TITLE: Mortalities, amyloidosis and other diseases in free-living red squirrels (Sciurus vulgaris) on Jersey, Channel Islands

AUTHORS: Tiffany Anne Blackett, Vic R Simpson, Sean Haugland, David J Everest, Clare F Muir, Kenneth C Smith and Aileen C Mill

JOURNAL TITLE: Veterinary Record

PUBLISHER: BMJ Publishing Group

PUBLICATION DATE: 4 September 2018 (online)

DOI: http://dx.doi.org/10.1136/vr.104779

Mortalities, amyloidosis and other diseases in free-living red squirrels (Sciurus vulgaris) on Jersey, Channel Islands

Tiffany Anne Blackett[1], Vic R Simpson[2], Sean Haugland[3], David J Everest[4], Clare F Muir[5], Kenneth C Smith5 and Aileen C Mill[6]

1. JSPCA Animals' Shelter, St Helier, Jersey
2. Wildlife Veterinary Investigation Centre, Truro, UK
3. Rest Associates, Diss, Norfolk, UK
4. Animal and Plant Health Agency Weybridge, Addlestone, UK
5. Department of Pathology and Pathogen Biology, Royal Veterinary College, Hatfield, UK
6. School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, UK

E-mail for correspondence: tblackett100@gmail.com

Abstract:

Between 2007 and 2014, 337 free-living red squirrels (*Sciurus vulgaris*) on Jersey, Channel Islands, were examined post mortem as part of a mortality and disease surveillance scheme. Road traffic accidents (RTAs) were attributable for 50.7 per cent (171/337) of the casualties, 34.4 per cent (116/337) succumbed to diseases including fatal exudative dermatitis (FED), 7.1 per cent (24/337) to predation, 6.5 per cent (22/337) to other trauma and 1.2 per cent (4/337) to suspected poisoning. Cat predation accounted for 5 per cent (17/337) of mortalities. Pathologies were diverse and individual animals were often identified with more than one disease process. Squirrelpox virus (SQPV) particles were not detected in selected cases examined by transmission electron microscopy (TEM). Amyloid was identified in 19.3 per cent (65/337) of squirrels, often in conjunction with inflammatory lesions like hepatic capillariasis. A consistent cause of amyloid accumulation was not identified, although there was a significant association of amyloidosis with hepatic capillariasis and FED. In addition to RTAs, amyloidosis and FED have been identified as important causes of squirrel morbidity and mortality on Jersey, while the underlying aetiology and predisposing factors for these two disease complexes are presently unclear. Disease, fragmented woodlands, an increasingly suburban habitat, along with various anthropogenic factors, may jeopardise the long-term viability of this island red squirrel population.

Introduction

The native red squirrel (*Sciurus vulgaris*) population in Great Britain (GB) is vulnerable to habitat fragmentation and destruction and resource competition by the invasive alien grey squirrel (*Sciurus carolinensis*)[1] first introduced to GB in 1876.[2] Red squirrels are also susceptible to a range of diseases (eg, Keymer,[3] Sainsbury and Gurnell,[4] Sainsbury and others,[5] Thomas and others,[6] Everest and others,[7] Simpson and others,[8–10] Meredith and others[11]), with squirrelpox virus (SQPV) being a major threat.[6]

Red squirrel introductions to Jersey started in 1885[2 12 13] and continued for 60 years.[13 14] It is understood the founders came from southern England and France[13] and the two genotypes identified within the present day population reinforce this.[15] Grey squirrels, the immune reservoir for SQPV,[5 16 17] are absent from Jersey and have never been introduced,[2 13] emphasising the Jersey population as an important island stronghold for red squirrel conservation. Prompted by the number of squirrels presenting in poor condition, the Jersey Society for the Prevention of Cruelty to Animals (JSPCA) Animals' Shelter commenced a disease and mortality surveillance study. The purpose was to identify disease prevalence and thereby enable targeted veterinary treatment and overall improvement of red squirrel welfare.

