

Epidemics of squirrelpox virus disease in red squirrels (*Sciurus vulgaris*):  
temporal and serological findings

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Abstract

Squirrelpox virus (SQPV) causes a fatal disease in free-living red squirrels (*Sciurus vulgaris*) and has contributed to their decline in the UK. A conservation programme was initiated in the 1990s to bolster the population of red squirrels in the coniferous woodland of Thetford Chase, East Anglia. In 1996, 24 red squirrels were reintroduced to Thetford from Northumberland or Cumbria, while in 1999 a captive breeding and release programme commenced, but in both years the success of the projects was hampered by an outbreak of SQPV-disease. The close monitoring of the red squirrels in these conservation initiatives allowed the collection of valuable information on the epidemiology of the disease. The disease characteristics were similar to other virulent poxviral infections: the incubation period was less than 15 days; the course of the disease an average of 10 days and younger animals were significantly more susceptible to disease. No red squirrels recovered from the disease which jeopardises the conservation of this species in the UK unless practical disease control methods can be identified.

Introduction

The native red squirrel, *Sciurus vulgaris*, population in the British Isles is in decline and is steadily being replaced by the alien grey squirrel (*Sciurus carolinensis*) which was introduced to Britain from North America in the late 19<sup>th</sup> Century. A major

contributory factor to red squirrel decline is a fatal disease caused by a squirrelpox virus (SQPV) (Family: Poxviridae, Sub-family: Chordopoxviridae) (Thomas *et al* 2003; Tompkins *et al* 2003; Rushton *et al*, 2000), for which the reservoir is probably the grey squirrel (Sainsbury *et al* 2000; McInnes *et al* 2006), characterised by erythematous exudative dermatitis, sometimes with haemorrhagic crusts, primarily on the lips, nose, eyelids, medial areas of the legs, toes, and ventral skin of the body, with high mortality (Sainsbury & Ward 1996; Sainsbury & Gurnell 1995). Sainsbury *et al* (2000) and Sainsbury *et al* (submitted) found that only one of 140 red squirrels and only eight of 525 red squirrels examined clinically or post mortem respectively had antibodies to SQPV without signs of SQPV disease, suggesting that only a small number of red squirrels mount a successful immune response to the virus.

The epidemiological characteristics of the disease are expected to be similar to other virulent poxviruses which induce acute diseases (Buller & Palumbo 1991). The rodent poxvirus, ectromelia (Sub-family: *Orthopoxviridae*), which causes heavy mortality in mice has a short incubation period of 7-8 days (Fenner 1994) and has been shown experimentally to cause death in lab mice in as little as seven-ten days (Roberts 1962a; Buller & Palumbo 1991). Younger mice are more susceptible although maternal antibody initially protects pups from disease (Fenner 1994). The transmission route of SQPV between red and grey squirrels is currently unknown, but possibilities include vector-borne (Shorten 1954; Edwards, 1962; Smits *et al* 2005) or direct transmission via abrasions during scent marking (Gurnell 1987, 1991 cited by Rushton *et al* 2000). Natural infection in captivity usually occurs via minor abrasions in the skin (Fenner 1947), through contaminated bedding or during manipulations by animal handlers (Fenner 1994).

The red squirrel population of East Anglia has been in notable decline since the 1960s (Lloyd 1983; Reynolds 1985) and the first recorded outbreak of skin disease in Thetford Chase was between 1963 and 1966 (Vizoso 1968). Further outbreaks of skin disease were recorded in East Anglia in the 1970s, 1980s and 1990s (Keymer 1974, 1976, Reynolds 1985, Sainsbury & Gurnell 1995). In 1995 and 1996, 36 of 49 (73%) grey squirrels tested from this area were seropositive for SQPV (Sainsbury *et al* 2000). As seen elsewhere, the rapid decline of red squirrels in East Anglia has occurred in parallel with the spread of the grey squirrel and at an observed rate that models predict can only be explained by outbreaks of SQPV disease coupled with

competition between the two species (Tompkins, White and Boots 2003; Rushton *et al* 2000). The last remaining population of red squirrels in East Anglia, estimated in 1995 to number less than 500 (Gurnell *et al* 1996), is isolated to the coniferous Thetford Chase, a managed forest on the Norfolk-Suffolk border and an ideal habitat for the red squirrel.

