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Short Communication

Rinderpest and peste des petits ruminants viruses exhibit neurovirulence in mice

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Members of the morbillivirus genus, canine distemper (CDV), phocine distemper virus (PDV), and the cetacean viruses of dolphins and porpoises exhibit high levels of CNS infection in their natural hosts. CNS complications are rare for measles virus (MV) and are not associated with rinderpest virus (RPV) and peste des petits ruminants virus (PPRV) infection. However, it is possible that all morbilliviruses infect the CNS but in some hosts are rapidly cleared by the immune response. In this study, we assessed whether RPV and PPRV have the potential to be neurovirulent. We describe the outcome of infection, of selected mouse strains, with isolates of RPV, PPRV, PDV, porpoise morbillivirus (PMV), dolphin morbillivirus (DMV), and a wild-type strain of MV. In the case of RPV virus, strains with different passage histories have been examined. The results of experiments with these viruses were compared with those using neuroadapted and vaccine strains of MV, which acted as positive and negative controls respectively. Intracerebral inoculation with RPV (Saudi/81) and PPRV (Nigeria75/1) strains produced infection in Balb/C and Cd1, but not C57 suckling mice, whereas the CAM/RB rodent-adapted strain of MV infected all three strains of mice. Weanling mice were only infected by CAM/RB. Intranasal and intraperitoneal inoculation failed to produce infection with any virus strains. We have shown that, both RPV and PPRV, in common with other morbilliviruses are neurovirulent in a permissive system. Transient infection of the CNS of cattle and goats with RPV and PPRV, respectively, remains a possibility, which could provide relevant models for the initial stages of MV infection in humans. *Journal of NeuroVirology* (2002) **8,** 45–52.

Keywords: rinderpest virus, peste des petits ruminants virus; measles virus; neurovirulence

Introduction

There is considerable variation among the members of the morbillivirus group of viruses, classified as a separate genus within the *Paramyxoviridae*, with regard to central nervous system (CNS) complications, following infection in their natural hosts. Canine distemper virus (CDV) leads in many cases to a fatal outcome as a result of CNS infection (Summers *et al*, 1984). Similarly, phocine distemper virus (PDV) and the cetacean viruses of dolphins (dolphin morbillivirus, DMV) and porpoises (porpoise morbillivirus, PMV) also exhibit high levels of CNS infection, which appears to be the main cause of death (Kennedy *et al*, 1988a, 1988b; Domingo *et al*, 1990). In contrast, rinderpest virus (RPV) and peste des petits ruminants virus (PPRV) are not associated with CNS complications in their respective hosts, cattle and goats.

Measles virus (MV) appears to occupy the middle of this spectrum, as under normal circumstances,

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infection in childhood produces an acute self-limiting infection, but occasionally CNS complications arise, namely, postinfectious measles encephalitis, measles inclusion body encephalitis, and subacute sclerosing panencephalitis. Nevertheless, it has been shown that pleocytosis in the cerebrospinal fluid occurs in 10% and electroencephalographic changes in 50% of children with normal measles infection (Hanninen *et al*, 1980), which suggests that transient infection of the CNS may be a normal occurrence. It is also possible that RPV (which is the most closely related morbillivirus to MV and from which MV may have entered the human population) and PPRV may also infect the CNS but be rapidly cleared by the immune response. Alternatively, features of the viruses may render them incapable of productively infecting neural tissues. Answers to these questions are crucial to our understanding of neurovirulence within the morbilliviruses. However, studies in the natural hosts of RPV and PPRV are both difficult and expensive to carry out and small animal models provide a means to initially assess whether these viruses have the potential to be neurovirulent.

A great deal of work has been published on MV and CDV infections of the CNS, using different virus isolates in a wide variety of rodents, including inbred mouse strains (Van Pottelsberghe *et al*, 1979; Rammohan *et al*, 1980, 1983; Lyons *et al*, 1980; Gilden *et al*, 1981; Bernard *et al*, 1983, 1991, 1993; Yoshikawa *et al*, 1983; Niewiesk *et al*, 1993). It has been reported for both viruses that virus origin, including passage history, host age, and genetics (particularly the major histocompatibility complex (MHC)) haplotype) affect the outcome of infection (Lyons *et al*, 1980; Yoshikawa *et al*, 1983; Morikawa *et al*, 1986; Niewiesk *et al*, 1993; Liebert and Finke, 1995).

There is only one report of RPV infection of suckling mouse brain (Imagawa, 1965). The Nakamura III strain of RPV, a lapinised vaccine subsequently adapted to tissue culture, was adapted over several passages for intracerebral growth in suckling mice by Imagawa (Imagawa, 1965), but adult mice were not susceptible to this neuroadapted RPV. Similar studies have not been carried out for PPRV, PDV, PMV, and DMV. Here we describe the outcome of infection, of selected mouse strains, with isolates of all known morbilliviruses, including wild-type strains of MV and RPV. No attempt was made to adapt these viruses to mice prior to these experiments being carried out. The results were compared with those obtained for a neuroadapted and a vaccine strain of MV, which acted as positive and negative controls, respectively.

Results

Intracerebral infection of suckling mice

Suckling mice were inoculated intracerebrally with the Hu2, CAM/RB, and MVP strains of MV, the Saudi/81, RBOK, and Kabete "O" strains of RPV, the Nigeria75/1 vaccine strain of PPRV, PDV, PMV, and DMV (details of these viruses are given in the Materials and methods). Animals were observed daily for clinical signs of infection and once apparent (between 4–8 d.p.i.) the mice were sacrificed. The remaining healthy mice were sacrificed from 10 to 22 d.p.i. Sections were examined by H&E for evidence of inflammation, and by immunohistochemistry for the presence of viral antigen.

