Papers

Epidemiological and postmortem findings in 262 red squirrels (*Sciurus vulgaris*) in Scotland, 2005 to 2009

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Postmortem and virological examinations for squirrelpox virus (SQPV) were carried out on 262 red squirrels (*Sciurus vulgaris*) found dead or moribund in Scotland between September 2005 and July 2009, to determine the likely causes of death and highlight factors that might be threats to the red squirrel population. Most of the squirrels were submitted from Dumfries and Galloway, and 71 per cent of them were adults. Road traffic accidents, squirrelpox, trauma or starvation were responsible for death in a large proportion (73 per cent) of the squirrels. Thin or emaciated body condition was associated with deaths resulting from pneumonia SQPV infection and starvation, and with the presence of external parasites. There were differences between age groups with regard to the cause of death; a large proportion of juveniles died of starvation, whereas a large proportion of subadults and adults died in road traffic accidents. SQPV infection was associated with the presence of external parasites, but was not associated with the sex of the animals.

THE red squirrel (*Sciurus vulgaris*) is considered a vulnerable species in the UK and is included as a priority species in the UK government's conservation initiative, the UK Biodiversity Action Plan. Red squirrels are no longer present in many parts of England and Wales, and many of the existing populations are declining (Harris and others 1995). The published estimate of the size of the red squirrel population in Great Britain is 161,000 animals, comprising approximately 30,000 in England, 10,000 in Wales and 121,000 in Scotland; the red squirrels in Scotland therefore represent 70 to 75 per cent of the surviving population of the species in Great Britain (Harris and others 1995). The protection of this last remaining substantial red squirrel population is a priority if the species is not to become extinct on the UK mainland.

The red squirrel's demise has been attributed mainly to the presence of the American grey squirrel (*Sciurus carolinensis*), a non-native species first introduced into the UK in the late 19th century (Usher and others 1992, Kenward and Holm 1993). Direct competition between red and grey squirrels for resources may partly account for the decline in the red squirrel population, with grey squirrels tending to outcompete red squirrels in some types of habitat (Kenward and Holm 1993, Kenward and others 1998). However, the most significant factor in this relationship between the decline in the number of red squirrels and the presence of grey squirrels is now believed to be squirrelpox virus (SQPV) (Tompkins and others 2002, Rushton and others 2006). SQPV, which belongs to the Poxviridae subfamily Chordopoxviridae (McInnes and others 2006), infects grey squirrels with no apparent adverse effects on their health, but causes a severe, usually lethal, disease in red squirrels (Tompkins and others 2002). There is only one reported case of clinically evident naturally occurring SQPV disease in a grey squirrel (Duff and others 1996). In red squirrels, the disease is characterised by severe, erythematous, exudative dermatitis around the face, feet and ventrum. Ulceration and infection of these lesions, along with lethargy, may lead to the death of infected red squirrels in the wild (Tompkins and others 2002), although the existence of seropositive red squirrels suggests that some may recover (Sainsbury and others 2008). SQPV is blamed for the extinction of many red squirrel populations in England and Wales, and the grey squirrel is likely to be the reservoir host for the virus (Sainsbury and others 2008). Indirect evidence for the likely existence of SQPV in Scotland was not recorded until 2005, when grey squirrels seropositive for the virus were first identified there. Two years later, the first affected red squirrels were detected (McInnes and others 2009), demonstrating that SQPV now presents a serious threat to red squirrels in Scotland.

Although SQPV appears to have contributed significantly to the decline in the red squirrel population in Great Britain, it is not the only cause of death associated with this decline, especially in Scotland, where squirrelpox is not yet widespread. Other previously reported causes of mortality in red squirrels include predation, starvation, extreme cold weather, parasitic disease, failure to thrive after weaning, stress after relocation, neoplasia, nutritional disease and human activities (road traffic casualties) (Keymer 1983, Kenward and Hodder