In 1992 a program was set up to look into the feasibility of translocating red squirrels into Thetford as a means of population reinforcement (Venning *et al* 1997) and a 1700 ha designated Red Squirrel Reserve was set up. In a second conservation initiative for the area, plans were made in 1998 to captive breed red squirrels for release. Captive breeding pens were built in the forest from which it was planned to release the young into Thetford Chase (Steele & Gurnell 2000). There was an outbreak of SQPV disease associated with each of these conservation initiatives, one outbreak in 1996 following a translocation and a second in 1999 after the captive breeding efforts had commenced (Venning *et al* 1997; Steele & Gurnell 2000).

Although epidemics of disease associated with SQPV have probably been occurring for over a century, the epidemiology of the disease is not well understood and empirical epidemiological data is very limited but valuable to improve our models of the infection in squirrel populations. The red squirrels involved in the translocation and captive breeding efforts were closely monitored and this gave us the opportunity to gather detailed epidemiological data on epidemics of disease. We predicted that the epidemiological characteristics of SQPV-disease would be similar to other virulent poxviral infections and specifically that i/ young red squirrels would be more susceptible to disease than older red squirrels, ii/ a proportion of red squirrels would mount an antibody response and survive infection, iii/ the incubation period of SQPV disease would be short, perhaps less than 10 days and iv/ in those animals which developed disease that the course of disease would be short (perhaps of one weeks duration). We tested these predictions through detailed serological and pathological investigations of the red squirrels involved in the epidemics.

## Materials and Methods

Thetford Chase covers an area greater than 20,000 ha. In 1992, a 1700ha area of this coniferous forest was designated a Red Squirrel Reserve, and a one hectare portion (NGR: TL8783) was fenced for use as a Pre-Release Pen (PRP) (Gurnell *et al* 1996). From September 1992, grey squirrels were regularly removed from the reserve by trapping (Gurnell *et al* 1996). Both red and grey squirrels could freely enter the PRP but were unable to leave unless a bridge was erected. An additional Holding Pen (HP) was constructed greater than 2 kms distant from the Red Squirrel Reserve.

In 1998 four captive breeding enclosures were built (CBE1, CBE2, CBE3, and CBE4) at a site close to the Red Squirrel Reserve.

#### Clinical examinations:

In 1996 health examinations were carried out on all red squirrels before release and at approximately monthly intervals thereafter assuming they could be trapped. In 1999 health examinations were carried out before release into the captive breeding enclosures. Blood samples were collected when necessary for diagnostic purposes during health examinations. If available, serum samples were stored at -20<sup>0</sup>C, and then tested for SQPV antibodies using an enzyme-linked immunosorbant assay (ELISA) (Sainsbury *et al* 2000). The optical density (OD) chosen as a cut-off point for a positive result was 0.2 (Sainsbury *et al* 2000). During clinical examination, body condition was assessed by the size of muscle mass and fat stores by palpation of the hind limb soft tissue at its proximal aspect, as emaciated, thin, normal or obese. The red squirrels were aged using evidence of reproduction and body weight (providing they were in good body condition). Adult females were those with evidence of breeding, such as pregnancy, mammary development or evidence of lactation (enlarged nipples and/or halos of alopecia around the nipples). Females without signs of breeding and with a body weight equal to or greater than 300g (indicating they may be capable of breeding (Wauters & Dhont 1989)), were classified as sub-adult/adult (S/A). Females without any sign of breeding and with a body weight less than 300g and greater than 150g were classified as sub-adults. Juvenile females had a body weight of less than 150g. Adult males had large testes (greater than 10mm length, usually with scrotal staining) (dark staining and large scrotal testes are indicative of reproductive activity (Gurnell 1987, Wauters & Dhondt 1989, Wauters *et al* 2001)). Sub-adult males had small scrotal (less than 10mm length) or abdominal testes and a body weight greater than 150g. Juvenile males had a body weight less than 150g.

