbit anti-reovirus anti-p15 antibody was used at 1:20,000 dilutions (dilution buffer, Prohisto, Cat # AAK1). Tissue slides were placed in a slide box (Prohisto, Cat # AAK1) and 3 ml of primary antibody solution was poured over each slide. The slides were gently rocked overnight at 4 °C. For Alphavirus and Flavivirus, following antigen retrieval, tissue sections were incubated with mouse anti-alphavirus monoclonal antibody (1:50 dilution) or mouse anti-flavivirus monoclonal antibody (1:100 dilution) for 1 hour at room temperature in a humidified chamber. Subsequent steps were common for all the three viruses. Following primary antibody incubation, slides were washed twice in washing buffer (Dako, Cat # S3006), incubated with horseradish peroxidase link (Dako, 20 min), and then washed twice in washing buffer. Next, slides were incubated with horseradish peroxidase enzyme (20 min, DAKO), washed twice, and then freshly prepared 3, 3'-diaminobenzidine-tetrahydrochloride (DAB) chromogen substrate solution (Dako) was added to develop the color. Finally, slides were washed, counterstained in Mayer's hematoxylin using a standard protocol, cover-slipped, and examined microscopically by two board certified pathologists (MAO and EJD) in a blind manner. Sections were identified as positive or negative based on the presence of discrete staining.

PCR

For BRV—A PCR based assay was designed as a test to detect BRV genome in formalin fixed tissues. Twenty-four cases were selected for this assay, which included four virus isolation (VI) positive controls, four negative controls, and 16 (out of total 68) unknown/suspect cases. Among the suspect cases, all cases which tested negative for BRV by IHC (n = 9) and seven cases which tested positive for BRV by IHC were selected. Total RNA was extracted from formalin-fixed, paraffin-embedded (FFPE) brain tissue sections using the RNeasy FFPE® kit (Qiagen Valencia, CA). Reverse transcription was done using the RETROscript® kit (Life Technologies, Carlsbad, CA). For amplification of viral DNA, PCR was carried out using primer sequences based on the BRV genome segment S4 (complete sequence; GenBank accession # AF406787). The primer sequences are as follows:

Forward primer: 5'-ATG GGT CAA AGA CAT TCA ATA G-3'

Reverse primer: 5'-TCA AAC GTT GAT ACT TCC ATC TGG-3'

Touchdown PCR conditions were used for the PCR. Briefly, following an initial denaturation step at 94°C for 2 minutes, the annealing temperature was set to 59°C for two cycles, and thereafter decreased by one degree for every 2 cycles until the temperature reached 52°C. At those conditions the samples were run for an additional 20 cycles. The PCR product was loaded on 1% agarose gels and electrophoresis was performed. They were then stained with ethidium bromide, visualized under UV illumination, and photo-documented. A 430 base pair band on a gel was determined as positive (Fig. 1). All reactions were run with positive and negative controls. PCR products from one sample were cloned into the pCR2.1-TOPO vector (Life Technologies, Carlsbad, CA) and sequenced to confirm if the PCR products were indeed from BRV (Table 1). In separate reactions, a 244 bp sequence of the 16S ribosomal RNA (rRNA) gene was amplified from the baboon samples as a DNA positive control that would make it possible to distinguish between PCR failure and truly negative results using the primer set L2513 and H2714 (data not shown)⁴⁵.

For Alphavirus and Flavivirus—The confirmatory tests for *Alphavirus* (Western Equine encephalitis (WEE)), and *Flavivirus* (West Nile virus (WNV) and St. Louis Encephalitis virus (SLE)) were done by qRT-PCR assay at a diagnostic laboratory as per established protocols^{46,48,49}.