Balb/C and Cd1, but not C57 suckling mice, showed signs of infection with RPV (Saudi/81 laboratory passaged), and the vaccine strain of PPRV (Nigeria75/1) (Diallo *et al*, 1989) Intracerebral infection with RPV produced clinical signs in 75% of Balb/C and 74% of Cd1 suckling mice. Intracerebral infection with PPRV produced clinical signs in 25% of Balb/C and 24% of Cd1 suckling mice. The clinical signs in RPV- and PPRV-infected mice occurred by 8 d.p.i. All three of the suckling mice strains examined showed clinical signs of infection with CAM/RB and all mice were dead by 4 d.p.i. Initial signs in mice, infected with CAM/RB, RPV, and PPRV, were hyperactivity, which was followed by disorientation, awkward gait, and finally, hind leg paralysis. The mice became moribund and death followed soon afterwards. Virus titres from selected, whole brains were between 10^3 and 10^4 $TCID_{50}$. Inoculations with all other virus strains (detailed in the materials and methods section) in suckling mice did not produce clinical signs.

All suckling C57, Cd1, and Balb/C mice, which exhibited clinical signs, suffered an acute infection with similar areas of the brain being involved. Balb/C suckling mice infected with CAM-RB, RPV Saudi/81, or PPRV Nigeria75/1 showed minimal perivascular inflammation (Figure 1A), yet Cd1 suckling mice showed foci of quite pronounced perivascular inflammation throughout the brain sections examined. Only CAM/RB produced clinical and pathological changes in C57 mice, which were similar to those seen in the Cd1 strain. In the case of each virus type, viral antigen was found in neurones and neuronal processes in the temporal, frontal, and olfactory cortices in both hemispheres (Figure 1B). Viral antigen was also found in neurones and their dendrite processes in the telencephalon layer of the hippocampus (Figure 1C). Virus-infected cells in the subpendymal layers were observed but the ependymal cells of the ventricle remained negative for viral antigen (Figure 1D). Virus was not detected in the cerebellum, meninges, or choroid plexus, but was observed in the neuronal cytoplasm and along the whole length of cortical axons (Figure 1E).

In some foci, viral antigen was detected along the entire length of the processes that connected two adjacent neurons (Figure 1F). Other cells, such as oligodendrocytes, astrocytes, microglia, and endothelial cells were consistently negative. Sections from antigen-positive brains gave no staining when

