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Identification and Prevalence of a Gammaherpesvirus in Free-ranging Northern Brown Bandicoots (*Isoodon macrourus*)

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ABSTRACT: Herpesviruses have been reported in several Australian marsupial species, with an overt, sometimes fatal disease described in macropods. Our study identifies a gammaherpesvirus in northern brown bandicoots (*Isoodon macrourus*) and provides virus prevalence data for bandicoots in southeast Queensland, Australia. Herpesvirus DNA was detected using pan-Herpesviridae family primers in a nested PCR format. Samples from 35 northern brown bandicoots were screened, including whole blood (n=29), oropharyngeal swabs (n=34), urine (n=22), and feces (n=23). Combining all sample types, herpesvirus DNA was detected at a total prevalence of 51% (18/35). Whole blood and oropharyngeal swabs proved to be the optimal samples for detection of this virus, with prevalences of 34% and 38%, respectively. Herpesvirus DNA was detected in 4.5% (1/22) of urine samples and not at all in fecal samples. Detection of herpesvirus was more likely in males than females. Animals were trapped at eight different locations, and at all but one location at least one herpesvirus positive animal was detected. This study indicates a high prevalence of the virus within northern brown bandicoot populations in southeast Queensland. Further research is required to understand the clinical manifestations, if any, of herpesvirus infection in this species and how this may affect populations in the face of stressors such as land clearing and habitat fragmentation.

Key words: Bandicoot, herpesvirus, *Isoodon*, marsupial.

The family *Herpesviridae* consists of enveloped, double-stranded DNA viruses that are classified into three subfamilies: *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae* (Pellett and Roizman 2013). The capacity to produce latency in endemic hosts is a key feature shared by viruses from all three subfamilies. This feature is a significant contributor to the survival and spread of the virus within populations (Pellett and Roizman 2013). Following infection, with or without overt disease, the virus becomes latent within host cells. Recrudescence may occur during stressful events or periods of immunocompromise, which may result in clinical disease and shedding (Ferrante et al. 2017).

Herpesviruses have been identified in several Australian marsupials including macropod species (*Macropus* spp.; Finnie et al. 1976; Callinan and Kefford 1981; Smith et al. 2008), koalas (*Phascolarctos cinereus*; Vaz et al. 2011, 2012), yellow-footed and agile antechinuses (*Antechinus flavipes* and *Antechinu agilis*; Amery-Gale et al. 2014), Tasmanian devils (*Sarcophilus harrisii*; Stalder et al. 2015), and common wombats (*Vombatus ursinus*; Stalder et al. 2015). Alpha- and gammaherpesviruses have been associated with disease outbreaks in macropods (Finnie et al. 1976; Callinan and Kefford 1981; Smith et al. 2008). Alphaherpesviruses have been reported to cause pyrexia, conjunctivitis, and respiratory disease, which in some cases has progressed to fatal systemic illness (Finnie et al. 1976; Callinan and Kefford 1981), while a gammaherpesvirus has been associated with ulcerative cloacitis (Smith et al. 2008).

While herpesvirus disease outbreaks have not been reported in bandicoots, herpesvirus DNA has been detected in two species: southern brown bandicoot (*Isoodon obesulus*) at a prevalence of 9% (1/11; Stalder et al. 2015) and northern brown bandicoot (*Isoodon macrourus*) at approximately 20% prevalence (Reiss et al. 2015). Bandicoots are terrestrial marsupials, endemic to Australia and Papua New Guinea (Gordon 2008). Despite dramatic declines in several bandicoot species since European arrival, the northern brown bandi-

coot remains common in many areas of southeast Queensland. Our study identifies a herpesvirus in northern brown bandicoots and presents prevalence data for southeast Queensland populations.

As part of a larger study, eight bandicoot trapping sites were selected in southeast Queensland, Australia. All sites were independent from each other, with a minimum of 10 km between sites. Trapping was conducted July 2018 to June 2020. Small wire cage traps were baited with bread and peanut butter, set at dusk, and checked the following morning at first light.