Results

Table 2 lists all of the cases, along with the signalment, year of necropsy, reported relevant clinical history, distribution of lesions in the CNS, histopathologic changes, BRV immunohistochemistry (IHC), and PCR results. Of the 68 suspect baboons, the average age at the time of necropsy was 9.27 years (range 0.01 to 25.33 years), 62 % (42 baboons) were less than 10 years old. The male to female ratio was 0.94. Few cases (21/68) had gross lesions, but when present, there were typically discrete foci of hemorrhage and necrosis, randomly scattered throughout the brain (Fig. 2) and spinal cord. The typical microscopic lesions of perivascular lymphocytic non-suppurative encephalitis and multifocal necrosis and hemorrhage were similar to those as previously reported⁵¹ (Fig. 3, Fig. 4); a few cases had primarily suppurative inflammation. Table 3 summarizes the IHC, PCR, and qRT-PCR testing results.

Immunohistochemistry for BRV (Fig. 5–7) demonstrated diffuse to granular cytoplasmic labeling of neurons multifocally within the cerebral cortex, brainstem (Fig. 5), ganglia (Fig. 6), and midbrain, labeling of small neurons in the cerebellar Purkinje cell layer, and few to many glial cells in the cerebrum (Fig. 7), with fewer numbers of labeled glial cells in the cerebellum, brainstem, and spinal cord. The type and number of positive cells varied between cases. IHC indicated that out of 68 unknown, 59 cases were positive for BRV; one was negative for BRV but positive for Flavivirus. The other eight tested negative by IHC for BRV, Alphavirus, and Flavivirus. Three out of four BRV VI positive controls were positive by IHC; one BRV VI positive control tested negative by IHC for BRV, and also for Alphavirus and Flavivirus. The ten cases (nine unknown and one VI positive control), which tested negative by IHC for BRV, all tested negative for West Nile virus (WNV), Western Equine encephalitis (WEE), and St. Louis Encephalitis (SLE) viruses by qRT-PCR. No mixed infections were detected.

Twenty-four cases were tested for BRV using PCR, which included four VI positive controls, four negative controls, and 16 unknown cases (nine BRV negative cases, and seven BRV positive cases, by immunohistochemistry). All four negative controls tested negative by PCR, three out of four VI positive controls were positive by PCR. One VI positive control which was positive by IHC tested negative, while one VI positive which was negative for BRV by IHC was positive by PCR. Out of 16 unknown cases, three (out of nine) cases which were negative for BRV by IHC tested positive by PCR, this included one case which was actually positive for Flavivirus by IHC but negative for WNV, SLE, and EEE by qRT-PCR. Three (out of seven) cases which were positive for BRV by IHC tested negative by PCR. The PCR products (Table 1) amplified using BRV specific primers, matched with 99 % sequence identity to previously published BRV S4 genome segment sequence (Genbank accession: AF406787).

Discussion

Using the recently developed FAST p15 polyclonal antibody, an IHC methodology was developed to detect BRV in archived formalin fixed baboon tissues revealing that 59 out of 68 cases (~ 87 %) of suspected viral MEM at the SNPRC were positive for BRV by IHC. A second assay (PCR) was also developed to detect BRV in formalin fixed tissues. Out of 16 unknown cases, three cases which were negative for BRV by IHC were positive by PCR, while three cases which were positive by IHC tested negative by PCR. One of the VI positive controls which tested negative by IHC was positive by PCR, while one other VI positive control which was positive by IHC was negative by PCR. All the negative controls tested negative by both IHC and PCR. If virus isolation is considered to be the gold standard then, on the basis of limited numbers of samples, both IHC and PCR have a sensitivity of 75 % and specificity of 100 %. The main objective of this study was to demonstrate that the majority of the cases of non-suppurative MEM in baboons are associated with BRV infection. The results from this study suggest that BRV is associated with non-suppurative viral MEM in baboons at the SNPRC, and that the presumptive diagnosis of BRV infection can be made by the characteristic lesions, pending further confirmation. The overall prevalence of central nervous system diseases in baboons is extremely low. In a recent publication which documented 10,883 macroscopic or microscopic morphologic diagnoses in 4297 baboons at SNPRC over a 20 year period, nervous system lesions accounted for only 4.21 percent, which included encephalitis (n = 57), meningitis (n = 27), meningoencephalitis (n = 17), scoliosis (n = 7), kyphosis (n = 6), myelitis (n = 5), encephalomyelitis (n = 3), porencephaly (n = 2), meningioma (n = 1), and glioblastoma (n = 1)¹⁴. This further emphasizes the importance of BRV among baboons.

