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The aetiology of wobbly possum disease: Reproduction of the disease with purified nidovirus

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ABSTRACT

The objective of this study was to investigate a role of a recently discovered marsupial nidovirus in the development of a neurological disease, termed wobbly possum disease (WPD), in the Australian brushtail possum (*Trichosurus vulpecula*). Four possums received 1 mL of a standard inoculum that had been prepared from tissues of WPD-affected possums, 4 possums received 1.8 mL (1×10^6 $TCID_{50}$) of a cell lysate from inoculated cultures, and 4 possums received 1 mL ($\times 10^7$ $TCID_{50}$) of a purified WPD isolate. All but one possum that received infectious inocula developed neurological disease and histopathological lesions characteristic for WPD. High levels of viral RNA were detected in livers from all possums that received infectious inocula, but not from control possums. Altogether, our data provide strong experimental evidence for the causative involvement of WPD virus in development of a neurological disease in infected animals.

Introduction

Wobbly possum disease (WPD) is a fatal neurological disease of the Australian brushtail possum (*Trichosurus vulpecula*) that has been identified in both captive (Mackintosh et al., 1995) and free-living (Perrott et al., 2000a) possum populations in New Zealand. The histopathological hallmark of the disease is perivascular infiltration with mononuclear cells in multiple organs including liver, spleen, brain and kidney (O'Keefe et al., 1997; Perrott et al., 2000b). Under experimental conditions the disease has been reproduced following inoculation of healthy possums with blood, urine, tissue homogenates and homogenized mites derived from WPD-affected possums, which were administered by multiple routes (Perrott et al., 2000b; O'Keefe et al., 1997). Transmission of the disease was also observed between possums in close contact (Perrott et al., 2000b). Early clinical signs of WPD in experimentally infected possums included inappetance, temperament changes and altered responsiveness to environmental stimuli. As the disease progressed, further neurological signs including ataxia, apparent blindness, cachexia and a fine head tremor were observed (Mackintosh et al., 1995; Perrott et al., 2000a; O'Keefe et al., 1997; Perrott et al., 2000b).

Based on the partial genomic sequence, the possum nidovirus is most closely related to current members of the family *Arteriviridae* (Dunowska et al., 2012). The aetiological involvement of the virus in the development of WPD was suggested based on the results of WPD-specific RT-PCR using liver DNA as template (Dunowska et al., 2012). The proportion of possums positive for the novel virus was significantly higher ($p < 0.0001$) among animals with WPD than among clinically healthy possums. More recently, high levels of WPD virus (WPDV) RNA were detected in a variety of tissues from WPD-affected possums (Dunowska et al., 2013), which was consistent with the multi-organ distribution of histopathological lesions (Perrott et al., 2000a; O'Keefe et al., 1997; Perrott et al., 2000b) and provided further support for an aetiological involvement of the virus in WPD.

We have recently described the *in-vitro* culture and purification of WPDV (Giles et al., 2015). The objective of the current study was to extend previous PCR-based data by providing experimental support for the hypothesis that infection of adult Australian brushtail possums with this nidovirus is aetiologically linked to development of WPD.

Results

Possums during acclimatization period

All possums acclimatized well to the captive environment as judged by their appetite and behaviour. Although 6/12 possums lost some weight during the acclimatization period, the remaining 6 possums gained weight in the same period (Table 1). The initial

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Table 1
Details of clinical signs observed in adult possums and % weight change over the duration of the study. All females had dependent joeys at the beginning of the study. Joeys of possums 6, 12 and 16 died during the period of the study, while joeys of possums 4, 10 and 13 appeared normal. Possums were inoculated with one of the following inocula: control inoculum (CI) comprising gradient material from uninfected cell culture lysates, standard inoculum (SI) prepared from tissues of WPD-affected possums, infected cell culture lysates (ICL), or WPDV (purified WPD virus isolate). Days post-inoculation for possums 1 and 2 were considered the same as for possums 3 and 4.

Possum number (F/M)[a]	Group	Inoculum type[b]	Onset of clinical signs (days post-inoculation)							% weight change		Euthanasia (days post-inoculation)
			Decreased appetite	Behavioural change	Tremours	Head Tilt	Crouched hindlimb gait	Ataxia	Decreased ability to climb	During acclimatization	Post-inoculation	
1 (M)	1	None	–	–	–	–	–	–	–	+29	−5	42
2 (M)	1	None	–	–	–	–	–	–	–	−0.3	+7	42
3 (M)	1	CI	–	–	–	–	-	–	–	−24	+14	41
4 (F)	1	CI	–	–	–	–	–	–	–	−3	+6	41
5 (M)	2	WPDV	1	19	19	19	–	19	19	0	−0.5	20
6 (F)	2	WPDV	1	8	–	–	8	–	–	−14	−2	8
7 (M)	2	WPDV	2	19	20	–	8	20	20	+13	−34	20
8 (M)	2	WPDV	5	19	–	–	8	19	19	+18	-26	27
9 (M)	3	ICL	5	8	8	-	19	8	8	−5	−27	19
10 (F)	3	ICL	5	8	8		8	–	9	+11	−17	9
11 (M)	3	ICL	6	22	22	19	-	19	22	+4	−30	22
12 (F)	3	ICL	6	27	27		–	27	27	−4	−49	27
13 (F)	4	SI	5	19	23	19	19	19	23	+12	−22	23
14 (M)	4	SI	5	8	8	–	8	–	8	+56	−28	9
15 (M)	4	SI	5	–	–	–		–	–	−9	–	6 (Died)
16 (F)	4	SI	1	19	8	–	19	9	19	+20	−45	20

