

squirrels (*Sciurus vulgaris*) in the coastal pine plantation of Newborough Forest on Anglesey (Everest and others 2008; D. J. Everest, S. S. Grierson, M. F. Stidworthy, C. Shuttleworth, unpublished observations). Following arrival on Anglesey in 2004, mortalities due to enteric adenovirus infection have been documented in captive-breeding enclosures (in 2005 and 2007) and in free-living forest squirrels (in 2006 and 2008). The virus has been recognised previously as a threat to populations in Norfolk (Sainsbury and others 2001) and Cumbria (Duff and others 2007), causing acute enteric infections with diarrhoea and death.

The source and route of infection for the adenovirus infecting the red squirrels remain uncertain. Wood mice (*Apodemus sylvaticus*) are known to harbour murine adenovirus, and are present in and around the forest enclosures on Anglesey. Another potential source is the grey squirrel, already identified as a reservoir for fatal squirrelpox infections in red squirrels (Rushton and others 2006). This species was resident in the forest before the red squirrel reintroductions and, despite intensive culling, has a continuing presence, including occasional observations of greys close to the red squirrel cages. A previous study detected seropositivity to murine adenovirus in grey squirrels removed from north Wales (Greenwood and Sanchez 2002).

PCR studies, undertaken at Veterinary Laboratories Agency (VLA) – Weybridge in 2008 had previously determined that adenovirus DNA could be amplified, retrospectively, from both frozen and formalin-fixed tissues from dead red squirrels from Anglesey and elsewhere. These included cases where the diagnosis of enteric adenovirus infection had been confirmed by negative contrast electron microscopy of faeces. PCR was undertaken using novel primers developed specifically against red squirrel adenovirus sequences, amplifying an internal region of the target sequence of the adenoviral DNA polymerase gene as described by Wellehan and others (2004).

In May 2006, a lactating adult female grey squirrel and three associated eight-week-old juveniles were found within a squirrel nest box, located approximately 90 m from the red squirrel enclosures. The adult and one juvenile female were presented for complete postmortem examination. No abnormalities were identified, but frozen tissue samples were retained. In 2009, as part of investigations into a potential source for red squirrel infections, frozen spleens from both animals were assayed at VLA – Weybridge using identical PCR methods and both were found to be positive for adenoviral DNA. We believe these Anglesey cases to be the first molecular confirmation of the presence of adenoviral DNA in body tissues from grey squirrels.

Further serological studies are ongoing on archived blood samples from grey squirrels that have since been culled from Newborough Forest to try to establish the

prevalence of exposure in this sympatric population. Prospective surveillance is also desirable for adenovirus infection in sympatric grey squirrels, in grey squirrels from areas of Anglesey without red squirrels, and in local wood mouse populations. Molecular investigation of genetic variation among any adenoviruses recovered from these populations may help to establish whether a common strain exists, and thereby implicate potential reservoirs and routes of infection for threatened red squirrel populations.

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References

- DUFF, J. P., HIGGINS, R. & FARRELLY, S. (2007) Enteric adenovirus infection in a red squirrel (*Sciurus vulgaris*). *Veterinary Record* **160**, 384
- EVEREST, D. J., STIDWORTHY, M. F. & SHUTTLEWORTH, C. (2008) Adenovirus-associated deaths in red squirrels on Anglesey. *Veterinary Record* **163**, 430
- GREENWOOD, A. G. & SANCHEZ, S. (2002) Serological evidence of murine pathogens in wild grey squirrels (*Sciurus carolinensis*) in North Wales. *Veterinary Record* **150**, 543-546
- RUSHTON, S. P., LURZ, P. W. W., GURNELL, J., NETTLETON, P., BRUEMMER, C., SHIRLEY, M. D. F. & SAINSBURY, A. W. (2006) Disease threats posed by alien species: the role of a poxvirus in the decline of the native red squirrel in Britain. *Epidemiology and Infection* **134**, 521-533
- SAINSBURY, A. W., ADAIR, B., GRAHAM, D., GURNELL, J., CUNNINGHAM, A. A., BENKO, M. & PAPP, T. (2001) Isolation of a novel adenovirus associated with splenitis, diarrhoea, and mortality in translocated red squirrels, *Sciurus vulgaris*. *Verhandlungsbericht über die Erkrankung der Zootiere* **40**, 265-270
- WELLEHAN, J. F., JOHNSON, A. J., HARRACH, B., BENKÖ, M., PESSIER, A. P., JOHNSON, C. M., GARNER, M. M., CHILDRESS, A. & JACOBSON, E. R. (2004) Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the adenoviruses. *Journal of Virology* **78**, 13366-13369

WILDLIFE HEALTH

PCR detection of adenovirus in grey squirrels on Anglesey

We wish to report the identification, using PCR techniques, of adenovirus in grey squirrels (*Sciurus carolinensis*) from Anglesey. To our knowledge, this is the first time that adenovirus DNA has been amplified from this species.

Retrospective analysis of postmortem tissues and ongoing prospective sampling have demonstrated that adenovirus infection is a significant cause of mortality for captive-bred and free-living reintroduced red

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