BRV is capable of being highly pathogenic, yet, the presence of BRV specific antibodies in asymptomatic animals suggests that the virus is often subclinical in baboons²⁸. Over the past 20 years at SNPRC, there have been sporadic cases of MEM which were suspected to be BRV induced, but could not be confirmed. These cases were more or less evenly distributed over the period of time (Table 2). Many of these suspected cases had clinical histories suggestive of CNS lesions, such as paralysis, ataxia, and seizures (Table 2). Gross lesions indicative of CNS disease were not present in most of the cases, but when present, were typically hemorrhage and necrosis; this is in contrast to the previous report, which reported

no gross lesions in infected baboons⁵¹. The majority of the cases had non-suppurative or lymphocytic meningoencephalitis, with occasional myelitis. It is possible that myelitis is more common than reported in Table 2, because spinal cord was not always taken for histology. In contrast to these more typical changes, a few cases had primarily suppurative inflammation, which is important because these would be more likely to be presumed bacterial in origin, and overlooked as potential BRV cases. Vasculitis was reported as a significant histologic lesion in a few cases and may account for the hemorrhage and necrosis seen histologically and on gross examination. Myocarditis was the most common non-CNS inflammatory lesion, occurring in nine animals, followed by pneumonia in six animals; these are common lesions in this baboon colony and the low number of cases suggests these are not major components of BRV infection and may be unrelated lesions¹⁴.

Besides BRV, other potential etiological agents of viral MEM in baboons exist, including members of the genera Flavivirus (WNV, SLE), Alphavirus (Eastern Equine Encephalitis virus [EEE], Venezuelan Equine Encephalitis virus [VEE], and Western Equine Encephalitis virus [WEE], Herpesvirus (*Herpesvirus papio* 2 [HVP2], simian agent 8 [SA8]), and Cardiovirus (Encephalomyocarditis virus, EMC70 strain). WNV specific antibodies were isolated from 36% of baboons tested, housed at Tulane National Primate Research Center (TNPRC), following an outbreak of WNV infection in the neighboring human population⁵⁸, however clinical signs or histopathological lesions of encephalitis were not reported among the baboon population⁵⁸. Filatenkov *et al* reported that virulent strains of VEE can induce clinical symptoms of MEM in baboons³⁴. Brack, *et al*, reported experimentally induced encephalitis in Kenya baboons due to herpesvirus simian agent 8 (SA 8)¹⁵. Dzhikidze, *et al*,³⁰ reported the occurrence of encephalomyocarditis in hamadryas baboons due to EMC70 strain of virus; however, during an outbreak of EMCV in the SNPRC baboon colony, the findings were all related to the cardiovascular system and no lesions were found in the brain⁴⁰. Isoun, *et al*, observed hemorrhagic encephalitis in a wild baboon; however, the etiology was not confirmed⁴¹. Benvineste, *et al*, did not observe any clinical signs of encephalitis in baboons due to experimental inoculation of simian immunodeficiency virus¹².

Out of these viruses, only few have epidemiological data suggestive of their prevalence in the state of Texas. WNV is relatively more frequent in both humans and animals in the geographical vicinity of SNPRC^{8,63}. West Nile virus affected Texas beginning in the year 2002, although 34 baboons in this study were necropsied between 2002 to 2010, only one was positive on IHC for Flavivirus (but was negative for WNV and SLE qRT-PCR), and in turn, was confirmed positive for BRV by PCR. SLE is very prevalent in Texas; from 1964 to 2010, among all 48 contiguous states, the highest number of confirmed cases have been reported from Texas (total = 1021, high incidence in 1964–68 followed by decreased annual incidences)^{7,39}. Though there are only sporadic reports of EEE among horses in Texas³⁸, it is much more prevalent among humans in the neighboring state of Louisiana (157 confirmed cases in humans between 2003–2011), compared to Texas (43 cases between 2003–2011)⁶. VEE is unlikely given that no recent confirmed cases have been reported in humans since the outbreak among humans and equines in 1971⁷⁰, although, due to close proximity with Mexico and frequent cross border transport of equids and reports of occurrence of VEE in