Red squirrel mortality is frequently caused by human-associated activities, such as road traffic accidents (RTAs) and predation by domestic cats and dogs,[9 18–20] which have also been identified as typical causes of Jersey squirrel mortality.[21 22] While SQPV is a major cause of red squirrel mortality in GB,[5 6 20] it has not been detected within the Jersey squirrel population. Yet on Jersey, numerous cases of a fatal exudative dermatitis (FED) have been recorded.[8] This skin condition, which is associated with infection by a specific clone of *Staphylococcus aureus*,[23] may appear clinically like SQPV infection but is distinct from it. FED is an important cause of Jersey squirrel mortality and based on investigations on Jersey and elsewhere,[23] FED has the potential to cause significant red squirrel mortality, especially in localised populations.[8 9 23]

Amyloidosis was not observed in a histopathological study of red squirrels in GB,[9] but multiple cases have been recorded in Jersey squirrels.[15] Reports of amyloidosis in free-living wildlife are uncommon and limited to sporadic cases, for example, in *Emberizidae* sparrows[24] and in a brown hare (*Lepus europaeus*).[25] Amyloidosis is the accumulation of protein organised into a beta-pleated sheet. Proteins arranged in this morphology resist degradation and subsequently accumulate in tissues causing loss of function.[26–29] Numerous factors may influence the occurrence of amyloidosis and in animals reactive amyloidosis is most frequently identified.[24 27] Reactive amyloidosis is characterised by structural changes in the acute phase protein serum amyloid A (SAA) following excess hepatic SAA production in reaction to chronic inflammation or neoplasia.[24 26 27 30 31] However, genetics and stress factors (eg, social, dietary and environmental) may also play a role in amyloid formation.[24 32 33] Severe systemic amyloidosis was

recorded in a single free-living red squirrel on the Isle of Wight (IOW) possibly associated with a degenerate neoplasm (V Simpson, unpublished observations), and cases of renal amyloidosis have been identified in occasional red squirrels in Lancashire (J Chantrey, personal communication).

This paper describes mortalities and disease prevalence in free-living squirrels on Jersey and identifies frequent amyloid deposition within multiple organs. The aims are to enhance understanding of causes of red squirrel mortality; determine the prevalence of specific diseases and assess their impact on the health of the local squirrel populations; aid development of future conservation management protocols and highlight how disease and genetics may affect red squirrel health, especially of small, isolated populations in fragmented, suburban habitats.

Materials and methods

An island-wide public appeal for the JSPCA Animals' Shelter to be notified of red squirrel carcase locations to enable collection for necropsy was launched in 2007. Casualty squirrels that died despite receiving clinical care, or were euthanased, were also included in this study. From 2007 to 2009, histological examinations were carried out by Rest Associates on behalf of Torrance Diamond Diagnostic Services, and from 2009 until 2014 at either The Wildlife Veterinary Investigation Centre or the Royal Veterinary College. Viral screening using transmission electron microscopy (TEM) or polymerase chain reaction (PCR) assays was undertaken at the Animal and Plant Health Agency (APHA) Weybridge. Statistical analysis of contingency tables using chi-squared and Fisher's exact tests were undertaken using R.[34]

The primary cause of death was recorded based on necropsy findings, finder observations and available clinical history. Pet predation incidents were inferred from submission history and physical wounds suggestive of attacks by cats (eg, characteristic small puncture wounds, bruising and limb fractures) or dogs (eg, skin trauma, bruising, and fractures (typically spinal)).

The squirrel's age (ie, juvenile, subadult, adult) was classified based on descriptions by Carroll and others[17] and Tittensor,[35] considering bodyweight, crown-tail base length (from crown middle (between the pinnae) to tail base), body condition, reproductive status, dentition and the observer's experience. Subjective assessments based on descriptions by Hickman and Swan[36] were undertaken to categorise body condition (ie, emaciated, thin, moderate, fat), considering musculature of the proximal hindlimb and lumbar region and degree of lumbar spine and pelvic bone prominence, as well as the level of intra-abdominal (eg, perirenal) fat.

Necropsies were performed within 24 hours of receipt or following frozen storage of the carcases at −20°C. Light microscopy enabled identification of mites and ticks based on descriptions by Simpson and others[8] and Urquhart and others[37] and the observer's experience. Tissue samples from the heart, lungs, liver, kidneys, adrenal glands, mesenteric lymph node, pancreas and spleen were routinely taken for histopathological examination, along with

any visible macroscopic lesions (eg, skin, gastrointestinal tract and uterine lesions). Tissue samples were preserved in 10 per cent buffered formal saline, embedded in paraffin wax, sectioned at 6 μm and stained by haematoxylin and eosin. Congo red, Masson's Trichrome and Ziehl-Neelsen stains were performed as appropriate. Selected Congo red stained sections were examined for birefringence under polarised light. Occasionally, not all required tissues were available for sampling due to the extent of both antemortem and postmortem damage, for example, in some RTA cases.

