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Short Communication

The wood mouse is a natural host for *Murid herpesvirus 4*

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Infection of laboratory mice by the *Murid herpesvirus 4* (MHV-4) is a much studied model system for gammaherpesvirus pathogenesis. Little, however, is known about its natural host range, epidemiology and pathogenesis outside the laboratory. We have studied MHV-4 infection in free-living murids in the UK. Using a combination of serology and PCR analysis, we found that MHV-4 was endemic in wood mice (*Apodemus sylvaticus*) but not in two species of voles (*Clethrionomys glareolus*, *Microtus agrestis*). The sites of detection of viral DNA were the lungs and, less commonly, the spleen, emphasizing the importance of the former in virus persistence during natural infection and confirming similar data in laboratory mice.

Infection of laboratory mice by *Murid herpesvirus 4* (MHV-4) is an excellent model system for the study of gammaherpesvirus pathogenesis and for the development of therapeutic strategies against these viruses (Nash *et al.*, 2001; Stewart, 1999). Following intranasal inoculation of mice with MHV-4 strain 68 (MHV-68), an initial productive infection occurs in the lungs (Sunil-Chandra *et al.*, 1992a). This is cleared from the lungs around day 10 p.i. by $CD8^+$ T-cells (Ehtisham *et al.*, 1993) but the virus then persists in a latent form in epithelial cells at this site (Stewart *et al.*, 1998). MHV-68 spreads to the spleen during the subsequent viraemia, where it becomes latent in B lymphocytes, macrophages and dendritic cells (Flano *et al.*, 2000; Sunil-Chandra *et al.*, 1992b; Usherwood *et al.*, 1996; Weck *et al.*, 1999).

In spite of our knowledge of its pathogenesis in laboratory mice, little is known about the epidemiology and diversity of MHV-4 in the wild. Field studies in Slovakia, originally aimed at identifying a vector for flaviviruses, resulted in the isolation of five herpesviruses from two species of murid rodents (Blaskovic *et al.*, 1980). Strains 60, 68 and 72 were isolated from the bank vole (*Clethrionomys glareolus*) and strains 76 and 78 from the yellow-necked wood mouse (*Apodemus flavicollis*). Further molecular studies of MHV-68 and sequencing of the genome showed that it belongs to the genus *Rhadinovirus* (often referred to as γ_2-herpesviruses), which includes *Human herpesvirus 8* (Kaposi's sarcoma-associated herpesvirus) and *Saimiriine herpesvirus* 2 (herpesvirus saimiri) (Efstathiou *et al.*, 1990; Nash *et al.*, 2001; Virgin *et al.*, 1997). Of the other murine herpesvirus strains isolated alongside MHV-68 (Blaskovic *et al.*, 1980), only strains 72 (MHV-72) and 76 (MHV-76) have been studied. Strain 72, which was isolated from the same host as MHV-68, appears to have similar biological properties in that it infects B lymphocytes (Mistrikova *et al.*, 1994; Raslova *et al.*, 2000) and long-term infection is associated with tumorigenesis (Mistrikova *et al.*, 1996). In contrast, strain 76 was isolated from a different murid species (Blaskovic *et al.*, 1980). Biological and molecular analyses of this strain, however, have shown that it is essentially identical to MHV-68 except for a deletion of 10 kb at the left-hand end of the unique portion of the genome and a subsequent attenuation *in vivo* (Macrae *et al.*, 2001). The nature of this deletion strongly suggested that it was generated by *in vitro* passage. Thus, all the strains studied so far appear to be extremely similar.

Field studies aimed at determining the presence of MHV-4-like viruses in the wild have been carried out in Slovakia and have reported a seroprevalence of between 1 and 12 % depending on the region (Kozuch *et al.*, 1993; Mistrikova & Blaskovic, 1985). However, it was not clear from these studies what the prevalence was in any given species of rodent. The aim of this study was to assess the prevalence of MHV-4 in the species of free-living murid rodents most likely to be its hosts in the UK.

Sera (5–10 µl per live animal and up to 500 µl from killed animals) were collected from wild populations of wood mice (*Apodemus sylvaticus*), bank voles and field voles (*Microtus agrestis*) as part of ongoing studies of the ecology of endemic infections of wild rodents (Begon *et al.*, 1999; Birtles *et al.*, 2001; Telfer *et al.*, 2002). Live voles and wood mice were sampled from the north of England and killed wood mice from Northern Ireland (Begon *et al.*, 1999; Chantrey *et al.*,

1999). Sera were tested for IgG antibody to MHV-4 in an immunofluorescence assay (IFA) essentially as described for cowpox antibody (Crouch *et al.*, 1995) except using MHV-68-infected Vero cells as antigen.