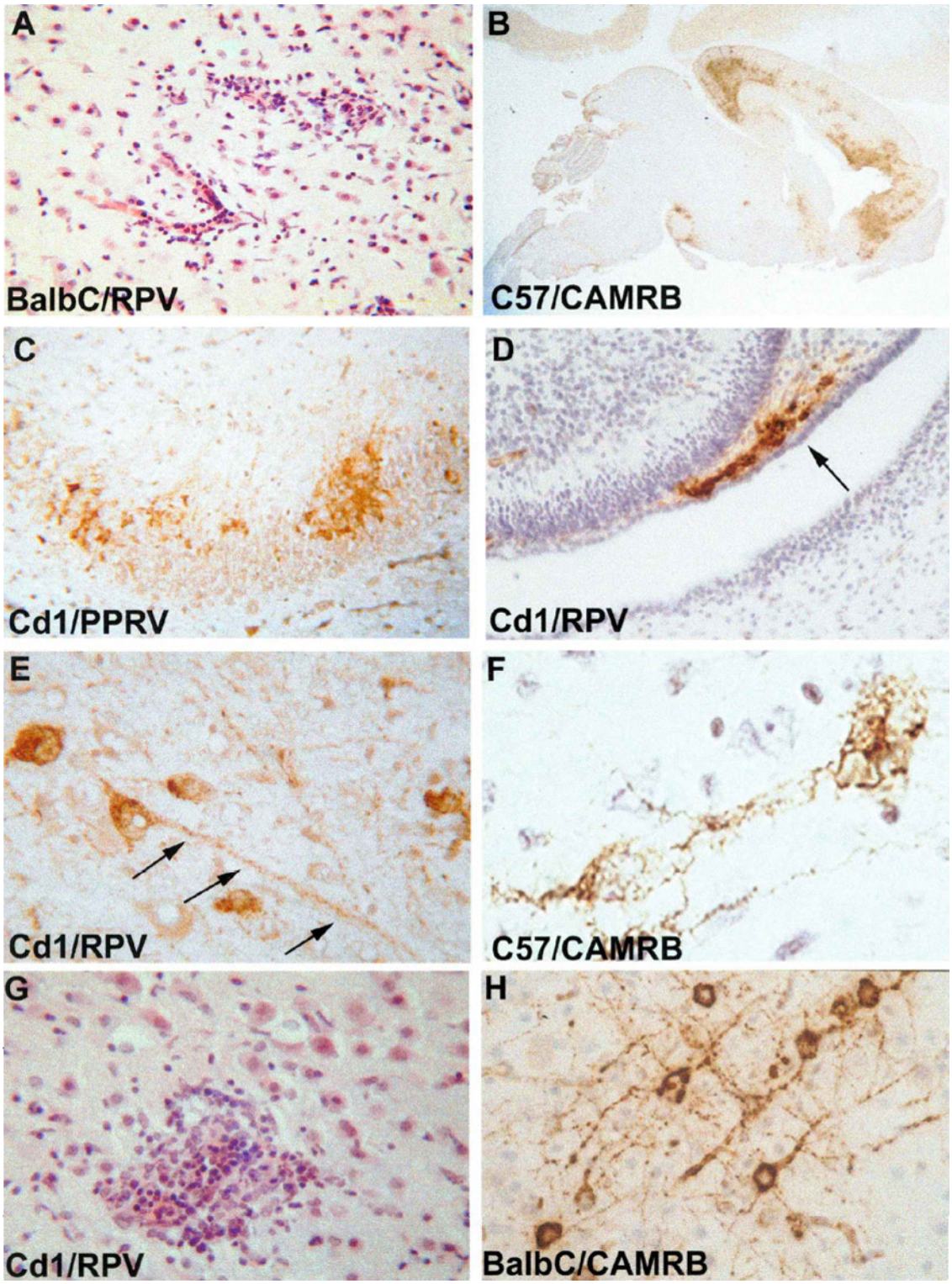

Figure 1 Histology and immunohistochemistry of brain sections from mice after intracerebral infection with morbilliviruses. Sections were formalin-fixed and antigens detected with an anti-(N) monoclonal antibody (measles) or an anti-RPV polyclonal antibody (other morbilliviruses); positive virus staining appears brown. (**A–G**) Suckling mice at 4 days postinfection. (**A**) Minimal inflammation; (**B**) extensive viral antigen throughout the cortex; (**C**) abundant viral antigen in the neurones and neuronal processes of the hippocampus; (**D**) virus in subependymal neurons. However, the ependymal cells lining the ventricle are negative (arrow); (**E**) the entire process of a neuron contains viral antigen (arrows); (**F**) two infected neuron cell bodies are connected by viral positive processes; (**G**) foci of perivascular inflammation; (**H**) pronounced viral antigen staining is present in neurons and processes in weanling mouse at 7 days postinfection. Sections were photographed on slide film and digitized with a Kodak RFS 2035 Plus Film scanner. The final resolution of all scanned images was 300 dots per in.

Table 1 Immunocytochemical results after CNS infection by different morbilliviruses in different murine strains

	Saudi/81(laboratory passaged RPV)		*Nigeria75/1 (PPRV)*		*CAM-RB (MV)*	
	Days postinfection					
	4–8	10–22	4–8	10–22	4–8	10–22
C57s	–	–	–	–	+++++	NS
C57w	–	–	–	–	++++	NS
Cd1s	++++	–	+++	–	+++++	NS
Cd1w	–	–	–	–	++*	+*
Balb/Cs	+++	–	++++	–	+++++	NS
Balb/Cw	–	–	–	–	+*	+*

The Hu2 and MVP strains of MV, PDV, PMV, DMV, and the RBOK and Kabete "O" strains of RPV were negative for viral antigen in the brain of all mice examined. Suckling (s) and weanling (w) C57, Cd1, and Balb/C mice were infected with the different morbilliviruses and viral antigen in the brain was detected by immunocytochemistry and results scored as follows: NS = non-surviving, – = no virus present, + = 1–2 foci of virus-infected cells, ++ = 3–4 foci of virus-infected cells, +++ = 6-many foci of virus-infected cells, ++++ = many discrete foci of virus-infected cells, +++++ = widespread virus-infected cells, and * = only a few virus-infected cells in each focus.

the primary antibody was omitted. The level of viral antigen staining in the brains of mice showing acute disease from 4 to 8 d.p.i. is indicated in Table 1. RT-PCR analysis of RNA from the brains of inoculated animals was carried out. Representative PCR products are shown in Figure 2.

The negative results for RPV Saudi/81 and PPRV Nigeria 75/1 in C57 mice (Figure 2a, 2b) and for Hu2 and MVP in all mouse strains at 4 to 8 d.p.i. (results not shown) were confirmed.

A beta actin cDNA product was amplified in all cases (Figure 2c), indicating that RNA isolation and reverse transcription was successful. The RPV, PPRV, and MV primers sets amplified products from infected mouse brains that were also positive for viral antigen (Figures 2a, b, c). All healthy surviving mice, including mock-infected and those inoculated with Hu2, MVP, PDV, PMV, and DMV, showed no evidence of viral antigen in the brain. However, perivascular inflammation, not related to trauma at the injection site, was observed in 50% of mice inoculated with RPV Saudi/81 and PPRV Nigeria 75/1, from 10 to 22 d.p.i. (Figure 1G). This was not observed in animals inoculated with the other viruses under test but these occasionally showed inflammation at the injection site. RNA was detected in the brains of surviving Cd1 and Balb/C sucklings at 10 to 22 d.p.i., in mice infected with RPV Saudi/81 (Figure 2a, lane 6) but not those infected with PPRV (Figure 2b, lane 7).