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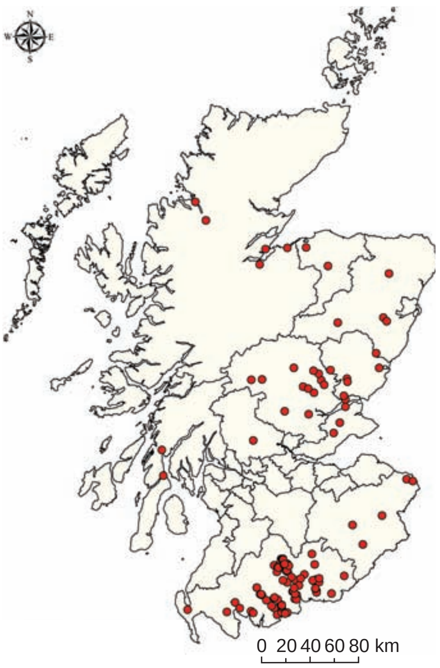

FIG 1: Grid reference number locations of 247 red squirrel carcases submitted from Scottish counties for postmortem examination between September 2005 and July 2009. Crown Copyright/database right 2009. An Ordnance Survey/EDINA supplied service

1998, Lurz and others 2005, Simpson and others 2006). There have also been occasional case studies on the causes of death and disease in individual red squirrels in the UK (Duff and others 2007, Simpson and others 2009). However, there has been no comprehensive study to examine and quantify the causes of death in a larger sample size of red squirrels in Scotland. The current threat to the red squirrel population in Scotland highlights the importance of gaining greater understanding of the factors responsible for mortality in this population. An ongoing scheme in Scotland has encouraged submission of red squirrel carcases found by members of the public and various organisations to the Royal (Dick) School of Veterinary Studies at the University of Edinburgh for postmortem examination. This is the first paper to use information obtained via this scheme. The aim of this study was to provide detailed information on the causes of death in red squirrels in Scotland, and the demographic variables associated with these squirrels, in order to highlight factors that might be threats to Scottish red squirrel populations.

Materials and methods

Study population and pathological examination

Two hundred and sixty-six dead red squirrels, found in Scotland between September 29, 2005 and July 24, 2009, were submitted to the Veterinary Pathology Unit at the University of Edinburgh by members of the public, red squirrel conservation organisations, ranger services and veterinary surgeries, for postmortem examination. Four of these carcases were omitted from the data set because their condition was too poor to allow meaningful assessment, leaving 262 squirrels.

The administrative region and Ordnance Survey grid reference where each squirrel was found were recorded, if known. The specific area in which each squirrel was found was recorded, if known, as in a garden, in a woodland, on/near a road, at the base of a tree, on/near a squirrel feeder, or in some other area; and it was also recorded, if known, whether the squirrel had been found dead, had died after having been found ill or injured, or was euthanased after having been found ill or injured. For some of the squirrel carcases there was incomplete or no information regarding the date of finding, location and/or situation in which they were found; squirrel carcases with no specific location information were assumed to have come from the area around the sender's address.

The carcases were frozen at –20°C on arrival if the postmortem examination was not scheduled to be carried out immediately. The postmortem condition (fresh, moderately decomposed or decomposed) at the time of the examination was assessed visually. Decomposition limited the completeness of the postmortem examination in some cases, and therefore the number of animals differs for some of the parameters assessed.

Assessment of the age of the squirrels was based on a previously reported method (Carroll and others 2009). Measurements were made of bodyweight and crown-rump length (the rostral margin of the pinna to the base of the tail). In females, the uterine horn length was measured, and pregnancy status and the degree of mammary development were assessed. In males, the presence or absence of scrotal pigment (which is prominent in mature males), position of the testes (scrotal or abdominal, with scrotal testes consistent with maturity) and length and width of the testes were recorded. Each squirrel was classified as adult, subadult or juvenile on the basis of its bodyweight, crown-rump length and the degree of maturity of the hair coat and reproductive organs. Body condition was determined by palpation of the hindlimb and lumbar musculature, and the squirrels were categorised as fat, normal, thin or emaciated. The presence of any fleas, ticks or lice was noted, along with the degree of infestation (mild, moderate or severe).