Antibody reactive with MHV-4 was detected in 36 of 273 (13 %) English and 36 of 149 (24 %) Northern Irish wood mice. In contrast, only 8 of 295 (2.7 %) bank vole sera contained detectable antibody to MHV-4, and no antibody was detected in sera from 135 field voles.

Thus, in spite of a number of strains of MHV-4 reported as being isolated from voles in the original studies (Blaskovic *et al.*, 1980), these serological data strongly suggest that MHV-4 is endemic in wood mice but not voles, at least in the UK.

To confirm and extend the serological data, we performed PCR analysis on infected mouse tissues. MHV-4 DNA can be readily detected by PCR analysis in the spleens and lungs of infected mice for the lifetime of the animal (Stewart *et al.*, 1998). Thus, DNA was extracted from the spleens and lungs of wood mice and bank voles using QIAamp tissue kits (Qiagen). To check the integrity of the DNA for amplification, extracted DNA (1 μg) was first amplified by PCR with primers specific for murine β-actin as described previously (Usherwood *et al.*, 2000). All samples were positive (not shown). Samples were next amplified by nested PCR with primers specific for the gp150 gene exactly as described previously (Usherwood *et al.*, 1996). The sensitivity of the assay was such that one copy of viral DNA could be detected in a background of 1 μg of negative cellular DNA as determined by limiting dilution of cloned target DNA. The results are shown in Fig. 1. No product was seen when negative cellular DNA alone was used, showing that there was no contamination of the PCR reactions. Products of the expected size (368 bp) were amplified from DNA derived from both spleen and lungs of one of five bank voles. In contrast, 13 lungs and 6 spleens from 14 wood mice were positive. One wood mouse had no detectable virus DNA in either spleen or lung tissue.

The PCR data support the serological data and the hypothesis that MHV-4 is endemic in wood mice and only rarely found in voles in the UK. Furthermore, since there are no voles in Northern Ireland, bank voles are not required for the infection of wood mice. Bank voles and wood mice share the same habitats (are sympatric) and share other microparasites such as cowpox virus and several species of bacteria, suggesting that transmission of agents between the two species is possible. Thus, either bank voles are less susceptible to infection with MHV-4 or this agent transmits less well between species.

This study is limited in its ability to describe the pathogenesis of MHV-4 in wild murids. However, viral DNA was detected more frequently in the lungs of wood mice than in their spleens. This backs up data on experimental infection of laboratory mice that show lungs as a major site of persistence and latency (Stewart *et al.*, 1998). There was a higher prevalence of MHV-4 as determined by PCR rather than serology. Potentially, this could be due to a limited sensitivity of the serological assay. However, published data from experimental infection of mice suggest that the antibody response to MHV-4 is weak (Sangster *et al.*, 2000). Thus, it is equally possible that the antibody response to a natural infection may be either weak or short-lived.

Longitudinal studies in several sympatric rodent populations including wood mice are currently under way in order to determine transmission rates and effects of infection with MHV-4 and other pathogens on population dynamics. This will give valuable information on how a gammaherpesvirus transmits within a population. Future experimental

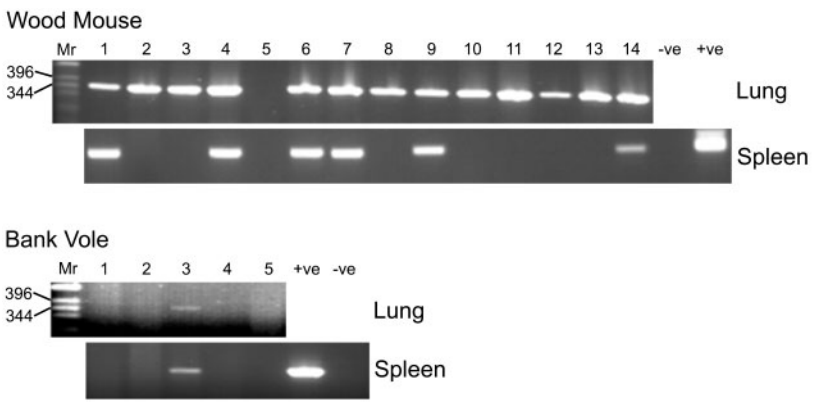

Fig. 1. PCR analysis of lungs and spleens from wood mice and bank voles. DNAs extracted from the lungs and spleens of wood mice and bank voles were amplified by a nested PCR assay specific for the MHV-68 gp150 gene. Products were electrophoresed through a 2 % agarose gel and visualized by ethidium bromide staining using a UV transilluminator. Results from wood mice are shown above those for bank voles and labelled accordingly. Results from lungs are shown above those from spleens for clarity. Molecular mass determinations were made relative to a 1 kb ladder (Life Technologies).

infection of captive-bred wood mice and bank voles will give information on the relative susceptibility of the two species to infection and the pathogenesis of MHV-4 in its natural host.

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