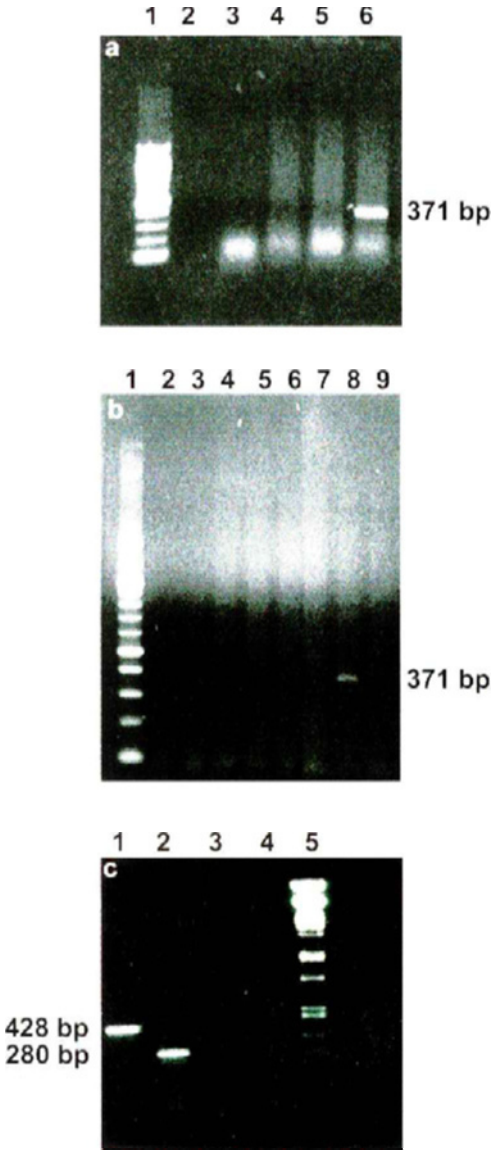

Figure 2 RT-PCR analysis of RNA derived from brains of mice after intracerebral inoculation with morbilliviruses. (**a**) Lane 1, λ DNA markers; lanes 2 to 6, RPV F primers: lane 2, water; lane 3, mock-infected C57 mouse; lane 4, mock-infected Balb/C mouse; lane 5, C57 mouse (6 d.p.i.) with RPV Saudi/81; lane 6, Balb/C mouse (20 d.p.i.) with RPV Saudi/81. (**b**) Lane 1, γ DNA markers; lanes 2–9, PPRV F primers: lane 2, water; lane 3, mock-infected Cd1 mouse; lane 4, mock-infected BalbC mouse; lane 5, mock-infected C57 mouse; lane 6, Balb/C mouse (20d.p.i.) with PPRV Nigeria 75/1, lane7, Cd1 mouse (20 d.p.i.) with PPRV Nigeria 75/1; lane 8, Cd1 mouse (8 d.p.i.) with PPRV Nigeria; lane 9, C57 mouse (6 d.p.i.) with PPRV Nigeria 75/1. (**c**) lane 1, B actin primers, Balb/C mouse (6 d.p.i.) with CAM /RB MV; lanes 2–4, MV P primers; lane 2, Balb/C mouse (6 d.p.i.) with CAM/RB MV; lane 3, water; lane 4, mock infected Balb/C mouse; lane 5, γ DNA markers.

Intracerebral infection of weanling mice

Only the three strains (CAM/RB MV, RPV Saudi/81, and PPRV Nigeria 75/1), which exhibited neurovirulence in suckling mice, were assessed for their ability to infect weanling mice. Balb/c mice were inoculated with the Hu2 strain of MV to act as an additional control to mock infection. Inoculated mice were observed daily for clinical signs of infection and, once apparent, the mice were sacrificed. Healthy mice were sacrificed between 10 to 22 d.p.i. Brain sections from these mice were analysed in the same way as those prepared from the suckling mice. C57 mice, infected with the CAM/RB strain of MV, developed

acute encephalitis at 6 to 8 d.p.i., with similar clinical signs to that produced in suckling mice. Virus titres of between 10^3 and $10^{4.5}$ $TCID_{50}$ were isolated from selected, whole brains. None of the weanling mice inoculated with RPV, PPRV, or Hu2 showed clinical signs and only the brain sections from Cd1 and Balb/c mice, infected with the CAM/RB strain of MV, showed positive viral antigen staining. The distribution of the viral antigen in these weanling mice brains was the same as described for the suckling mice. However, brain sections showed minimal perivascular inflammation and there were only a few virus positive neuronal bodies. Staining of neuronal processes was observed (Figure 1H). Control sections where the primary antibody was omitted showed no virus antigen staining. No infectious virus was recovered from these mice.

Intranasal and intraperitoneal infection in Cd1 suckling mice

The three viruses that were found to be neurovirulent by i.c. infection were tested for infection by peripheral routes in CD1 mice. C57 mice were also examined after inoculation with CAM-RB. Intranasal inoculation of suckling mice with the CAM/RB strain of MV, the Saudi/81 laboratory passaged strain of RPV, and the Nigeria75/1 vaccine strain of PPRV produced no clinical signs. There was no viral antigen staining and no evidence of inflammation in the brain sections taken from these mice. Intraperitoneal (i.p.) inoculation of groups of C57 and Cd1 mice with CAM/RB and Cd1 mice with RPV Saudi/81 or PPRV Nigeria 75/1 also produced no clinical signs or evidence of CNS infection. Peripheral organs (lungs, spleen, liver, intestine) from 2 C57 mice inoculated i.p. with CAM/RB were examined by immunohistochemistry and found to be negative.

