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

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SUMMARY

Squirrelpox virus (SQPV) causes a fatal disease in free-living red squirrels (Sciurus vulgaris) which has contributed to their decline in the United Kingdom. Given the difficulty of carrying out and funding experimental investigations on free-living wild mammals, data collected from closely monitored natural outbreaks of disease is crucial to our understanding of disease epidemiology. 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 and 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 in which seven and four red squirrels died respectively. Valuable information on the host–pathogen dynamics of SQPV disease was gathered by telemetric and mark–recapture monitoring of the red squirrels. SQPV disease characteristics were comparable to other virulent poxviral infections: the incubation period was <15 days; the course of the disease an average of 10 days and younger animals were significantly more susceptible to disease. SQPV disease places the conservation of the red squirrel in jeopardy in the United Kingdom unless practical disease control methods can be identified.

INTRODUCTION

Squirrelpox virus (SQPV) (Family: Poxviridae; Subfamily: Chordopoxviridae) causes a fatal disease in red squirrels, Sciurus vulgaris [1–3], characterized by erythematous exudative dermatitis, with haemorrhagic crusts, primarily on the lips, nose, eyelids, medial areas of the legs, toes, and ventral skin of the body [4, 5]. The disease has made a major contribution to the decline of the native red squirrel in the British Isles, and its steady replacement by the alien grey squirrel (Sciurus carolinensis), introduced to Britain from North America in the late 19th century, which is also believed to be the reservoir of SQPV [6, 7]. Sainsbury et al. [6] found that 1/140 red squirrels and 8/525 red squirrels, examined clinically or post mortem respectively, had antibodies to SQPV without signs of SQPV disease, suggesting that only a small proportion of red squirrels mount a successful immune response to the virus. The transmission route of SQPV between red and grey squirrels is currently unknown, but it has been proposed that either a vector-borne [8, 9] or a direct route via abrasions which occur during scent marking are possible [3].
It is expected that the epidemiological characteristics of SQPV infection in red squirrels, our knowledge of which is limited, is comparable to other virulent poxviruses which induce acute diseases. The ectromelia virus (Subfamily: Orthopoxviridae) causes an acute disease in mice with high mortality and has been investigated in detail [10]. It has been shown experimentally to cause death in laboratory mice in as little as 7–10 days [11, 12] and has a short incubation period of 7–8 days [10]. Younger mice are more susceptible although maternal antibody initially protects pups from disease [10]. Natural infection in captivity usually occurs via minor abrasions in the skin [13], through contaminated bedding or during manipulations by animal handlers [10].

The red squirrel population of East Anglia has been in notable decline since the 1960s [14–16]. The first recorded outbreak of skin disease in Thetford Chase was between 1963 and 1966 [17], and further outbreaks were recorded in East Anglia in the 1970s, 1980s and 1990s [5, 15, 18, 19] and the aetiology of those which occurred after 1980 was confirmed as SQPV. In 1995 and 1996, 36/49 (73%) grey squirrels tested from this area were seropositive for SQPV [6]. 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 [3, 20]. The last remaining population of red squirrels in East Anglia, estimated in 1995 to number less than 500 [21], was 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 programme was set up to look into the feasibility of translocating red squirrels into Thetford as a means of population reinforcement [22] 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 [23]. 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 [22, 23]. The red squirrels were closely monitored as a part of efforts to assess these conservation initiatives, and in so doing detailed epidemiological data on epidemics of SQPV disease was gathered.

There is a grave lack of empirical data on the epidemiological characteristics of disease in free-living wild animals [24, 25] and studies are hindered by the enormous time and monetary resource required, and logistical difficulties. Where disease outbreaks occur in closely monitored populations of wild animals, we can take advantage of these circumstances to gather crucial data to aid our understanding of the epidemiology.

Although epidemics of disease associated with SQPV have probably been occurring for over a century, empirical epidemiological data is very limited but valuable to improve our models of the infection in squirrel populations. 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 immune response and survive infection, (iii) the incubation period of SQPV disease would be short, perhaps <10 days and (iv) in those animals which developed disease that the course of disease would be short (perhaps of 1 week’s duration). We were able to assess these predictions by examining the findings from the detailed serological and pathological investigations of the red squirrels involved in the epidemics at Thetford.

**MATERIALS AND METHODS**

Thetford Chase covers an area >20000 ha. In 1992, a 1700 ha area of this coniferous forest was designated a Red Squirrel Reserve, and a 1 ha portion (NGR: TL8783) was fenced for use as a pre-release pen (PRP) [21]. From September 1992, grey squirrels were regularly removed from the reserve by trapping [21]. 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 >2 km distant from the Red Squirrel Reserve.