Squirrels found dead were examined post mortem as soon as possible and stored at +4<sup>0</sup>C in the interim (or frozen at -20<sup>0</sup>C if they could not be examined within five days).

#### Post mortem examination:

The carcasses were weighed, and the skin, eyes, ears and orifices assessed for abnormalities of colour, consistency, size or shape. Body condition was assessed as for clinical examination. An incision was made down the midline to examine the internal organs for abnormalities of size, consistency, colour or shape. Animals were separated into the following age classes: juvenile, subadult female, adult female and subadult/adult male. Juvenile animals had a deciduous second upper premolar or were equal to or less than 150g in body weight. Adult females were either pregnant or had signs of mammary development consistent with a previous lactation, for example enlarged nipples or halos of alopecia encircling the nipples. All other females were classed as subadult and all other males as subadult / adult. Where possible body fluid was taken, stored at -20<sup>0</sup>C and submitted for ELISA for antibodies to SQPV. Where the carcass showed signs of skin disease, a sample of each skin lesion was collected, stored at -20<sup>0</sup>C, and subsequently examined by transmission electronmicroscopy (TEM).

### Translocation of red squirrels and results

#### Translocation of red squirrels in 1996:

Twenty-four red squirrels were trapped at Kielder Forest, Northumberland (NGR: NY6690) and Foulshaw Moss, Cumbria (NGR: SD4682) on 24<sup>th</sup> and 25<sup>th</sup> July 1996 (Venning *et al* 1997). The squirrels were transported to Thetford Chase, where they were sexed, aged, fitted with a radio-collar, and given a health examination. There were no significant findings on health examination and all 24 animals were seronegative for SQPV. Twenty-two of these red squirrels were released into the PRP and remained there for at least 38 days; the remaining two red squirrels were placed into the HP (Venning *et al* 1997). On 1<sup>st</sup> September, bridges were erected between the trees inside and outside the PRP to allow the red squirrels to enter the surrounding forest, from where they were monitored by radiotelemetry. On 21<sup>st</sup> September the bridges connecting the PRP to the surrounding forest were removed and in so doing

three red squirrels were retained in the PRP. On 24<sup>th</sup> September three captive-bred female red squirrels and the two red squirrels from the HP, were transferred to the PRP. For more details on the translocation methods see Venning et al (1997). In Figure 1 the movements of red squirrels in 1996 are set out in a flow diagram.

All eight red squirrels present in the PRP after 24<sup>th</sup> September died between 15 and 49 days later, and seven of these eight (three sub-adult males, one S/A female and three captive-bred females aged between four and six months) had gross post-mortem signs of SQPV disease (Table 1). The eighth animal was too decomposed to determine cause of death. The red squirrel which died 15 days after release was captive-bred, was not believed to have had contact with grey squirrels when in captivity, and the captive-breeder had suffered no previous losses to SQPV disease, and so it is probable that this squirrel was first exposed to the virus soon after arrival at Thetford and that the incubation period of the viral disease is less than 15 days. Five of the eight animals were found alive with clinical signs of SQPV disease and of these five, three were euthanased for welfare reasons, and two died, three and nine days after transfer to a veterinary hospital. Three of the seven cases of confirmed SQPV disease were seropositive (ODs were 1.65, 1.33 and 0.78) and two seronegative (ODs were 0.00 and 0.08), and the remaining two were not tested.

#### Captive breeding of red squirrels in 1999:

On 1<sup>st</sup> March 1999, 12 red squirrels were trapped in Bellart How Moss, Cumbria (NGR = SD4583) and translocated to Thetford Chase. The males and females were initially kept separate but on 15<sup>th</sup> April, health examinations were carried out, and breeding pairs selected and placed into each of the four CBEs.