Where amyloid deposits were present in only one organ or tissue type this was categorised as localised amyloidosis, with generalised amyloidosis defining cases where amyloid deposits were present in more than one organ or tissue type. Ulcerative, pustular and bacterial dermatitis cases were attributed to the condition termed FED based on the appearance of macroscopic skin lesions, antemortem clinical observations and histological findings, and in six cases by bacterial culture and multilocus sequence typing (MLST) of *S aureus*. Skin lesions from only a limited number of cases with severe lesions attributed to FED were examined for viral presence by TEM. Intracytoplasmic inclusion bodies and ballooning degeneration typical of SQPV infection have not been documented at histological examination of FED cases and grey squirrels, which have been implicated in SQPV transmission,[38] have never been present on the island. Further SQPV-specific screening using ELISA and PCR was not undertaken.

Intestinal samples or faeces were collected from selected ill casualty squirrels and selected cases with macroscopic evidence suggestive of gastrointestinal disease at necropsy (ie, perineal staining, gastrointestinal tract inflammation, diarrhoea) for direct microscopy and/or TEM or PCR analyses. TEM and PCR analyses were performed following Everest and others.[39 40]

Results

Between 2007 and 2014, 337 squirrels were examined, of which 54.3 per cent (n=183) were male. Casualties that died in veterinary care or were euthanased accounted for 35.9 per cent (n=121). Carcase preservation was variable, with autolysis precluding histological examination of all sampled tissues in 7.7 per cent (n=26) of cases.

RTAs caused the deaths of 50.7 per cent (n=171). A further 34.4 per cent (n=116) died from disease (inferred from antemortem clinical observations, gross necropsy lesions and histology results) and 6.5 per cent (n=22) from other trauma (eg, entrapment injuries, witnessed falls from trees, trauma in animals not found on or at the roadside, etc). Twenty-four squirrels (7.1 per cent) had physical wounds suggestive of predation and/or the finder had observed the attack (cat, n=8; dog, n=3; crow, n=1; magpie, n=1). Pets accounted for 91.7 per cent of predation incidents (ie, 17/24 (70.8 per cent) attacks were attributed to cats and 5/24 (20.8 per cent) dogs). In none of the cases attributed to predation or other trauma was there any evidence of carcase consumption suggestive of a scavenging incident. Four individuals (1.2 per cent) displayed gross lesions suggestive of poisoning (table 1).

General body condition and ectoparasitic burden

The average adult bodyweight for males (n=134) and females (n=117) was 281.7 and 282.4 g, respectively. Most squirrels were in moderate body condition. Twenty-eight squirrels were emaciated and all died from disease, except two that succumbed to non-vehicularrelated trauma. Sixty-one squirrels were thin, of which 78.7 per cent (48/61) died from disease.

Ectoparasites including fleas and/or mites (ie, hypopi of *Dermacarus sciurinus*, *Metalistrophorus pagenstecheri*) were recorded on 126 (37.4 per cent) squirrels, of which 80/126 (63.5 per cent) perished from disease. The majority of mite burdens involved *D sciurinus* (table 2). In six squirrels, the *D sciurinus* burden was so great that mites were macroscopically visible throughout the coat resembling thick scurf. These six animals were either thin or emaciated and all died from disease. *Ixodes* species ticks were recorded on three individuals that all died from disease, had concurrent flea burdens and presented either thin (n=2) or emaciated (n=1).

Disease

Individual squirrels were often identified with concurrent pathology due to more than one disease process, which may have had a combined effect on the animal's overall health. Data are summarised in table 2.

Histopathology identified toxoplasmosis in 2.1 per cent of squirrels (7/337), all of which had splenic lymphoid necrosis. Interstitial pneumonia was noted in 71.4 per cent (5/7) of toxoplasmosis cases and hepatic necrosis in 85.7 per cent (6/7). Necrotic lesions associated with *Toxoplasma gondii* infection were identified in the mesenteric lymph node (4/7), myocardium (4/7) and pancreas (1/7).

Lesions morphologically consistent with those of exudative, pustular dermatitis associated with FED were recorded in 61.3 per cent of skin samples (49/80). Gross lesions were typically seen around the mouth, nose and on the feet (figure 1), occasionally on the eyelids and more infrequently on the scrotum and pinnae. Several cases had accompanying alopecia and sloughing of the digital and/or metacarpal pads with necrosis of the digits. Bacteriological examination and MLST in six cases with lesions consistent with FED identified the presence of *S aureus* ST49 possessing the leukotoxin M encoding gene (lukM). Lesions from 2/49 (4.1 per cent) FED attributed cases were examined for SQPV by TEM and were virus particle negative.