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

Contact between grey and red squirrels was possible through the mesh walls of the HP and the CBEs. In both 1996 and 1999 the various enclosures mentioned above were inspected daily to monitor the squirrels but it was not possible to view every red squirrel on every day.
Clinical examinations

In 1996 health examinations were carried out on all red squirrels, and diagnostic blood samples taken, before release and at approximately monthly intervals thereafter assuming they could be trapped. In 1999 health examinations were carried out before the red squirrels were placed into the CBEs. Blood samples were collected when necessary for diagnostic purposes during health examinations. If available, serum samples were stored at $-20\, ^\circ\text{C}$, and then tested for SQPV antibodies using an enzyme-linked immunosorbent assay (ELISA) [6]. The optical density (OD) chosen as a cut-off point for a positive result was 0.2 [6]. During clinical examination, body condition was assessed by palpation of the size of muscle mass and fat stores 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 $\geq 300\, g$ (indicating they may be capable of breeding [26]) were classified as subadult/adult (SA/A). Females without any sign of breeding and with a body weight $< 300\, g$ and $> 150\, g$ were classified as subadults (SA). Juvenile females had a body weight of $< 150\, g$. Adult males had large testes ($> 10\, mm$ length, usually with scrotal staining) (dark staining and large scrotal testes are indicative of reproductive activity [26–28]). SA males had small scrotal testes ($< 10\, mm$ length) or abdominal testes and a body weight $> 150\, g$. Juvenile males had a body weight $< 150\, g$.

Squirrels found dead were examined post mortem as soon as possible and stored at $+4\, ^\circ\text{C}$ in the interim (or frozen at $-20\, ^\circ\text{C}$ if they could not be examined within 5 days).

Post-mortem examination

The carcases 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 male or female, SA female, adult female and SA/A male. Juvenile animals had a deciduous second upper premolar or were $\leq 150\, g$ 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 SA and all other males as SA/A. Where possible body fluid was taken, stored at $-20\, ^\circ\text{C}$ and submitted for ELISA for antibodies to SQPV. Where the carcase showed signs of skin disease, a sample of each skin lesion was collected, stored at $-20\, ^\circ\text{C}$, 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 Foulsaw Moss, Cumbria (NGR: SD4682) on 24 and 25 July 1996 [22]. 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 [22]. On 1 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 22 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 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. [22]. In Figure 1 the movements of red squirrels in 1996 are set out in a timeline.

All eight red squirrels present in the PRP after 24 September died between 15 and 48 days later, and seven of these eight (three SA males, one SA female and three captive-bred females aged between 4 and 6 months) had gross post-mortem signs of SQPV disease and virus presence was confirmed by TEM (Fig. 1 and Table 1). The eighth animal was too decomposed to determine cause of death. The red squirrel which died 15 days after transfer to the PRP 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 this is an indicator that the incubation period of the viral disease is <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, 3 and 9 days after transfer to a veterinary hospital for treatment. Three of the seven cases of confirmed SQPV disease were seropositive (ODs 1.65, 1.33 and 0.78) at death, two seronegative (ODs 0.01 and 0.08), and the remaining two were not tested.

No signs of SQPV disease were detected in the 19 red squirrels released into the surrounding forest on 1 September during post-release monitoring.

Captive breeding of red squirrels in 1999

On 1 March 1999, 12 red squirrels were trapped in Bellart How Moss, Cumbria (NGR: SD4583) and nine of these translocated to Thetford Chase and three to Alice Holt Lodge, Surrey. The males and females were initially kept separate but on 15 April, health examinations were carried out, and breeding pairs selected and one pair placed into each of three of the CBES (the fourth CBE housed a single male) and another pair into the PRP. On 14 July 1999, the three red squirrels (R815, R817, R820) at Alice Holt Lodge were transferred to the HP at Thetford Chase.

In August one female was euthanised due to a spinal injury, and on 9 September a new pair was created: R820 (from the HP) was paired with a male, R813 in CBE3. The remaining two red squirrels in the HP, R815 and R817 were moved to the PRP on 28 September [23] and six days later (4 October) they were fitted with radio-collars with the aid of a handling cone that had previously been used to handle R820 in CBE3 [23]. The HP feeding areas and nest boxes were disinfected on 29 September and two captive-bred adult males (R826, R827) were housed there. Four red squirrels, R820, R815, R817, R813 developed clinical signs consistent with SQPV disease on 30 September, and 19, 19 and 21 October respectively, were hospitalized, and survived for 15, 10, 10 and 1 days respectively, and 22, 12, 12 and 1 days respectively after clinical signs were first observed. Figure 2 shows the movements and dates of the death of red squirrels in 1999. R815 and R817 were found to be seropositive 3 days after clinical signs were detected (R815: OD 0.80; R817: OD 1.71), and R820, 13 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 (Table 2). 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.