Samples were collected from 35 individual northern brown bandicoots. Bandicoots were anesthetized via a facemask using 4% isoflurane in oxygen (1.0 L/min). Following induction, animals were maintained on 1.5–2% isoflurane. A brief physical examination was performed on each animal, body condition score graded on a five-point scale, body weight recorded, and pes length measured. A blood sample not exceeding 1% of total body weight was taken from the jugular vein (Department of Environment, Water and Natural Resources 2013) or the middle coccygeal vein. Blood was transferred into 0.5 mL ethylenediaminetetraacetic acid tubes (Greiner Bio-One, 450530, Kremsmünster, Austria). An oropharyngeal swab was collected and stored in 0.5 mL of phosphate buffered saline. Where possible, a urine sample was collected by gentle expression of the urinary bladder, and fecal samples were collected from the floor of the trap or from the transport bag. All samples were stored at –20 C. Animals were monitored until fully recovered from anesthesia and released at the capture site at dusk on the day of capture.

A Companion F3 (MSD Animal Health, New South Wales, Australia, batch no. 1813506) trivalent live vaccine containing feline alphaherpesvirus 1 was used as a positive control for the herpesvirus PCR. Vaccine and genomic DNA extraction from whole blood, oropharyngeal swabs, and urine were performed using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany, catalog no. 51304) and per the manufacturer's guidelines, with the exception that buffer AL was substituted for buffer AVL for urine extractions. From fecal samples, DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, catalog no. 51504) per the manufacturer's guidelines.

The presence of herpesvirus DNA was detected using pan-*Herpesviridae* family primers in a nested PCR format (VanDevanter et al. 1996). Each 25 µL PCR reaction was performed using TopTaq™ DNA Polymerase Master Mix Kit (Qiagen, catalog no. 200203) according to the manufacturer's guidelines, with 0.2 µM primers and 10^{-1} purified DNA 5 µL. Cycling was performed as described by VanDevanter et al. (1996), except with the initial denaturation and extension times each extended to 3 min and the final extension extended to 10 min. The secondary PCR thermocycling protocol was the same as the primary PCR, except that the initial extension phase was reduced to 72 C for 1 min. PCR products were submitted to the Australian Genome Research Facility, Brisbane, Queensland for direct sequencing.

Direct sequencing of the 218 base pair PCR products for blood samples, oropharyngeal swabs, and urine sample resulted in identical DNA sequences. Primer sequences were removed, and the 165 base pair consensus sequence was compared with sequences on the GenBank database using the BLAST search engine (National Center for Biotechnology Information 2020). Consensus sequences were phylogenetically analyzed using MEGA-X software (Kumar et al. 2018). Phylogeny was inferred by using the maximum likelihood method and Tamura-Nei model (Tamura and Nei 1993), and demonstrated a close relationship to Peramelid gammaherpesvirus 1 from southern brown bandicoots characterized by Stalder et al. (2015) as distinct from other gammaherpesviruses of Australian native fauna species and known alpha- and betaherpesviruses (Fig. 1). Based on current nomenclature the virus has been tentatively designated *Peramelid herpesvirus 2* and has been lodged with GenBank, accession number MW455460.

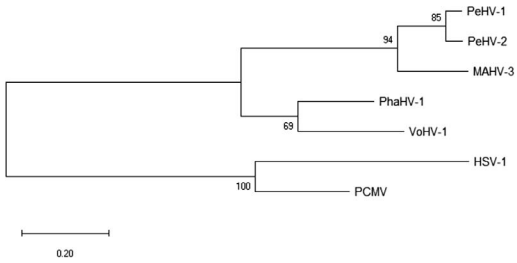

FIGURE 1. Maximum likelihood phylogenetic tree generated from the alignment of nucleotide sequences of the partial herpesvirus polymerase gene from northern brown bandicoots (*Isoodon macrourus*), other Australian wildlife species, pigs, and humans. PeHV-1=*Peramelid gammaherpesvirus 1* (P. Vaz pers. comm.); PeHV-2=*Peramelid gammaherpesvirus 2* (MW455460); MaHV-3=*Macropodid gammaherpesvirus 3* (EF467663.1); PhaHV-1=*Phascolarctid gammaherpesvirus 1* (JN585829.1); VoHV-1=*Vombatid gammaherpesvirus 1* (MG452721.1); PCMV=*Porcine cytomegalovirus*, betaherpesvirus (JF418940.1); HSV-1=herpes simplex virus type 1, alphaherpesvirus (NC_001806). Bootstrap values are displayed on the tree branches.