In September one female was euthanased due to a spinal injury, and on the 9<sup>th</sup> September a new pair was created: R820 was paired with a male, R813 in CBE3. R815 and R817 were moved to the PRP from the HP on 28<sup>th</sup> September (Steele & Gurnell 2000) and six days later they were fitted with radio collars with the aid of a handling cone that had previously been used to handle R820 in CBE3 (Steele & Gurnell 2000). The HP feeding areas and nest boxes were disinfected on 29<sup>th</sup> September and two captive bred adult males (R826, R827) were housed there. Four red squirrels (R813, R815, R817, R820), developed clinical signs consistent with

SQPV disease between 1<sup>st</sup> and 21<sup>st</sup> October, were hospitalised, and survived for one, 10, 10, 15 days respectively, and one, 12, 12, 22 days respectively after clinical signs were first observed. R815 and R817 were found to be seropositive three days after clinical signs were detected (R815: OD 0.80 and R817: 1.71), and R820, 12 days after clinical signs were detected (OD 1.07). Gross post-mortem examination findings were consistent with SQPV disease in all four cases and the presence of SQPV was confirmed by TEM. Samples of body fluid collected post mortem from R813 (OD 1.44), R817 (OD 1.73) and R820 (OD 1.28) were seropositive for SQPV. R826 and R827, which were transferred into the HP the day after R815 and R817 had been moved out, survived until January (R827) and September (R826) 2001, and at death were seronegative for SQPV. Figure 2 shows the movements of red squirrels in 1999.

## Discussion

Given our knowledge of the epidemiology of SQPV disease, much of it researched and collated since these epidemics occurred, it is not surprising that red squirrels, translocated into an area co-inhabited by seropositive grey squirrels (Sainsbury *et al* 2000), succumbed to SQPV disease. Although grey squirrels were periodically removed from the designated Red Squirrel Reserve in Thetford between September 1992 and the time of the disease outbreaks described in this paper, the Reserve was continuously repopulated through immigration of grey squirrels. Grey squirrels were present in the Reserve and in the PRP during the translocations of red squirrels in 1996 and 1999, therefore could have come into close contact with the red squirrels described here. As already noted by Venning *et al* (1997) translocations of red squirrels into regions with seropositive grey squirrels are to be strongly discouraged unless novel disease control methods can be developed.

Although red squirrels are known to be susceptible to stress (Kenward & Hodder 1998, Wauters *et al* 1996), and stressors such as translocation might increase susceptibility to disease, many of the animals had been at Thetford Chase for several months, and adapted without difficulty, before they succumbed to disease (Venning *et al* 1997). Indeed SQPV has been shown experimentally to cause death in red squirrels apparently without the influence of any secondary infections or other stressors

(Tompkins *et al* 2002). Of greater influence on the development of these epidemics is likely to have been the relatively high density of red squirrels at the release and captive breeding sites, increasing the likelihood of virus transmission. Free-living red squirrels tend to be solitary animals for much of the year and live at densities of between 0.3 and 1.1 squirrels / ha (Gurnell 1987). At the time of the first outbreak in the PRP, the density of red squirrels was 8 / ha, and there were also an unknown number of grey squirrels in the PRP.

These closely monitored outbreaks of natural SQPV disease provide crucial epidemiological information, including our first clues to the incubation period of natural infection, the duration of the disease and the age at which red squirrels are most susceptible. Two events provide consistent information on the incubation period: firstly, one of the three captive-bred red squirrels translocated to Thetford on 20<sup>th</sup> September 1996 died of SQPV disease 15 days after release into the PRP; and secondly, in 1999, disease was detected in R815 and R817 15 days after they had been in contact with a potentially contaminated handling cone. Immediately after R815 and R817 had been moved out of the HP, to the PRP, using the suspected contaminated handling cone, two red squirrels (R826, R827) were moved into the HP and did not develop signs of SQPV disease which provides circumstantial evidence that R815 and R817 had not been exposed to SQPV until they left the HP and were in contact with the handling cone, although, alternatively, it could simply mean that the disinfection of the HP, before R826/R827 moved in, was effective. However, there is sufficient evidence to conclude that the incubation period for natural infection is probably less than 15 days and this is broadly comparable with the five to eight day period recorded by Tompkins *et al* (2002) for experimental infection of red squirrels (n=4) with SQPV. The apparent link between the movement of red squirrels and the occurrence of cases of disease suggests that the transmission of SQPV is more likely to be by direct contact than through a vector.