Discussion

Neurovirulence of a virus for its host may depend on a number of different factors, including the presence of the necessary receptors and/or intracellular factors in CNS cells, the ability of the virus to cross the blood–brain barrier and the effectiveness of the immune response in clearing virus from the brain. In this study, the ability of a number of different morbilliviruses, not previously adapted to mouse brain, to replicate in the murine CNS was compared to that of a virus known to replicate efficiently in the mouse brain (CAM/RB neuroadapted MV) and one that was shown previously not to infect mouse brain (Hu2 MV). Following i.c. inoculation, the same clinical signs were observed in suckling mice infected with MV (CAM/RB), RPV (Saudi/81 laboratory passaged strain), and PPRV (Nigeria75/1). All three strains showed similar viral antigen distribution in the mouse brain to that described in previously published studies of CDV and MV infections in mice (Imagawa, 1968; Van Pottelsberghe *et al*, 1979; Rammohan *et al*, 1980, 1983; Bernard *et al*, 1991, 1993). These morbilliviruses were found to preferentially infect neurones of the limbic system, especially in the hippocampus. The predilection for specific brain regions may either reflect localisation of receptor(s) or more likely spread along common neuronal pathways. However, which of these alternatives is the case cannot be decided until a neuronal receptor for at least one of the viruses has been identified and specific monoclonal antibody to this molecule is available.

The RBOK and virulent Kabete "O" strains of RPV, the Hu2 and MVP strains of MV, PDV, DMV, and PMV did not infect any of the suckling mice strains. Furthermore, only Balb/C and Cd1, but not C57, suckling mice were susceptible to infection with RPV Saudi/81 and PPRV/Nigeria, while CAM/RB produced acute disease in all three mouse strains. It is possible that C57 mice lack neuronal receptors for Vero cell–adapted RPVSaudi/81 and PPRV Nigeria 75/1, but have the receptor for neuroadapted MV. However, this does not explain why the RBOK vaccine strain of RPV, which was derived from the Kabete "O" wild-type strain (Plowright and Ferris, 1962) did not infect the CNS of Balb/C and Cd1 mice, as this virus was also passaged many times in Vero cells. Growth in Vero cells evidently does not adapt MV strains for the murine CNS. On the contrary, only the rodent adapted CAM-RB and hamster neurotropic (HNT) strains of MV are neurovirulent for mice and not the Edmonston vaccine or Edmonston-derived Hu2 strain, both of which have been extensively passaged in Vero cells. The MVP strain that is a wild-type isolate has also been given a number of passages in Vero cells and is also not neurovirulent for mice. We have shown recently that the haemagglutinin (H) gene of the CAM/RB strain confers partial neurovirulence for mice to the Edmonston vaccine strain (Duprex *et al*, 1999). Vero cell adaptation could alter the H protein, allowing infection of the murine CNS by some strains of RPV and PPRV but other determinants of neurovirulence (perhaps on other viral proteins) may also be necessary. The situation may be different to that of MV as our initial results indicate that RPV does not use the same receptor as MV in Vero cells (Galbraith *et al*, 1998).

The outbred strain of Cd1 mice appears to be the most susceptible to RPV and PPRV, as suckling mice showed more extensive inflammation than Balb/C mice. It is possible to adapt MV and CDV strains to growth in weanling mice by passage in suckling rodent brains (Bernard *et al*, 1983; Liebert and ter Meulen, 1987). The Nakamura III vaccine strain of RPV, adapted to growth in suckling mouse brains, did not produce signs of infection in CFW weanling brains (Imagawa, 1965). However, these workers did not carry out a pathological study of the brains. Therefore, it is possible that some replication did occur. Further virus passage in suckling Cd1 mice

would have to be performed to determine if it is possible to select for replication of RPV and PPRV in weanling mouse brains.

Intraperitoneal (i.p.) inoculations, with the CAM/RB MV, RPV Saudi/81 laboratory-passaged and the PPRV Nigeria75/1 strains, did not cause CNS infection. Immunohistochemical examination of peripheral organs of mice inoculated with CAM-RB MV by the i.p. route showed that no infection had occurred, in agreement with a previous study (Liebert and Finke, 1995). Similarly, inoculation of mice with CAM-RB, RPV Saudi 81, or PRPV Nigeria 75/1 by the olfactory route did not cause infection. Olfactory neurones in the nasal cavity are connected to limbic and monoaminergic cells in the brain and olfactory pathways are an important route for infection of the murine brain with other viruses (Tyler and Fields, 1996). In this study, no antigen was detected in olfactory neurones. It has been previously shown that intranasal inoculation with the HNT strain of MV causes encephalitis in suckling hamsters, with viral antigen being detected in lung and brain tissue (Parhad *et al*, 1981), however it does not infect mice by this route.

The degree of neurovirulence for mice shown by the morbillivirus strains tested ranges from acute encephalitis, with virus production, to what appears to be total refractivity to infection/replication. However, between these two extremes there is evidence of subacute infection with viral antigen production. As was found in previous studies, the outcome of infection is dependent on many factors, including the strain of virus and the genetic background and age of the mice. Although neurovirulence in mice does not correlate with the ability of the virus to cause encephalitis in the natural host (the naturally highly neurovirulent phocine and cetacean viruses did not exhibit this property in mice), we have shown that both RPV and PPRV, in common with the other morbilliviruses can be neurovirulent in a permissive mouse model. In this study, no viral antigen was detected in the brains of surviving mice between 10 and 22 days d.p.i., inoculated with RPV Saudi/81 (25%) and PPRV Nigeria 75/1 (75%), However, low levels of perivascular inflammation were detected in approximately half of the animals (as well as RNA in the case of RPV Saudi/81). Because this was not observed with the other test viruses, it is possible that virus infects the brain but is rapidly cleared from the CNS in these surviving animals. A similar situation may occur in the natural host. Although finding low levels of virus replication in young cattle or goats, as previously suggested (Bergeon, 1952; MacOwen, 1956; Plowright, 1964) would not have any practical implications with regard to RPV and PPRV, it may give insight into the events during the initial stages of measles virus infection in humans. These experiments are currently underway and may help to determine why certain individuals, including those with a defective immune response, are susceptible to CNS complications.