A full postmortem examination was undertaken where the condition of the carcase permitted this, and all macroscopic lesions were recorded. In most squirrels, a PCR for SQPV was carried out on the eyelid, forefoot digital skin and lip skin. In cases where blood or body cavity fluid was obtainable, an ELISA for SQPV antibody was also performed, as described by McInnes and others (2009). When SQPV infection was suspected, confirmation was obtained by electron microscopy of scabs to demonstrate the characteristic virus particles (McInnes and others 2009). In two cases, histopathological examination was undertaken of haematoxylin and eosin-stained sections of buffered formalin-fixed, paraffin wax-embedded tissues, using standard methods. The postmortem findings, history, results of the virological examination described and, in the two cases specified, histopathological examination, were used to determine, where possible, the predominant cause of death (or cause of signs that led to euthanasia) for each of the 262 squirrels.

Data analysis

Minitab 15 was used for statistical analysis of the data. Associations between categorical variables were examined using chi-squared analysis where appropriate. Fisher's exact test was performed if expected counts were less than five. In all cases $P<0.05$ was taken to indicate statistical significance.

Results

Sample population

The dead red squirrels submitted for examination were from various regions

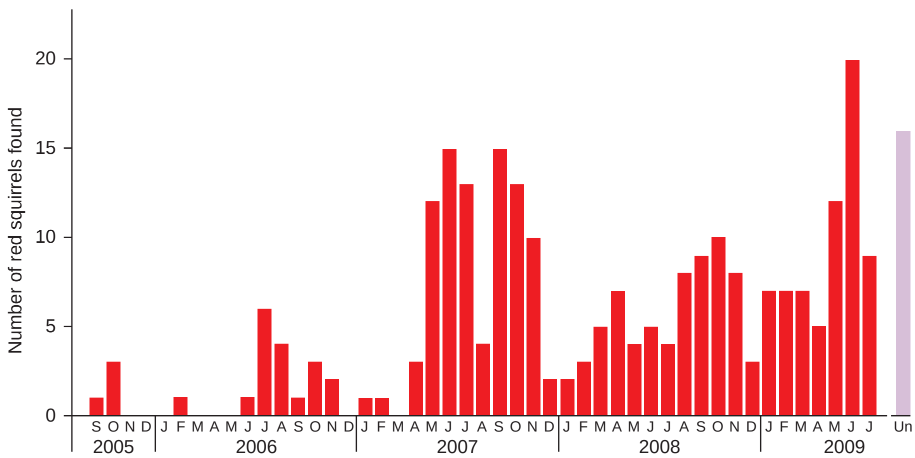

FIG 2: Numbers of Scottish red squirrel carcases (n=262) submitted for postmortem examination between September 2005 and July 2009, categorised by the date on which they were found. Un Month of submission was unknown

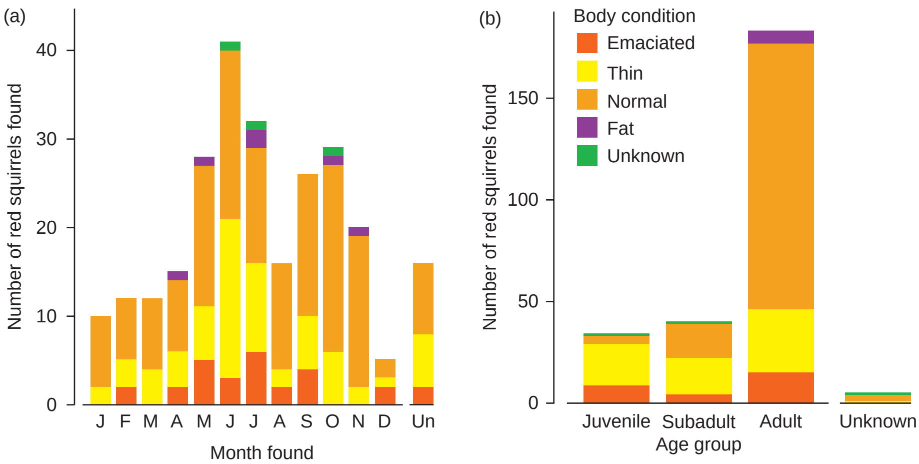

FIG 3: Body condition of 262 red squirrel carcases from Scotland (a) in terms of percentage by month, and (b) within each of three age groups, reported between September 2005 and July 2009. Un Month of submission unknown

throughout Scotland; however, a majority (175 of 262; 66.8 per cent) came from Dumfries and Galloway. The precise locations, based upon grid reference numbers, were known for 247 of the squirrels (Fig 1); the majority of the squirrels were found associated with the road network (data not shown).