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 [6], 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, and therefore could have come into close contact with the red squirrels described here. As already noted by Venning et al. [22], 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 [29, 30]; and stressors such as translocation might increase their susceptibility to disease, many of the animals had been at Thetford Chase for several months, and adapted without difficulty, before they succumbed [22]. Indeed, SQPV has been shown experimentally to cause death in red squirrels apparently without the influence of any secondary infections or other stressors [2]. 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 [27]. 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: first, one of the three captive-bred red squirrels translocated to Thetford on 20 September 1996 died of SQPV disease 15 days after release into the PRP; and second, in 1999, disease was detected in R815 and R817 15 days after they had been in contact with a handling cone, potentially contaminated through contact with R820. 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 or R815 and R817 became infected with SQPV between 28 September and 4 October through contact with a grey squirrel in the PRP. However, there is sufficient evidence to conclude that the incubation period for natural infection is probably <15 days and this is broadly comparable with the period of 5–8 days recorded by Tompkins et al. [2] for experimental infection of red squirrels (n = 4) with SQPV. The source of infection for R820 was probably through direct or indirect contact with a grey squirrel infected 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 rather than through a vector.

When Edwards [9] 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 (average = 9.8 days) although veterinary treatment may have prolonged the course of the disease. When red squirrels (n = 4) were experimentally infected with SQPV [2], three of the four animals died 13, 16 and 17 days after infection (one animal survived); a similar period for the course of the disease. The survival of red squirrels for a period of 10 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. Our knowledge that other poxviruses of rodents such as cowpox virus [31] and ectromelia virus [10] can survive for extended periods outside the host, and poxviruses which induce skin lesions, such as orf virus, are transmissible via shed scab material [32], suggests that the survival of virus in material deposited from the exudative lesions of red squirrels could be important in the transmission of SQPV between red squirrels.

Eight of the 11 red squirrels, across 1996 and 1999, with pathological signs consistent with SQPV disease and which were TEM positive, were seropositive at death or up to 10 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. When Tompkins et al. [2] experimentally infected four red squirrels with SQPV, one survived despite showing clinical signs of disease and was found to have mounted a larger antibody response (OD 3.0) than the other three animals. It may be that the size of the antibody response was critical in our cases. The eight free-living SQPV disease-free seropositive red squirrels identified by Sainsbury et al. (unpublished observations) had a mean OD of 0.80 (S.D. = 0.51) but their OD might have waned post-infection. However, previous work on the immune response to smallpox and squirrel fibroma viruses has indicated that although antibodies have several mechanisms to destroy poxviruses, such as facilitating complement-mediated lysis, they are unable to fully protect the host against primary poxvirus infection, which is reliant on a cytotoxic T-cell response [12, 33]. For example, although rabbits infected with squirrel fibroma virus responded with neutralizing antibody, the fibromas continued to develop.

Overall in 1996 and 1999, one adult male, three SA males, one SA/A and one SA female, two of unknown age, and three female captive-bred red squirrels aged between 4 and 6 months, died of confirmed SQPV disease. SA males and females and SA/A females are likely to have been aged < 1 year [27]. The proportion of non-adults (8/11, 73%) with SQPV disease was significantly greater than the proportion of non-adults in the population of red squirrels as a whole (16/36, 44%) (χ² = 40.3, P < 0.0001), and so it would initially appear that younger animals are at significantly greater risk from contracting SQPV disease. However, in 1996 all the red squirrels which contracted SQPV disease, as far as we are aware, were confined to the PRP, and therefore the red squirrels at risk of SQPV disease may have included fewer adults than non-adults, and these results must be viewed with caution.

Our studies of these epidemics have revealed valuable epidemiological information on SQPV disease: an incubation period of < 15 days, a disease course on average of ~10 days, and apparent increased susceptibility in younger animals which were unable to mount an effective immune response to the virus. We also provide further circumstantial evidence that the route of transmission of SQPV is direct. This empirical data will facilitate further investigation of the role that SQPV plays in the apparent competition between red squirrels and invading grey squirrels, and the continuing demise of the red squirrel.
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DECLARATION OF INTEREST

None.

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