Mexico, it is always a concern^{33,56}. There is not much epidemiological data to suggest the prevalence of WEE in Texas (no confirmed cases in humans between 2000–2011)⁵. HVP2 has previously been shown to be commonly present in both captive and wild caught baboon populations^{31,32}. HVP2, originally reported as SA8, has been shown to be present in our colony; however, lesions were mostly restricted to mucocutaneous herpetic lesions and it was not associated with MEM⁵⁵.

Our testing of other potential causes of MEM was limited to the use of a pan-Flavivirus antibody and a pan-Alphavirus antibody on all cases, as well as qRT-PCR for WNV, SLE, and WEE, on the cases that tested negative for BRV by IHC (all of them tested negative by qRT-PCR). One case tested positive for Flavivirus by IHC, but we were unable to determine if this was a true positive or a false positive, as the most likely flaviviruses (WNV and SLE) were excluded by the qRT-PCR testing. Overall, in this report, 68 suspected cases of BRV induced MEM over a period of 20 years in the captive baboon population housed at SNPRC were tested using a novel IHC based assay to identify BRV in formalin fixed paraffin-embedded tissues. The results indicate that 87% of the suspected cases were positive for BRV by IHC. A conventional PCR based assay was designed as a confirmatory test to detect BRV, however, both IHC and PCR assays failed to pick one out of four VI positive controls (one false negative, sensitivity :75 %). Thus, neither IHC nor PCR based assay seem to be perfect and both have some limitations. However, a negative result does not completely rule-out infection. Also, in retrospective studies like the present study, variations in fixation conditions can result in false negatives. This study has two major implications; first, it presents two diagnostic assays (IHC and PCR) which (with some reservations) can be used for the detection of BRV infection in nervous tissues. Second, the results from this study suggest that BRV is associated with the majority of MEM in baboons and BRV should be considered as an important differential for cases of non-suppurative MEM in baboons. To prove whether BRV is the causative agent or an innocent bystander in these lesions would be a subject of future investigations.

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Fig. 1.

Agar gel electrophoresis image of the amplified sequence of S4 genome segment of Baboon orthoreovirus. A 430 base pair band on gel was considered as positive. Columns with case numbers: A: 23, B: 63, C: 44, D: VI positive control, E: 32, F: 58, G: 20, H: 1, I: 27, J: 61, Control: 49 (sequenced), MK: Size marker.

Fig. 2.

Brain; Baboon, case No. 43. Gross pathology of a baboon brain positive for Baboon orthoreovirus. Note multifocal hemorrhage and malacia.

Fig. 3.

Brain; Baboon, case No. 46. Histopathology of a baboon brain positive for Baboon orthoreovirus. Note hemorrhage, necrosis, and mononuclear inflammatory cell infiltration. HE.

Fig. 4.

Brain; Baboon, case No. 46. Histopathology of a baboon brain positive for Baboon orthoreovirus. Note perivascular infiltration by lymphocytes and gliosis. HE.

Fig. 5.

Brainstem; Baboon, case No. 19. Immunohistochemistry for Baboon orthoreovirus p15 Fusion Associated Transmembrane (FAST) protein intracytoplasmic domain. Note strong cytoplasmic labeling of neurons.

Fig. 6.

Ganglion; Baboon, case No. 19. Immunohistochemistry for Baboon orthoreovirus p15 Fusion Associated Transmembrane (FAST) protein intracytoplasmic domain. Note strong cytoplasmic labeling of neurons.

Fig. 7.

Cerebrum; Baboon, Case No. 6. Immunohistochemistry for Baboon orthoreovirus p15 Fusion Associated Transmembrane (FAST) protein intracytoplasmic domain. Note weak to moderate cytoplasmic labeling of glial cells.