When Edwards (1962) described an outbreak of skin disease in red squirrels (believed to be SQPV disease) he found the ‘course of the disease is usually one week’. During the 1996 and 1999 outbreaks, six red squirrels survived between 1 and 22 days ( $\bar{X}$ =9.8 n=6) although veterinary treatment may have prolonged the course of the disease. Following experimental infection of red squirrels (n=4) with SQPV, as mentioned above, death of these animals occurred 13, 16 and 17 days after infection



(one animal survived) (Tompkins *et al* 2002) suggesting a similar period for the course of the disease. The survival of red squirrels for an average period of ten days while they are exhibiting exudative lesions of the skin provides an opportunity for transmission of the virus to other red squirrels sharing feeding, scent marking and possibly nesting sites. Five of nine (56%) of red squirrels positive for SQPV-disease were in good body condition suggesting that they were active, had managed to feed, and might possibly have been in contact with other red squirrels, until close to death.

Eight of the eleven red squirrels with pathological signs consistent with SQPV disease and TEM positive were seropositive at death or up to ten days beforehand, with OD values ranging between 0.44 and 1.81. Five of these seropositive animals had previously been seronegative (the other animals were not tested) suggesting that an antibody response to current infection had been detected. However, despite mounting this response, these red squirrels succumbed to the disease. Previous work on the immune response to poxviruses has indicated that although antibodies have several mechanisms to destroy poxviruses, such as facilitating complement-mediated lysis, they are unable to protect the host against primary poxvirus infection (Buller and Palumbo 1991; Smith and Kotwal 2002).

Overall in 1996 and 1999, one adult male, three sub adult males, one S/A and one sub-adult female, two of unknown age, and three female captive bred squirrels between four and six months old died of confirmed SQPV-disease. Sub-adult males and females and S/A females are likely to have been less than one year old (Gurnell 1987). The proportion of non-adults (8/11; 73%) with SQPV-disease was significantly greater than the proportion of non-adults in the population as a whole (16/36; 44%) ( $\chi^2 = 40.3$ ;  $p < 0.0001$ ), and so younger animals are at significantly greater risk from contracting SQPV disease. However, in 1996 because all the red squirrels which contracted SQPV disease were confined to the PRP, the red squirrels at risk of SQPV-disease may have included fewer adults than non-adults, and so these results must be evaluated with caution.

Our studies of these SQPV-disease epidemics have revealed valuable epidemiological information: an incubation period of less than 15 days, a disease course on average approximately nine days, and increased susceptibility in younger animals which were unable to mount an effective immune response to the virus. These characteristics are

similar to another virulent poxviral disease in rodents: ectromelia virus infection. SQPV infection in red squirrels causes a severe disease which is of great significance to the conservation of red squirrels in the UK.

### Acknowledgments:

We would like to thank Janie Steele and Tim Venning for carrying out trapping, managing and detecting ill health in the red squirrels; Janice Gilray at the Moredun Research Institute for carrying out the ELISA testing; D W (Bill) Marriott, Brian Skinner and the veterinary nursing staff of Old Golf House Veterinary Group for allowing the use of their facilities and for technical assistance; Michael Stack, Tony Scott and Stefan Farrelly, Veterinary Laboratories Agency for carrying out electron microscopy; Shaheed K Macgregor for bacteriological and parasitological examinations; Christine Dean, Tracy Howard, Gill Bell and Gill Ahearne for technical assistance; and Tai Strike and Sue Thornton for carrying out some of the post-mortem examinations.

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