Materials and methods

Cells and viruses

Vero cells were grown in DMEM-Hepes containing 5% (v/v) FCS, 100 U/ml benzylpenicillin and 100 U/ml streptomycin. B95a cells were obtained from Professor Kobune, National Institute of Health, Tokyo, Japan, and grown in RPMI-Hepes containing 5% (v/v) FCS, 100 U/ml benzylpenicillin, and 100 U/ml streptomycin (Kobune *et al*, 1990). Primary bovine skin fibroblast cells were prepared from superficial bovine skin biopsies as previously described (Lund and Barrett, 2000), and grown in fresh DMEM containing 20% FCS, 200 U/ml benzylpenicillin, 200 U/ml streptomycin, 3% fungizone, and 2 ng/ml EGF.

The Human 2 (Hu2) strain of MV was derived from the Edmonston vaccine strain (Mawhinney *et al*, 1971) and propagated in Vero cells. The rodent-adapted neurotrophic CAM/RB strain of MV was the kind gift of Dr UG Liebert, Würzburg, Germany (Liebert and ter Meulen, 1987). This virus was further adapted to growth in the murine CNS by two passages in suckling mouse brains. The MVP strain of MV was isolated from a child with primary measles (Rima *et al*, 1983) and was subsequently given several passages in Vero cells. The Saudi/81 strain of RPV was originally isolated from infected bovine tissues, using primary bovine kidney cells and subsequently passaged on both B95a and Vero cells to produce laboratory-passaged stocks. The RBOK (rinderpest bovine old Kabete) vaccine strain of RPV (Plowright and Ferris, 1962) was derived from the virulent Kabete "O" strain of RPV, which was attenuated by 95 passages on primary bovine kidney cells and for laboratory use it was adapted to growth on Vero cells. The Kabete "O" wild-type strain of RPV (Plowright and Ferris, 1962) was maintained by animal passage. The viruses used in these experiments was isolated from freeze-dried lymphoid tissue on primary bovine skin fibroblast cells and a high-titre virus stock was prepared on B95a cells (Lund *et al*, 2000). The Nigeria 75/1 vaccine strain of PPRV was attenuated by multiple passages on Vero cells (Diallo *et al*, 1989). PDV (Cosby *et al*, 1988), PMV (Kennedy *et al*, 1988b) and DMV (Domingo *et al*, 1990) isolates were grown on Vero cells.

Mice

C57 and Balb/C mice were obtained from in-house breeding colonies in the Laboratory Service Unit, Medical Biology Centre, Queens University Belfast and from breeding colonies at the Small Animal Unit, the Institute for Animal Health, Pirbright Laboratory. Cd1 mice were obtained from breeding colonies at the Institute for Animal Health, Pirbright Laboratory. The mice were kept in a barrier system with light, negative pressure and 12-h day (artificial light). They had a plentiful supply of food and water and air

conditioning kept the temperature at 22°C ± 2°C and the humidity at 50% ± 5%.

Virus infections
Routinely, at least 10 mice were inoculated with each virus strain. Suckling mice (3–5 days) and weanling mice (21–25 days) were injected under mild halothane anaesthesia, either intraperitoneally or intracerebrally, in the temporal region of the right cerebral hemisphere, with approximately 10^4 $TCID_{50}$ of each virus in 2-μl (suckling mice) or 50-μl (weanling mice) volumes. Virus was introduced intranasally by inhalation of a 50-μl volume. Control mice were inoculated, using each of the preceding routes, with equivalent volumes of tissue culture medium. Mice were sacrificed under ether narcosis at the time points shown in Table 1. Brain tissue was removed from the skull immediately following death and frozen at −70°C for isolation of virus and RT-PCR or fixed in 5% formal saline at room temperature for 24 h and then embedded in paraffin wax for immunohistochemistry. RNA was extracted from at least two mouse brains for RT-PCR analysis (for MV, RPV, and PPRV inoculated suckling mice) and two for assay of infectious virus at 4 to 8 days and in surviving mice at 10 to 20 days. At least three mice were examined by immunohistochemistry at each of the two time points (suckling and weanling mice). A minimum of three sections at each of two levels through the brain was examined in each case. Foci of infected cells (determined by immunohistochemistry) were scored in each section with average values shown in Table 1.

Virus isolation and titration
Virus was isolated by homogenisation of brain tissue and collection of the supernatant, following centrifugation at 2000 rpm in a bench centrifuge. The supernatant was titred on Vero cells, by $TCID_{50}$ assay, using the method of Reed and Muench (1938).