Table 1

Sequence of the amplified PCR product of S4 genome segment of Baboon orthoreovirus

>842C
atgggtcaaagacattcaatagttcaaccaccagccccaccgcc
aaatgcttttgtgaaattgtgagcagttctactggcattataatcgtgttgcatatt
tgcattatattcttattttatataagttgctgcagtggtacaatcgtagccaagaa
taagaaacgtaaagagcaaattagagaacaaattgagctgggtttatcatatgggtgc
tggagtagcatcactctcttctcaacgttattgcacataatcctggatcagttatctc
ggctatccctatctataaaggctccgtgcactggtgtacctaattcgcgcctacttcaat
cacgagcgggactgcagaaggagacactagaatttgaatcatgatggaagaaccaga
tggaggtatcaacgtttgaatcgaattcccgcgcc

Table 2

Clinical, pathology, IHC, and PCR results of all cases tested for Baboon orthoreovirus

Case #	Age (years)	Year of necropsy	Locations			Histologic lesions	Reovirus IHC	Reovirus PCR	Clinical history - CNS or other **
			M	E	My				
1	17.17	1997	X	X	-	Eos	-	-	Ataxia
2	0.42	1989	X	X	-	Lym	+		Found Dead
3	0.92	1990	X	X	-	Lym	+		Found Dead
4	0.33	1992	X	X	-	Lym	+		Found Dead
5	14.83	1997	X	X	-	Lym	+	-	Stroke, Weakness
6	5.92	1997	X	X	-	Lym	+		Azotemia, Bloody discharge
7	13.5	2000	X	-	X	Lym	+		Paralysis
8	9.33	2001	X	X	X	Lym	+		Paralysis
9	7.17	2007	X	-	-	Lym	+		Colony management
10	7.75	2008	-	X	-	Lym	+		Seizures, Lacerations
11	4.33	2008	X	X	-	Lym, Abs	+		Fracture
12	7.67	2008	-	X	-	Lym, Gli	+		Laceration
13	10.42	2009	-	X	-	Lym, Gli	+		Dermatitis (<i>Histoplasmosis Duboisii</i>)
14	1	2009	X	X	-	Lym, Gli	+		CNS, Nasal discharge, Diarrhea
15	5.42	2010	-	X	-	Lym, Gli	+	+	CNS, Paralysis
16	18.42	2010	-	X	-	Lym, Gli	+		Pneumonia
17	23.58	2004	X	X	-	Lym, Gra	+		Paralysis
18	7.92	2006	X	X	-	Lym, Hem	+		Weakness
19	9	2009	X	-	-	Lym, Neu	+		Seizures
20	0.67	1997	X	X	-	Lym, Neu	-	-	Weakness
21	22.25	1997	X	X	-	Lym, Neu, Hem, Vasculitis	+		Disoriented
22	9d	2005	X	-	-	Lym, Neu, Macrophages	+	+	Found Dead
23	1.25	1990	-	X	-	Nec	-	-	Weakness
24	0.5	1999	-	X	-	Nec, Gli	+		Weakness
25	18.33	1992	-	X	-	Nec, Lym	+		Paralysis (One sided)
26	0.83	1993	X	X	-	Nec, Lym	+		Disoriented, Head tilt