The month of discovery was known for 246 of the 262 squirrels. The numbers of squirrels submitted for examination increased overall throughout the study period (Fig 2).

Information with regard to the exact location of the animal was available for 154 of the 262 squirrels. Of these, 41 per cent were known to have been found on or near a road, 18 per cent in a garden, 12 per cent within a wooded area, 9 per cent at the base of a tree and 4 per cent in or near a feeder; 16 per cent had been found in a variety of other locations. Information on whether the squirrel was found dead, died after being found ill or injured, or was euthanased after being found ill or injured was available for 226 of the 262 squirrels. Seventy per cent of these 226 squirrels had been found dead, 15 per cent had died after being found ill or injured, and 15 per cent had been euthanased as a result of illness or injury.

The age groups of 257 of the squirrels and the sex of 259 of them could be determined. The majority of the squirrels were adult (71 per cent) and 48 per cent were female (Table 1); six of the females were pregnant. Among the carcases examined, there was no statistically significant association between age group and sex (P=0.603). Body condition was determined in 259 of the 262 squirrels. Of these, most were in normal body condition (60 per cent), and 38 per cent were either thin or emaciated; emaciated animals were mainly found from May to October (Fig 3a). There was a statistically significant association between age group and body condition ($P<0.001$) (Fig 3b), with a higher proportion of juvenile and subadult squirrels being thin or emaciated (88 per cent), and a much lower proportion of adults in these categories (25 per cent).

TABLE 1: Numbers of red squirrel carcases found in Scotland, categorised by age group and sex, which were submitted for postmortem examination between September 2005 and July 2009

	Juvenile	Subadult	Adult	Age group unknown	Total
Female	14	17	91	1	123
Male	20	21	92	3	136
Sex unknown	0	2	0	1	3
Total	34	40	183	5	262

Assigned causes of death

On the basis of the gross postmortem findings, and in two cases histopathological examination, it was possible to assign a likely predominant cause of death in 245 (94 per cent) of the 262 squirrels. Of the remaining 17 carcases, two were in too poor a condition for a predominant cause of death to be determined, and 15 appeared healthy with no significant abnormalities found at postmortem examination. The assigned causes of death are shown in Table 2 and Fig 4a; road traffic

TABLE 2: Summary of the causes of death of 245 red squirrels for which a predominant cause of death could be assigned

Assigned cause of death	Description	Number (%) of squirrels
Road traffic accident	Clear evidence of trauma and found on or near a road	105 (42.9)
Squirrelpox virus infection	Typical skin ulceration and scab formation, confirmed by electron microscopy	35 (14.3)
Trauma	Clear evidence of trauma but not found near a road	27 (11.0)
Starvation	Emaciated body condition and no other predominant cause of death	24 (9.8)
Pneumonia	Consolidation of lung lobes with or without pleuritis	18 (7.3)
Enteropathy		12 (4.9)
Intussusception	Colonic or colorectal intussusception	5 (2.0)
Enteritis	Mucosal congestion and/or accumulation of fluid ingesta or gas in the intestine. In some cases, diarrhoea present	4 (1.6)
Small intestinal foreign body	Vegetable foreign body penetrating the intestinal wall	1 (0.4)
Unspecified	Accumulation of fluid ingesta or gas in the intestine without congestion or diarrhoea	2 (0.8)
Abscess	Digit (1); kidney (1); submandibular (1); intrathoracic (2)	5 (2.0)
Ectoparasitism	Severe louse infestation with grossly evident anaemia	4 (1.6)
Other infections		4 (1.6)
Balantitis	Prepuce severely inflamed with purulent discharge	1 (0.4)
Pyometra	Pus accumulation in distended uterus	1 (0.4)
Severe conjunctivitis	Conjunctivitis with keratitis in one eye and bilateral purulent ocular discharge	1 (0.4)
Septicaemia	Enlarged lymph nodes and spleen, epicardial haemorrhages	1 (0.4)
Died/euthanased for other reasons		11 (4.5)
Neoplasia	Suspected soft tissue sarcoma with pulmonary metastases; multicentric lymphoma	2 (0.8)
Electrocution	Found under powerline with subcutaneous haemorrhages/oedema and internal haemorrhage	2 (0.8)
Cardiac failure	Cardiomegaly with left ventricular hypertrophy; cardiomegaly with atrial dilation	2 (0.8)
Suspected fatal allergic reaction	Severe subcutaneous oedema of pinnae, muzzle, eyelids, limbs, scrotum and tail	1 (0.4)
Stress	No gross abnormalities but squirrel recently caught for translocation	1 (0.4)
Urinary obstruction	Severe bladder distension and apparent urethral obstruction	1 (0.4)
Congenital hindlimb contracture	Partially contracted hindlimbs with abnormal angulation	1 (0.4)
Suspected anticoagulant rodenticide poisoning	Multiple internal and external haemorrhages, history of possible access to rodenticide	1 (0.4)