Immunohistochemistry
Except where specified, all of the secondary antibodies and normal sera for blocking nonspecific immunoglobulin binding were obtained from Dako (Denmark). Sections were dewaxed through two changes of xylene and rehydrated through graded alcohols. Endogenous peroxidase was blocked by 10-min incubation in 3% H_2O_2 in methanol. Immunoreactivity was enhanced by microwaving at 700 W for 30 min in 0.01 M sodium citrate at pH 6.0. Normal rabbit serum (NRS 5%, MV) or normal swine serum (NSS, 5%, all other morbilliviruses) was applied to sections for 10 min at RT and they were then incubated with a 1/1000 dilution of monoclonal antibody against the MV nucleocapsid (N; Harlan Sera-lab clone 49.21, MV) or anti-RPV polyclonal antibody (produced in rabbits at the Institute for Animal Health, Pirbright Laboratory, all other morbilliviruses) at a dilution of 1/600 overnight at 4°C. For immunoperoxidase staining, sections (MV) were incubated at RT for 30 min with biotinylated rabbit anti-mouse immunoglobulins at a dilution of 1/400 or with biotinylated swine anti-rabbit immunoglobulins (other morbilliviruses) at a dilution of 1/400. All sections were then incubated with streptavidin-biotin-peroxidase complex at RT for 30 min. Antigen-antibody reactions were visualized by incubation with diaminobenzidine (DAB) at RT for 3 min. Sections were counterstained in haematoxylin, dehydrated through graded alcohols, and mounted in DPX. Sections from normal mouse brain were treated in the same way and each experiment included negative controls, where sections were treated with buffer, rather than primary antibody.

RT-PCR analysis
Total RNA was extracted from mouse brain by one of two methods, either (a) homogenisation in guanadine isothiocyanate , followed by centrifugation on a CsCl (Sambrook *et al*, 1989) or (b) guanidinium-thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi, 1987). Reverse transcription was carried out using random hexanucleotide primers (in house). PCR to amplify β-actin cDNA was performed on all mouse brains tested to confirm the integrity of the RNA. The sequence of these primers has been previously published (Patterson and Fann, 1992). The primers for the genes, their position in the genome and product size (in brackets) are as follows:

MVP (428)	ATGTTTATGATCACAGCGGT	2138–2157
	ATTGGGTTGCACCACTTGTC	2547–2566
RPVF (371)	GGGACAGTGCTTCAGCCTATTAAGG	818–842
	CAGCCCTAGCTTCTGACCCACGATA	1165–1189
PPRVF (371)	ATCACAGTGTTAAAGCCTGTAGAGG	777–801
	GAGACTGAGTTTGTGACCTACAAGC	1124–1148

The PCR reaction conditions were denaturation at 94°C for 30 s, primer annealing at 50°C for 30 s, and primer extension at 72°C for 2 min for 40 cycles. A final extension step of 72°C for 10 min was also included.

References

Bergeon P (1952). Peste bovine richesse en virus pestique des tissus nerveux et de la moelle osseuse de veaux atteints de peste bovine experimentale. *Bull Soc Pathol Exotique* **12:** 148–152.

Bernard A, Fevre-Montange M, Bencsik A, Giraudon P, Wild F, Confavreux C, Belin M-F (1993). Brains structures selectively targeted by canine distemper virus in a mouse model infection. *J Neuropathol Exp Neurol* **52:** 471–480.

Bernard A, Fevre-Montange M, Giraudon P, Hardin H, Wild F-B, Belin M-F (1991). Demonstration of viral proteins and RNA in hypothalamus of mice infected by canine

distemper virus. *Comptes Rendus Academy of Science III (Paris)* **313:** 545–551.

Bernard A, Wild T-F, Tripier MF (1983). Canine distemper virus infection in mice: characterisation of a neuroadapted virus strain and its long-term evolution in the mouse. *J Gen Virol* **64:** 1571–1579.

Chomczynski P, Sacchi N (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-choroform extraction. *Anal Biochem* **162:** 156–159

Cosby SL, McQuaid S, Duffy N, Lyons C, Rima BK, Allen GM, McCullough JM, Kennedy S, Smith JA, McNeilly F, Craig C, Orvell C (1988). Characterisation of a seal morbillivirus. *Nature* **336:** 115–116.

Diallo A, Taylor WP, Lefevre PC, Provost A (1989). Attenuation of a virulent PPRV strain: potential homologous live vaccine. *Rev d'Elevage Med Vet Pays Trop* **42:** 311–319.

Domingo M, Ferrier L, Pumarola M, Marco A, Plana J, Kennedy S, McAlisky M, Rima BK (1990). Morbillivirus in dolphins. *Nature* **348:** 21.

Duprex WP, Duffy I, McQuaid S, Hamill L, Cosby SL, Billeter MA, Schneider- Schaulies J, ter Meulen V, Rima BK (1999). The H gene of rodent brain-adapted measles virus confers neurovirulence to the Edmonston vaccine strain. *J Virol* **73:** 6916–6922.

Galbraith SE, Tiwari A, Baron MD, Lund BT, Barrett T, Cosby SL (1998). Morbillivirus downregulation of CD46. *J Virol* **72:** 10292–10297.

Gilden DH, Wellish M, Rorke LB, Wroblewska Z (1981). Canine distemper virus infection of weanling mice. Pathogenesis of CNS disease. *J Neuro Sci* **52:** 327–339.

Hanninen P, Artstila P, Lang H, Salmi A, Panelius M (1980). Involvement of the central nervous system in acute uncomplicated measles virus infection. *J Clin Microbiol* **11:** 610–613.

Imagawa DT (1965). Propagation of rinderpest virus in suckling mice and its comparison to murine adapted strains of measles and distemper. *Arch Gesamte Virusforsch* **17:** 203–215.

Imagawa DT (1968). Relationships amongst measles, canine distemper and rinderpest virus. *Prog Med Virol* **10:** 160–193.