Scattered apicomplexan schizonts identified as *Hepatozoon* species were present within the alveolar interstitium in 16.1 per cent (49/304) of squirrels. *Hepatozoon* species were identified in six subadults and no juveniles. This infection was considered incidental; there was no associated necrosis or inflammation. In only three squirrels with *Hepatozoon* species infection were concurrent respiratory pathologies detected (alveolar amyloid deposition, n=1; bronchopneumonia, n=1; bronchointerstitial pneumonia, n=1).

Capillaria hepatica infection was detected in 33.5 per cent of animals (106/316). Typically, infection induced chronic granulomatous hepatitis with associated necrosis and fibrosis. Infections ranged from mild to severe, with extensive bridging fibrosis and cirrhosis observed in severe cases. In severe cases (7/106), the liver pathology was considered fatal. Hepatic infection with only adult nematodes was observed in four squirrels, three of which showed no inflammatory response and were attributed to capillariasis in the prepatent phase. The fourth squirrel had mild acute necrotic hepatitis associated with the nematodes.

Amyloidosis was the most common renal pathology, recorded in 16.6 per cent of squirrels (54/325) (figure 2), and was the predominant splenic and pancreatic pathology, occurring in 13.6 per cent (40/295) and 7.1 per cent (16/226) of squirrels, respectively.

Pyelonephritis was identified in 5.5 per cent of animals (18/325). In 13 squirrels with pyelonephritis (72.2 per cent), renal amyloidosis was simultaneously observed. Interstitial nephritis was recorded in 3.1 per cent of squirrels (10/325), four of which had renal amyloid deposits. In one animal, interstitial nephritis and numerous tubular and interstitial oxalate deposits occurred concurrently with renal amyloidosis.

Dental abnormalities were identified in 3.7 per cent (9/246) of squirrels. Incisor malocclusion and overgrowth occurred in seven individuals, one of which also had a discharging lower incisor abscess. Two squirrels had maxillary abscesses associated with the upper molars.

Faecal sample examinations from eight squirrels revealed small numbers of coccidian oocysts, which were not considered clinically significant, and nematode larvae in one sample. All eight samples were TEM negative for adenovirus (ADV), rotavirus, coronavirus, parvovirus and enterovirus. However, 50 per cent (4/8) were positive for amplified ADV DNA via
PCR screening. In two of these ADV-positive animals, there was associated gastrointestinal pathology: one juvenile had a mesenteric root intestinal torsion, another necrotic enteritis. Pooled gastrointestinal and splenic samples from another four squirrels were examined for ADV by PCR, resulting in ADV screening of 12 squirrels in total. From these further four animals, an additional ADV-positive result was obtained from a splenic sample from a squirrel without gastrointestinal pathology, totalling 41.7 per cent (5/12) of squirrels positive for ADV by PCR.

A single case of granulomatous lymphadenitis and lymphangitis was recorded. No microorganisms were seen in the histologically examined sections, including with Ziehl-Neelsen stain, although a faecal sample proved positive using PCR for amplified ADV DNA.

Amyloid distribution and occurrence

Amyloid deposits were identified in 65 squirrels, of which 40 per cent were female (26/65) and 96.9 per cent were adults (63/65). Disease was considered the predominant cause of death in 67.7 per cent (44/65) of these squirrels,

while RTAs accounted for 26.2 per cent (17/65) of mortalities, predation 1.5 per cent (1/65) and other trauma 4.6 per cent (3/65). Various concurrent pathologies were recorded (table 3). Amyloid deposits were identified more frequently with coexisting *C hepatica* infection; amyloidosis occurred in 26/106 animals with concurrent hepatic capillariasis, compared with 31 amyloidosis cases identified in 210 squirrels with examined liver samples negative for hepatic capillariasis (chi-squared=4.54; P=0.033). Amyloidosis was also strongly associated with FED, occurring in 18/49 animals with FED-positive skin samples, compared with 3/31 skin samples negative for FED (Fisher's exact OR=5.31; P<0.001).

Tissue distribution of amyloid deposits was variable, but predominantly involved the kidneys (54/65), spleen (40/65) and pancreas (16/65). In some individuals, amyloid deposits were also identified in the liver (4/65), mesenteric lymph node (3/65), adrenal glands (2/65), lungs (1/65), blood vessel walls (1/65) and small intestine (1/65). Generalised amyloidosis occurred in 55.4 per cent (36/65) of cases, of which 91.7 per cent (33/36) had renal amyloid deposits. Multiple pale creamcoloured, irregular-sized foci were often macroscopically visible over the surface of amyloid-affected organs.