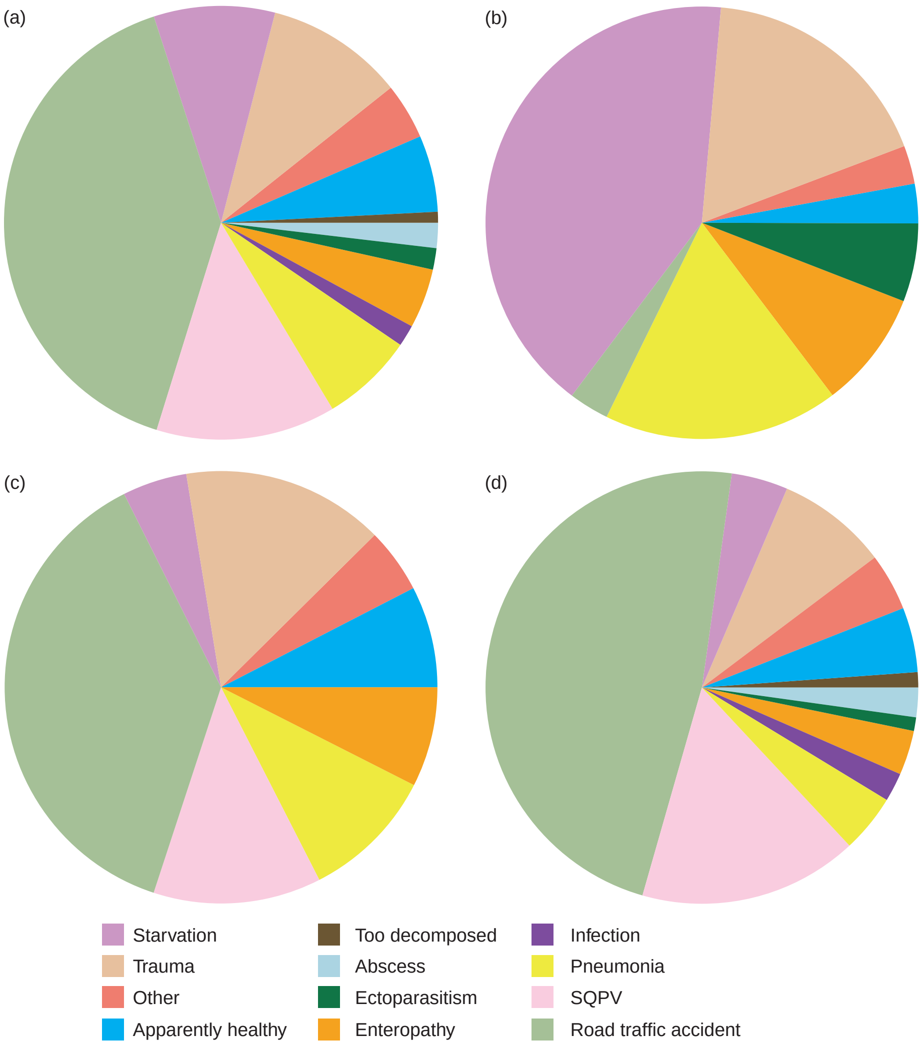

FIG 4: Predominant assigned causes of death in red squirrel carcases found in Scotland between September 2005 and July 2009 and submitted for postmortem examination. (a) All squirrels in the study (n=262), (b) juveniles (n=34), (c) subadults (n=40), (d) adults (n=183)