Kennedy S, Smyth JA, McCullough SJ, Allan GM, McNeilly F, McQuaid S (1988a). Confirmation of cause of recent seal deaths. *Nature* **335:** 404.

Kennedy S, Smyth JA, McCullough SJ, Allan GM, McQuaid S (1988b). Viral distemper now found in porpoises. *Nature* **336:** 21.

Kobune F, Sakata H, Sigiura A (1990). Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. *J Virol* **64:** 700–705.

Liebert UG, Finke D (1995). Measles virus infections in rodents. *Curr Top Micro Immunobiol* **191:** 149–166.

Liebert UG, ter Meulen V (1987). Virological aspects of measles virus-induced encephalomyelitis in Lewis and BN rats. *J Gen Virol* **68:** 1486–1493.

Lund BT, Barrett T (2000). Rinderpest virus infection in primary bovine skin fibroblasts. *Arch Virol* **145:** 1231–1237.

Lund BT, Tiwari A, Galbraith SE, Baron MD, Morrison WI, Barrett T (2000). Vaccination of cattle with attenuated rinderpest virus stimulates CD4+ T cell responses with broad viral specificity. *J Gen Virol* **81:** 2137–2146.

Lyons MJ, Hall WW, Petito C, Cam V, Zabriskie JB (1980). Induction of chronic neurologic disease in mice with canine distemper virus. *Neurology* **30:** 92–98.

MacOwen KDS (1956). *Annual Report of Department of Veterinary Services for Kenya for 1955*. 26–31.

Mawhinney H, Allen IV, Beare JM, Bridges JM, Connolly JH, Haire M, Nevin C, Neill DW, Hobbs JR (1971). Dysgammaglobulinaemia complicated by disseminated measles. *Br Med J* **2:** 380–381.

Morikawa Y, Matsubara Y, Yoshikawa Y, Kai C, Yamanouchi K (1986). Neurovirulence in mice of neural cell adapted canine distemper virus. *Micro Immunobiol* **30:** 225–236.

Niewiesk S, Brinckmann U, Bankamp B, Sirak S, Liebert U, ter Meulen V (1993). Susceptibility to measles virus-induced encephalitis in mice correlates with impaired antigen presentation to cytotoxic T lymphocytes. *J Virol* **67:** 75–81.

Parhad IM, Johnson KP, Wolinsky JS, Swoveland P (1981). Encephalitis after inhalation of measles virus: a pathogenetic study in hamsters. *Ann Neurol* **91:** 21–27.

Patterson, PH, Fann, M-J (1992). Further studies of the distribution of CDF/LIF mRNA. In: *Polyfunctional Cytokines: Il-6 and LIF*, vol 167. Ciba Foundation Symposium, Wiley, Chichester, pp 125–140.

Plowright W (1964). Rinderpest virus. *Ann NY Acad Sci* **101:** 548–563.

Plowright W, Ferris RD (1962). Studies with Rinderpest virus in tissue culture. The use of an attenuated culture virus as a vaccine for cattle. *Res Vet Sci* **3:** 172–182.

Rall GF, Manchester M, Daniels LR, Callahan EM, Belman AR, Oldstone MB (1997). A transgenic mouse model for measles virus infection of the brain. *Proc Natl Acad Sci USA* **94:** 4659–4663.

Rammohan KW, Dubois-Dalcq M, Rentier B, Paul J (1983). Experimental models to study measles virus persistence in the nervous system. *Prog Neuropathol* **5:** 113–137.

Rammohan KW, McFarlin DE, McFarlin HF (1980). Chronic measles encephalitis in mice. *J Inf Dis* **142:** 608–613.

Reed LJ, Muench H (1938). A simple method for estimating fifty percent end points. *Am J Hyg* **27:** 493–497.

Rima BK, Roberts MW, Martin SJ (1983). Comparison of morbillivirus proteins by limited proteolysis. *Med Microbiol Immunol (Berl)* **171:** 203–213.

Sambrook J, Fritsch EF, Maniatis T (1989). *Molecular Cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY.

Schneider-Schaulies J, Dunster LM, Schwartz-Albiez R, Krohne G, ter Meulen V (1995). Physical association of moesin and CD46 as a receptor complex for measles virus. *J Virol* **69:** 2248–2256.

Summers BA, Greisen HA, Appel M (1984). Canine distemper encephalomyelitis variation with virus strain. *J Comp Pathol* **94:** 65–75.

Tyler KL, Fields BN (1996). Pathogenesis of virus infections. In: *Fields Virology*, 3rd ed. Fields BN, Knipe DM, Howley PM (eds). Lippincott-Raven Publishers: Philadelphia, pp 173–218.

Urbanska EM, Chambers BJ, Ljunggren HG, Norrby E, Kristensson K (1997). Spread of measles virus through axonal pathways into limbic structures in the brain of TAP1 −/− mice. *J Med Virol* **52:** 362–369.

Van Pottelsberghe C, Rammohan KW, McFarland HF, Dubois-Dalcq M (1979). Selective neuronal, dendritic, and postsynaptic localisation of viral antigen in measles-infected mice. *Lab Invest* **40:** 99–108.

Yoshikawa Y, Yamanouchi K, Morikawa Y, Sakaguchi M (1983). Characterisation of canine distemper viruses adapted to neural cells and their neurovirulence in mice. *Microbiol Immunobiol* **27:** 503–518.