Case #	Age (years)	Year of necropsy	Locations			Histologic lesions	Reovirus IHC	Reovirus PCR	Clinical history - CNS or other **
			M	E	My				
27	3.75	1997	X	X	-	Nec, Lym	-	-	Ataxia
28	16.92	1998	X	X	X	Nec, Lym	+		Abnormal gait
29	25.33	2002	-	X	X	Nec, Lym	+	-	Paralysis
30	9.92	2009	-	X	-	Nec, Lym	+		Arthritis, Spondylosis
31	11.42	2009	-	X	-	Nec, Lym	+		Seizures, Spondylosis, Weight loss
32	0.42	1995	X	X	X	Nec, Lym, Gli	-	+	Paralysis
33	22.83	1995	X	X	X	Nec, Lym, Vasculitis	+		Paralysis
34	6	2000	X	X	X	Nec, Non-sup	+		CNS
35	1.75	2003	X	X	X	Nec, Non-sup	+		Weakness
36	16	2004	X	X	-	Nec, Non-sup	+		Ataxia
37	13.67	2006	X	X	-	Nec, Supp	+		Seizures, Respiratory distress
38	22.5	2008	-	X	-	Nec, Supp	+	-	Paralysis, Foreign body
39	15.83	1996	X	X	-	Nec, Supp, Vasculitis	+		Shock, C-section
40	17	1996	X	X	-	Neu, Hem, Vasculitis	+		Weakness
41	17.5	1996	X	X	X	Non-sup	+		Seizure
42	15.92	1999	-	X	-	Non-sup	+		Found Dead
43	0.08	1999	-	X	-	Non-sup	+	+	Pneumonia
44	0.83	2002	X	X	X	Non-sup	-	+	Paralysis, Weakness
45	1.33	2003	X	X	X	Non-sup	+		Disoriented, Weakness
46	21.08	2003	-	X	X	Non-sup	+		Paralysis
47	6.17	2003	X	X	X	Non-sup	+		Paralysis
48	17.92	2003	-	X	-	Non-sup	+		Paralysis
49	1.17	2005	X	X	X	Non-sup	+	+	Ataxia, Seizure, Weakness
50	0.83	2006	X	X	-	Non-sup	+		Paralysis
51	6.67	2007	-	X	X	Non-sup	+		Ataxia, Lacerations
52	7.83	2007	-	X	-	Non-sup	+		Vaginal constriction (mass)
53	7.75	2007	-	X	-	Non-sup	+		Colony management
54	1.08	2008	-	X	-	Non-sup	+		Stomatitis

Case #	Age (years)	Year of necropsy	Locations			Histologic lesions	Reovirus IHC	Reovirus PCR	Clinical history - CNS or other **
			M	E	My				
55	1.33	1993	X	X	X	Non-sup, Gli	+		Seizure, Weakness
56	6.33	1995	X	X	-	Non-sup, Gli	+		Ataxia
57	1.42	2003	X	X	X	Non-sup, Supp (Meninges)	+		CNS, Weakness
58	4d	1995	X	-	-	Supp	-	-	Blind
59	6.67	1997	X	X	-	Supp	+		Found Dead
60	22.83	1997	X	-	-	Supp	+		Ataxia, Otitis
61	1.25	1999	X	X	-	Supp	-	-	Blind, Weakness
62	24.83	1999	X	-	-	Supp	+		Found Dead
63	0.25	2000	X	X	-	Supp	-	+	Found Dead
64	3.17	2001	X	X	-	Supp	+		Found Dead
65	20.25	2005	-	X	-	Supp	+		Weakness, Laceration
66	24.83	2005	X	-	-	Supp	+		Weakness, Nasal discharge
67	3.83	2008	X	X	-	Supp	+		Found Dead
68	15.42	1997	X	-	-	Supp, Gra	+		Found Dead

Key Locations inflammation present: M= Meningitis, E= Encephalitis, My= Myelitis; 'X'= present, '-' = not present or not examined

Histologic Lesions: Abs = Abscess, Eos = Eosinophilic, Gra = Granulomatous, Gli = Gliosis, Hem = Hemorrhage, Lym = Lymphocytic, Neu = Neutrophilic, Nec = Necrosis, Non-sup = Non-suppurative, Supp = Suppurative

Table 3

Summary of immunohistochemistry and PCR based results.

BRV status	Total Tested IHC (BRV, Flavi, Alpha)	Result BRV IHC		Tested PCR (BRV)		Result Flavi IHC		Result Alpha IHC		Tested RTPCR (WNV, SLE, WEE)	
		+	-	Tested	+	-	+	-	+	Tested	+
Suspect	68	59	9	16	7	9	1	67	0	9	0
Negative control	4	0	4	4	0	4	0	4	0	0	0
Positive control by VI*	4	3	1	4	3	1	0	4	0	1	0
											1

VI* = Virus isolation