Discussion

The most common cause of mortality was RTAs (50.7 per cent). This is comparable to the 48 per cent Anglesey red squirrel RTA mortality documented by Shuttleworth and others,[41] but is higher than the 38.8 per cent RTA mortality recorded on the IOW[9] and the 36 per cent Jersey RTA mortality reported by Magris and Gurnell.[22] However, this study involved opportunistic sampling, hence the reported RTA mortality may not be truly demonstrative; animals that succumb to RTA trauma are typically more readily found. Jersey has a high human population density, which may increase the risk of traffic mortalities as well as pet predation pressures.

Predation incidents (7.1 per cent) mainly involved domestic pets. Attacks attributed to cats were responsible for 5 per cent of squirrel deaths, while Magris and Gurnell[22] recorded 36 per cent of Jersey squirrels as cat attack victims. Predation by pets has an important impact on squirrel mortality in GB[9 19] and on Jersey.[22]

Magris and Gurnell[22] reported higher bodyweights for males and females, 344 and 352 g, respectively. In this study, the lower recorded average adult bodyweight may be because just over one-quarter of squirrels were either thin or emaciated and disease accounted for approximately one-third of mortalities. Large ectoparasitic burdens were typically recorded on thin or emaciated squirrels. No lice (eg, *Neohaematopinus sciuri*) were recorded, similar to the findings of Simpson and others[9] for squirrels from the IOW.

Overall, the most prevalent disease was hepatic capillariasis (31.5 per cent, 106/337), followed by amyloidosis (19.3 per cent, 65/337) and lesions consistent with FED (14.5 per cent, 49/337). *Hepatozoon* species infection was relatively common in sampled lung tissue (16.1 per cent, 49/304), although it was less prevalent than in IOW squirrels (32.7 per cent, 38/116).9 As in other studies, a low prevalence of fungal infections, reproductive disease,

neoplasia and dental abnormalities were recorded.[9 20 42] Mycobacterial dermatitis (squirrel leprosy) was not identified at histological examination, despite recent identification of clinical cases of *Mycobacterium lepromatosis* in red squirrels from Scotland and the IOW, and of *Mycobacterium leprae* in red squirrels from Brownsea Island.[10 11 43 44]

Almost 20 per cent of the total number of squirrels had either localised or generalised amyloidosis. All amyloidosis cases, except two, occurred in adult animals. Mature animals are more likely to have encountered factors that may predispose to amyloid deposition.[24]

C hepatica is a nematode that occupies the liver, commonly infecting wild rodents and, less frequently, other species.[45–48] It is possible that *C hepatica* infection may predispose to amyloidosis development due to the nature of the lesions typically observed in infected red squirrels. Glomerular amyloidosis in an Italian domestic dog was reported associated with canine *Capillaria plica* infection in which the parasitic infection was thought to play a role in the amyloidosis development.[49] Amyloidosis occurred in a higher than expected proportion of squirrels with hepatic capillariasis (24.5 per cent, 26/106), yet severity of infection does not appear to relate to amyloid deposition and hepatic capillariasis was not observed in the four squirrels with hepatic amyloid deposits. Furthermore, hepatic capillariasis is frequently identified in British red squirrels at histology,[9 46] but no other cases concurrently involving amyloidosis are reported.

Another important disease on Jersey, and to a lesser extent on the IOW,[8 9 23] is a form of exudative dermatitis known as FED. FED may appear macroscopically comparable to SQPV, but where lesions were examined by TEM for viral presence from FED cases in this study, and from IOW FED cases,[8] they were negative. FED is linked with infection by *S aureus* ST49 with the lukM gene, yet the pathogenesis of FED is unclear.[8 23] Many Jersey FED cases had a variety of coexisting pathologies, including amyloidosis, but none was regularly identified, although a significant proportion of animals with examined skin samples positive for FED had concurrent amyloidosis. Unknown factors or pathogens may exist that predispose red squirrels to FED, which may also trigger amyloid deposition. However, the observation of FED in squirrels with amyloidosis may reflect general debility associated with the organ dysfunction accompanying the amyloid deposition, which could enable FED development.

Toxoplasmosis was recorded in 2.1 per cent squirrels, compared with the 15.6 per cent (12/77) detected by Simpson and others[9] on the IOW. Both the IOW and Jersey are islands with large human population densities, and, in association, potentially a big domestic cat population. Also, supplementary feeding of red squirrels by the public on both islands is commonplace. Squirrels that range into suburbia are at increased risk of traffic-associated mortalities, and may be at greater risk from direct or indirect effects associated with pet encounters. Squirrels foraging in suburban areas may be more exposed to *T gondii* infection.[9] None of the squirrels with toxoplasmosis had concurrent amyloidosis.