accidents, squirrelpox, trauma and starvation together represented 78 per cent of the assigned causes of death.

There was a statistically significant association between age group and predominant cause of death (P<0.001). Road traffic accidents were rare in the juvenile group (Fig 4b), and no juveniles had died of squirrelpox. Starvation was the most common cause of death in juveniles, followed by pneumonia and trauma (Fig 4b). This difference was less marked between subadults (Fig 4c) and adults (Fig 4d), with road traffic accidents being the most common in both age groups, followed by trauma, squirrelpox and pneumonia in the subadults, and squirrelpox and trauma in the adults (Fig 4d). There was a statistically significant association between body condition and predominant cause of death (P<0.001); 77 per cent of thin or emaciated squirrels died from pneumonia, squirrelpox or starvation, compared with 17 per cent of normal/fat squirrels, whereas 83 per cent of normal/fat squirrels died from road traffic accidents or trauma.

The number of submissions per region (Fig 1) was a confounding factor, and the predominant cause of death varied according to the location in which they had been found (Fig 5). No SQPV-associated disease was reported outside Dumfries and Galloway, and the majority of submissions from central Scotland related to road traffic accidents. Road traffic accidents were also the largest grouping in the animals submitted from the Borders and Dumfries and Galloway.

External parasites

Among the 262 squirrels examined, external parasites (fleas, ticks and/or lice) were observed on 29 per cent. Fifty-six per cent of the squirrels with external parasites were found to have fleas alone, 12 per cent had ticks alone, and 12 per cent had lice alone; 15 per cent had both fleas and ticks, 4 per cent had both ticks and lice, and 1 per cent had both fleas and lice. There was a statistically significant association between body condition and the presence of external parasites (P=0.027), with 38 per cent of thin or emaciated squirrels having external parasites, compared with 25 per cent of normal/fat squirrels. In only four cases was ectoparasitism considered the likely cause of death; all four were juveniles with many hundreds of lice and severe pallor of the carcase, consistent with anaemia.

SQPV

No squirrelpox cases were seen from the start of the study period (September 2005) until May 2007, when two cases were submitted. In July 2007 there was one case, in March 2008 and June 2008 there were two cases each, and in August 2008 there was one case. In 2009 there was at least one case every month, with one case each month from January through to April, then seven cases in May, 13 cases in June, and three cases in July. Of the 35 squirrels confirmed positive for SQPV infection, 33 had shown obvious clinical or postmortem signs of the disease.

There was no statistically significant association between SQPV infection and sex (P=0.340). However, there was a statistically significant association between the presence of SQPV infection and age group (P=0.015), with 86 per cent of confirmed squirrelpox cases being adults and no cases in juveniles. In addition, there was also a statistically significant association between SQPV infection and body condition (P=0.011), with 57 per cent of SQPV-infected squirrels being thin or emaciated. There was a statistically significant association between SQPV infection and the presence of external parasites (P=0.007), with 49 per cent of SQPV-infected squirrels carrying external parasites, compared with only 26 per cent of uninfected squirrels. Specifically, there was a statistically significant association between SQPV infection and the presence of fleas (P=0.001), with 43 per cent of SQPV-infected squirrels carrying fleas, compared with 18 per cent of the uninfected squirrels; however, there was no significant association between SQPV infection and the presence of ticks or lice (P>0.226).