Herpesvirus infection was determined by the presence of herpesvirus DNA in any of the four sample types, giving an overall herpesvirus prevalence of 51% (18/35), significantly greater than the 9% (1/11) reported for free-ranging southern brown bandicoots (Stalder et al. 2015) and the approximately 20% reported for northern brown bandicoots trapped in the Northern Territory, Australia (Reiss et al. 2015). We found that whole blood and oropharyngeal swabs were the two most useful samples for detection of herpesvirus DNA, with prevalences of 34% and 38%, respectively (Table 1). Similar detection rates for both sample types indicate the usefulness of the less invasive oropharyngeal swab for future research of this virus. The single positive urine sample was identified in an animal that also tested positive on whole blood and oropharyngeal swab.

TABLE 1. Prevalence of herpesvirus DNA detected by PCR in whole blood, oropharyngeal swabs, urine, and fecal samples from northern brown bandicoots (*Isoodon macrourus*) in southeast Queensland, Australia.

Sample	No. samples	PCR positive	% Prevalence
Whole blood	29	10	34
Oropharyngeal swab	34	13	38
Urine	22	1	4.5
Feces	23	0	0

Paired blood and oropharyngeal swab samples were available for 13 of the positive animals. Four animals positive for herpesvirus DNA in blood were negative on oropharyngeal swab, and four animals positive on oropharyngeal swab returned a negative result for blood. Detection of the virus in blood may indicate acute, active infection; however, gammaherpesviruses often become latent in lymphocytes, thus detection could also be capturing latent infection. Detection of DNA in oropharyngeal swab samples is considered most probably due to active shedding of virus through the respiratory pathway. It is also important to consider that an animal with a negative detection may not only be a nonviremic, nonshedding animal, but may be an exposed animal with a latent infection that was not detected, as a serologic study was not performed. It is plausible that our variable observations reflect different states of viremia or latency, and that employing both blood and oropharyngeal swab PCRs may provide useful information in future studies.

In our study, seven out of eight locations returned at least one positive herpesvirus result, with prevalences of 40–100%; only one animal was trapped at the location at which herpesvirus was not detected. Reiss et al. (2015) reported a significant location effect when investigating herpesvirus in northern brown bandicoots: at one site, the Cobourg Peninsula, no herpesvirus was detected in northern brown bandicoots despite a sample size of 29 individuals, the largest of any of the five sites studied. The majority of sites in our study had herpesvirus present, which may indicate that the virus is widespread in bandicoot populations in southeast Queensland. All our study sites were isolated from each other, and positives were recorded at sites with as few as two individuals sampled.

We did not note any clinical signs consistent with respiratory herpesvirus. However, given

that overt disease has been reported with gammaherpesvirus infection in macropods (Smith et al. 2008), this virus may have the potential to cause clinical disease in bandicoots, especially in the face of environmental stressors such as extreme weather, bushfires, or land clearing. Further research is required to determine how widespread the virus is within bandicoot populations and how stress may cause recrudescence of the latent virus.

A chi-square test was performed to determine any significant difference in infection between male and female bandicoots. Males were significantly more likely to be infected than females (P=0.027). At the pan-species level Stalder et al. (2015) found that being male was a positive predictor for the presence of herpesvirus DNA, with males of several species having higher prevalence than females, including eastern grey kangaroos (*Macropus giganteus*), koalas, and Tasmanian devils. Male northern brown bandicoots appear to have larger home ranges than females, and in environments where food sources are abundant bandicoots are known to tolerate overlapping home ranges (Fitzgibbon et al. 2011). These factors may explain why prevalence was higher in males, as having a larger home range may lead to increased interaction with other bandicoots. Nevertheless, our study may underestimate the number of latent infections because no serological study was performed; a larger sample size and serological testing would be required to determine if prevalence of this virus shows a true sex difference.

Our unique phylogenetic classification of gammaherpesvirus sequences in northern brown bandicoots adds to current knowledge of herpesviruses within Australian marsupials. We found a high prevalence of herpesvirus within sampled bandicoot populations; this may be of concern for small, isolated bandicoot populations, especially those confronted with stressors such as habitat loss.

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