Enteric disease was uncommon and of the squirrels examined for ADV presence,[50] 41.7 per cent (5/12) were positive for amplified ADV DNA by PCR. However, there was little associated enteric pathology and the lack of virus particle detection in the faecal samples examined by TEM leads the authors to determine that the PCR detection of ADV DNA reflected a subclinical presence.[7] ADV infection of red squirrels has been documented over a large geographical area in both captive and free-living animals and is often asymptomatic,[7] yet the origins of the ADV presence are unclear. In the absence of grey squirrels, other small woodland rodents, such as wood mice (*Apodemus sylvaticus*), which can harbour murine ADV, may be one possible transmission route,[7 50 51] as well as intraspecific transmission between red squirrels. Amyloid was not observed in any squirrels that proved positive for amplified ADV DNA.

Stress may be a factor in the pathogenesis of amyloidosis.[24 32 33] For example, reactive amyloidosis recorded in captive domestic Pekin ducks may be influenced by nutritional and disease issues and social stress.[24] On Jersey, squirrels live in woodland patches dotted within a largely suburban environment, increasing the likelihood of squirrels encountering people, traffic and pets. Also, the widespread, islandwide practice of providing supplemental food via numerous artificial feeders may boost squirrel numbers.[22] Such feeding and ranging behaviours, in conjunction with high local squirrel densities, may influence health and intensify social stress through the likelihood of increased intra-specific agonistic interactions at feeders and within home ranges.

Stress may also play a role in red squirrel ADV infection, triggering clinical disease.[52 53] ADV has been confirmed as a cause of cluster mortalities in some British captive red squirrel collections.[51] However, while Jersey squirrels live in a habitat that may more expose to stress, ADV infection in the local populations seems to be typically subclinical and may be endemic. Yet the number sampled was small (n=12), hence further ADV screening is warranted to more accurately assess both subclinical and pathogenic ADV infection prevalence in the Jersey squirrel population.

Reactive amyloidosis can be a consequence of chronic inflammation, yet it is reported that amyloidosis only occurs in 5 per cent–15 per cent of animals with chronic inflammatory conditions, indicating that other elements, including genetics, may play a role in the pathogenesis.[30] For example, while captive cheetahs (*Acinonyx jubatus*) seem susceptible to amyloidosis, which is thought to be associated with various triggers including chronic infections, the high incidence may also indicate a genetic predisposition.[27 32]

The island population of Jersey squirrels consists of approximately 500 adult animals[54] and the local populations live in small woodland fragments, increasing the risk of genetic threats.[22] Simpson and others[15] illustrated that although there was some inbreeding within the Jersey squirrels, there was no link between the degree of inbreeding and incidence of disease in general or, specifically, cases of amyloidosis. However, a genetic peculiarity may still exist, such as a heritable allele that predisposes the individual to amyloid deposition. One or more of the founding squirrels may have carried such a susceptibility allele which, through stochastic processes, has dispersed through the island's squirrels.[15]

Conclusion

No evidence of SQPV presence has been detected in Jersey squirrels, supporting the observations of McInnes and others [55] [56] that in the absence of grey squirrels neither pathogenic nor subclinical cases were found. Yet the population experiences mortality from a variety of causes, including those associated with human activities and suburban living (ie, pet predation, RTAs) and disease. A diverse range of pathologies were detected and it was not uncommon to observe individual animals with coexisting pathology due to more than one disease process. Amyloidosis, as well as FED and RTAs, are major causes of morbidity and mortality in Jersey squirrels, with multiple cases influencing population health dynamics. No consistent concurrent pathology and the underlying aetiologies are presently unknown. Yet the presence of FED and hepatic capillariasis appears to be associated with amyloidosis. Amyloidosis is a multifaceted disease and the predisposing factors and epidemiology in free-living Jersey squirrels are unclear. Due to the divided nature of the woodlands and general suburban character of the island, Jersey squirrels may be at greater risk from genetic threats and exposure to a variety of pathogens and stressors that may have a combined cumulative effect in predisposing individuals to amyloidosis development. Therefore, further research, to explore the possibility that a heritable susceptibility to develop amyloidosis may exist in the Jersey squirrels, and to closely examine how behaviour, feeding patterns and specific pathogenic agents may influence the health status of this Channel Island squirrel population, is vital. Future studies into the pathogenesis of FED and ongoing disease surveillance are necessary. These actions are imperative to inform conservation measures to help ensure the future survival of this important red squirrel-only island stronghold.