Discussion

Convenience sampling was the only practical way to obtain the squirrels examined in this study. As a consequence, the results are unlikely to be representative of the whole Scottish red squirrel population. Bias was undoubtedly introduced in the way in which the squirrels were collected, because squirrels that had died within areas visible to the public were more likely to be discovered. Similarly, more squirrels are likely to be submitted during times of the year when members of the public are more active outdoors. Therefore the numbers of squirrels submitted by month do not necessarily reflect actual fluctuations in mortality. In addition, the majority of the squirrel carcases were submitted from Dumfries and Galloway. This is most likely because of the greater concern about SQPV infection among the public and countryside rangers in this region. Nevertheless, this study is the first to document a (non-exhaustive) list of likely causes of death from a large sample size of red squirrels in Scotland, and to indicate how some of these causes relate to the

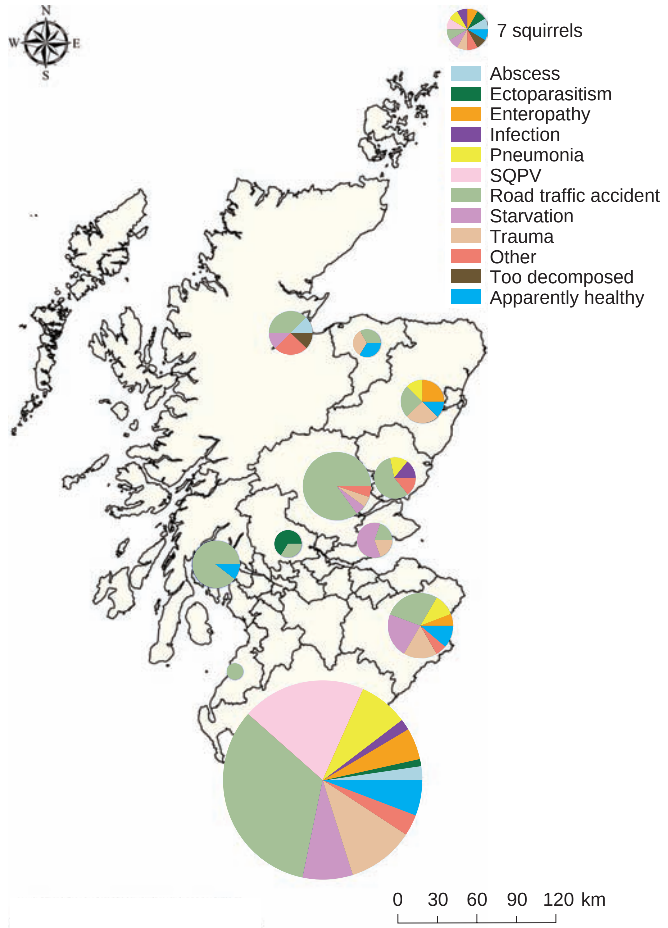

FIG 5: Cause of death, assigned from findings at postmortem examination of 258 red squirrels found between September 2005 and July 2009, categorised according to the county from which the submissions came. The size of each pie chart represents the number of squirrels submitted from that county, and the location of the county is the average location for squirrels submitted from that county. Crown Copyright/database right 2009. An Ordnance Survey/EDINA supplied service

age, body condition and sex of the animals, and the presence of external parasites.

Road traffic accidents were the most common assigned cause of death in the Scottish red squirrels examined in this study, followed by SQPV-associated disease, trauma other than by road traffic accident and starvation. The predominance of road traffic accidents as an apparent cause of death remained when adults and subadults were considered separately, but not in juveniles, in which starvation was the predominant cause of death. Adults and subadults range more widely, and this may result in them crossing roads, whereas juveniles are more likely to be found near the nest area. Efforts to decrease the number of red squirrel road casualties have been incorporated into conservation efforts in some areas; for example, aerial squirrel crossings have been built over roads on the island of Jersey; however, the true impact of these crossings on the squirrel population is not known (Gurnell and Magris 2002). The majority of the juveniles that had apparently died of starvation were found during the late summer or early autumn, corresponding to the season in which summer-born young are weaned (Lurz and others 2005). This newly weaned status was frequently corroborated by postmortem examination, and presumably reflects failed adaption to independent life. Starvation of this type does not apply to subadults or adults because they have long since been weaned, and therefore an apparent inability to use food sources, rather than any actual shortage of food sources, could explain why starvation was one of the more significant causes of death. Provision of supplementary food has been shown in some cases to increase the population density of squirrels in a given area (Gurnell and Magris 2002); however, increased population density also has the potential to support a higher level of disease and spread of parasites. Since the spread of SQPV in particular is known to be a threat to red squirrel populations (Tompkins and others 2002, Rushton and others 2006), any attempt to increase squirrel population density through food supplementation may be detrimental rather than helpful in areas where there is a risk of SQPV being present.