[a] F=female, M=male.
[b] CI=control inoculum, WPDV= purified WPD virus isolate, ICL=infected cell culture lysates, SI=standard inoculum.

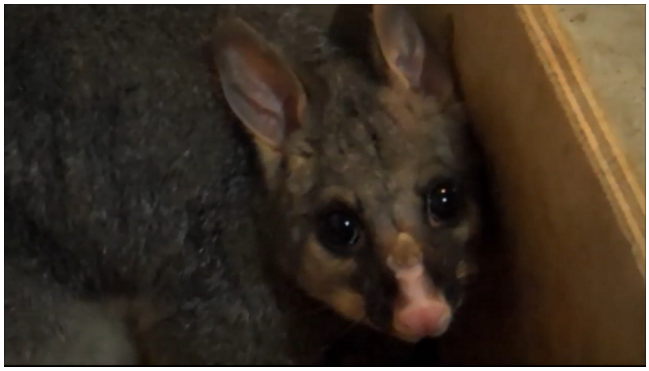
weight loss was less than 5% for 4 possums, with the highest weight loss (24%) recorded for one of the control possums (#3). This possum, however, regained its appetite during the course of the study, which was evidenced by a 12% weight gain recorded at the time of euthanasia.

None of the possums displayed any overt signs of external disease on clinical examination prior to inoculations. Group 1 comprised 3 males and 1 female with a dependent joey. Group 2 comprised 3 males and 1 female with a dependent joey. Group 3 comprised 2 males and 2 females, each with a dependent joey. Possum 12 was lacking a tail. However, the tail stump was covered in fur, without any signs of recent trauma. Group 4 comprised 2 males and 2 females, each with a dependent joey.

Clinical signs

All but one infected possums developed neurological signs consistent with WPD between 8 and 19 days post-inoculations and were subsequently euthanized (Table 1). Decreased appetite was observed in 12/12, weight loss in 11/12, and neurological signs in 11/12 of infected possums. Possum 15 (group 4) was polydipsic prior to inoculation and this continued post-inoculation. This possum died prior to the development of neurological signs 6 days post-inoculation. Three (6j, 12j, and 16j) of the 5 dependant joeys of WPDV-inoculated possums died. The 2 remaining joeys (10j and 13j) appeared clinically normal at the time of euthanasia. As joeys were still pouch-bound, this assessment was based on vigorousness of movement when removed from the pouch and external body condition.

A decreased appetite was first observed in all possums that received infectious inocula between days 1 and 6 post-infection, with an average onset of 3 days post-inoculation. Neurological signs (Table 2) observed in infected possums were indistinguishable between groups 2–4 and consisted of: decreased ability to climb (11/12), behavioural changes (11/12), tremors (9/12), ataxia (8/12), a crouched hindlimb gait (8/12), head tilt (4/12), and circling (2/12). Neurological signs were first observed between days 8 and 27, with an average onset of 9 days post-inoculation. Ataxia was characterized by a stumbling, rolling, swaying gait with or without hypermetria. Tremors were fine, generalized skeletal and often worsened with movement. Behavioural changes included loss of pre-existing fear response towards humans. A video (Video S1) showing examples of neurological deficits observed in experimentally infected possums is provided as supplementary data on-line. Control possums (group 1) including one dependent joey did not develop any clinical signs of WPD.



Video S1. Examples of the neurological deficits observed in experimentally infected possums. Head tremors, rolling, ataxia, decreased ability to climb and a decreased fear response to humans are demonstrated. A video clip is available online. Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.virol.2016.01.005.

Post-mortem examination

Gross pathological changes observed in infected adult possums included: poor body condition and renomegaly (all possums), hepatomegaly (possums 5, 7, 9, 16), splenomegaly (possums 5, 7, 9), cardiomegaly (possum 9), cloudy cerebrospinal fluid (possum 7), localized lymph node enlargement (possum 13) and petechiation of the gastrointestinal serosa, meninges, and renal capsule