References

1. Skelcher G. The ecological replacement of red by grey squirrels. In: Gurnell J, Lurz PWW, eds. Conservation of red squirrels *S. vulgaris L.* London: People's Trust for Endangered Species, 1997:67–78.
2. Middleton AD. The Grey Squirrel. London: Sidgwick and Jackson Ltd, 1931:14–35.
3. Keymer IF. Diseases of squirrels in Britain. *Mamm Rev* 1983;13:155–8.
4. Sainsbury AW, Gurnell J. An investigation into the health and welfare of red squirrels, *Sciurus vulgaris*, involved in reintroduction studies. *Vet Rec* 1995;137:367–70.
5. Sainsbury AW, Nettleton P, Gurnell J. Recent developments in the study of parapoxvirus in red and grey squirrels. In: Gurnell J, Lurz PWW, eds. Conservation of red squirrels *S. vulgaris L.* London: People's Trust for Endangered Species, 1997:105–8.
6. Thomas K, Tompkins DM, Sainsbury AW, *et al*. A novel pox virus lethal to red squirrels (*Sciurus vulgaris*). *Journal of Virology* 2003;84:3337–41.
7. Everest DJ, Shuttleworth CM, Stidworthy MF, *et al*. Adenovirus: an emerging factor in red squirrel *Sciurus vulgaris* conservation. *Mamm Rev* 2014;44:225–33
8. Simpson VR, Hargreaves J, Everest DJ, *et al*. Mortality in red squirrels (*Sciurus vulgaris*) associated with exudative dermatitis. *Vet Rec* 2010;167:59–62.
9. Simpson VR, Hargreaves J, Butler HM, *et al*. Causes of mortality and pathological lesions observed post-mortem in red squirrels (*Sciurus vulgaris*) in Great Britain. *BMC Vet Res* 2013;9:229.
10. Simpson V, Hargreaves J, Butler H, *et al*. Leprosy in red squirrels on the Isle of Wight and Brownsea Island. *Vet Rec* 2015;177:206–7.
11. Meredith A, Del Pozo J, Smith S, *et al*. Leprosy in red squirrels in Scotland. *Vet Rec* 2014;175:285–6.
12. Shorten M. The New Naturalist - Squirrels. London: Collins, 1954:71.
13. Le Sueur F. The Natural History of Jersey. Chichester: Phillamore, 1976:95–8.
14. Baal HJ. Zoological section. Bulletin Annual Societe Jeriaise, Volume XV 1949-1952, 1949:106.
15. Simpson S, Blampied N, Peniche G, *et al*. Genetic structure of introduced populations: 120-year-old DNA footprint of historic introduction in an insular small mammal population. *Ecol Evol* 2013;3:614–28.
16. Sainsbury AW, Deaville R, Lawson B, *et al*. Poxviral disease in red squirrels *Sciurus vulgaris* in the UK: spatial and temporal trends of an emerging threat. *Ecohealth* 2008;5:305–16.
17. Carroll B, Russell P, Gurnell J, *et al*. Epidemics of squirrelpox virus disease in red squirrels (*Sciurus vulgaris*): temporal and serological findings. *Epidemiol Infect* 2009;137:257–65.
18. Shuttleworth CM. Traffic related mortality in a red squirrel (*Sciurus vulgaris*) population receiving supplemental feeding. *Urban Ecosyst* 2001;5:109–18.
19. Duff JP, Haley P, Wood R, *et al*. Causes of red squirrel (*Sciurus vulgaris*) mortality in England. *Vet Rec* 2010;167:461.
20. LaRose JP, Meredith AL, Everest DJ, *et al*. Epidemiological and postmortem findings in 262 red squirrels (*Sciurus vulgaris*) in Scotland, 2005 to 2009. *Vet Rec* 2010;167:297–302.
21. Magris L, Morris P, Gurnell J. Human impacts on red squirrel (*Sciurus vulgaris*) ecology on the Island of Jersey. In: Gurnell J, Lurz PWW, eds. Conservation of red squirrels *S. vulgaris L.* London: People's Trust for Endangered Species, 1997:49–60.
22. Magris L, Gurnell J. Population ecology of the red squirrel (*Sciurus vulgaris*) in a fragmented woodland ecosystem on the Island of Jersey, Channel Islands. *J Zool* 2002;256:99–112.
23. Simpson VR, Davison NJ, Kearns AM, *et al*. Association of a lukM-positive clone of *Staphylococcus aureus* with fatal exudative dermatitis in red squirrels (*Sciurus vulgaris*). *Vet Microbiol* 2013;162:987–91.
24. Roertgen KE, Johnson KH. Amyloidosis. In: Faribrother A, Locke LN, Hoff GL, eds. Non infectious diseases of wildlife: Manson Publishing, 1996:194–202.
25. Veterinary Laboratories Agency. VLA Surveillance Report. *Veterinary Record* 2008;163:560.
26. Ménsua C, Carrasco L, Bautista MJ, *et al*. Pathology of AA amyloidosis in domestic sheep and goats. *Vet Pathol* 2003;40:71–80.
27. Woldemeskel M. A concise review of amyloidosis in animals. *Vet Med Int* 2012;2012:1–11.
28. Murakami T, Ishiguro N, Higuchi K. Transmission of systemic AA amyloidosis in animals. *Vet Pathol* 2014;51:363–71.
29. Watanabe K, Uchida K, Chambers JK, *et al*. Deposition, Clearance, and Reinduction of Amyloid A Amyloid in Interleukin 1 Receptor Antagonist Knockout Mice. *Vet Pathol* 2017;54:99–110.
30. Grauer GF, Dibartola SP. Section XIV – the Urinary System. In: Ettinger SJ, Feldman EC, Saunders WB, eds. Textbook of Veterinary Internal medicine, Volume two. Chapter 170 - Glomerular Disease, 2000:1662–78.
31. Ludlage E, Murphy CL, Davern SM, *et al*. Systemic AA amyloidosis in the common marmoset. *Vet Pathol* 2005;42:117–24.
32. Caughey B, Baron GS. Are cheetahs on the run from prion-like amyloidosis? *Proc Natl Acad Sci U S A* 2008;105:7113–4.
33. Veterinary Laboratories Agency. VLA Surveillance Report. *Veterinary Record* 2011;168:237.
34. Core Team R. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2016.
35. Tittensor AM. The red squirrel (*Sciurus vulgaris*) in relation to its food resource: University of Edinburgh, 1970.
36. Hickman DL, Swan M. Use of a body condition score technique to assess health status in a rat model of polycystic kidney disease. *Journal of the American Association of Laboratory Animal Science* 2010;49:155–9.
37. Urquhart GM, Armour J, Duncan JL, *et al*. Class Arachnida. Veterinary Parasitology: Longman Group, Scientific and Technical,