A higher than expected proportion of thin or emaciated squirrels was found to have died, apparently from pneumonia, squirrelpox or starvation. By definition, any squirrel that had died of starvation would have been in poor body condition at the time of death. Squirrels that died of debilitating disease conditions, such as squirrelpox or severe ectoparasitism, would reasonably be expected to have lost body condition before death. The possibility also exists that squirrels in thin or emaciated condition are more susceptible to disease. Demas and others (2003) found that a decrease in total body fat can reduce humoral immunity in Siberian hamsters (*Phodopus sungorus*) and prairie voles (*Microtus ochrogaster*); a later study of Siberian hamsters (Demas and Sakaria 2005) found this change in immunity to be regulated through levels of leptin, an adipose tissue hormone. In the present study, it was not known whether the squirrels that died of disease were in poor body condition before or after succumbing to disease, but poor body condition is likely to have been a predisposing factor to disease in at least some of the squirrels.

There was also an association between body condition and the presence of external parasites, with more thin or emaciated animals having external parasites compared with animals in normal or fat body condition. It is not known what proportion of these squirrels were in poor body condition before becoming infested with external parasites, and what proportion lost body condition as a result of already established ectoparasitism. Depression of immunity as a result of poor body condition may have contributed to the establishment of ectoparasitism in some of these squirrels; however, the infestation was severe enough to be the suspected cause of death only in four of the animals.

The presence of SQPV disease was first detected during the present study in May 2007; there had been no previous reported cases of SQPV in red squirrels in Scotland before that date (McInnes and others 2009). It is notable that, since the study period described by McInnes and others (2009), SQPV has increased in importance as a cause of mortality among red squirrels in Scotland. Nearly all of the squirrels that were confirmed as infected with SQPV had clearly visible pox lesions at postmortem examination, although in two cases SQPV infection was not suspected on postmortem examination. These two cases were considered to be early cases, and highlight the fact that infection may be overlooked in the absence of laboratory investigation. There was a significant association between SQPV infection and thin or emaciated body condition. It has been shown that affected red squirrels can lose weight as a result of SQPV disease (Tompkins and others 2002). However, it is not yet known whether squirrels in poor body condition when they come into contact with the virus are more susceptible to squirrelpox disease than squirrels in normal body condition.

It is still uncertain whether SQPV can be carried by vectors such as fleas, ticks or lice; Sainsbury and others (2008) concluded that epidemiological patterns of SQPV infection in red squirrels did not indicate that the disease was vectorborne. In the present study, SQPV-infected squirrels were more likely to have external parasites, specifically fleas. However, the converse was not true; that is, squirrels with parasitic infestation were not more likely to be infected with SQPV. This could suggest that SQPV infection might predispose squirrels to external parasitism, rather than that the presence of external parasites predisposes to SQPV infection. However, the association between fleas and squirrelpox must be treated with caution because it is possible that more squirrels with squirrelpox were euthanased by veterinary surgeons and immediately placed in a bag, preventing loss of fleas from the carcases.

Finally, there was no statistically significant association between sex and SQPV infection. This finding is contrary to findings of a previous study which examined past squirrelpox epidemics outside Scotland, and concluded that males may be more likely than females to be infected (Sainsbury and others 2008).

In conclusion, this study brings to light many different causes of mortality in red squirrels in Scotland, and the demographic factors associated with these. It demonstrates the value of schemes that gather information about causes of red squirrel mortality, some of which may not be as obvious as SQPV disease. Information gathered from wildlife submission schemes and studies such as this have the potential to aid in the detection of new disease patterns or other threats to populations, and may ultimately help to direct conservation efforts.

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