1992:176–7, 199.
38. Collins LM, Warnock ND, Tosh DG, *et al*. Squirrelpox virus: assessing prevalence, transmission and environmental degradation. *PLoS One* 2014;9:e89521.
39. Everest DJ, Stidworthy MF, Milne EM, *et al*. Retrospective detection by negative contrast electron microscopy of faecal viral particles in free-living wild red squirrels (*Sciurus vulgaris*) with suspected enteropathy in Great Britain. *Vet Rec* 2010;167:1007–10.
40. Everest DJ, Shuttleworth CM, Grierson SS, *et al*. Systematic assessment of the impact of adenovirus infection on a captive reintroduction project for red squirrels (*Sciurus vulgaris*). *Vet Rec* 2012;171:176.
41. Shuttleworth CM, Signorile AL, Everest DJ, *et al*. Assessing causes and significance of red squirrel (*Sciurus vulgaris*) mortality during regional population restoration: an applied conservation perspective. *Hystrix, the Italian Journal of Mammology* 2015;26:69–75.
42. Sainsbury AW, Kountouri A, DuBoulay G, *et al*. Oral disease in free-living red squirrels (*Sciurus vulgaris*) in the United Kingdom. *J Wildl Dis* 2004;40:185–96.
43. Meredith A, Del-Pozo J, Stevenson K, *et al*. Mycobacterial dermatitis of red squirrels in Scotland: a case series. *Proceedings of the European Wildlife Disease Association (EWDA) Conference: Conservation Medicine*, Edinburgh;25-29 August 2014.
44. Avanzi C, Del-Pozo J, Benjak A, *et al*. Red squirrels in the British Isles are infected with leprosy bacilli. *Science* 2016;354:744–7.
45. Urquhart GM, Armour J, Duncan JL, *et al*. Class Nematoda, superfamily Trichuroidea. Veterinary Parasitology: Longman Group, Scientific and Technical, 1992:93–4.
46. Simpson V. Wildlife as reservoirs of zoonotic diseases in the UK. *In Pract* 2008;30:486–94.
47. Stidworthy MF, Lewis JC, Masters NJ, *et al*. *Capillaria hepatica* in primates in zoological collections in the British Isles. *Vet Rec* 2009;164:66.
48. Stidworthy MF. Rodent-associated infections in zoo animals. Proceedings of the British Veterinary Zoological Society, Torquay;24-25 April 2010; 30–2
49. Callegari D, Kramer L, Cantoni AM, *et al*. Canine bladderworm (*Capillaria plica*) infection associated with glomerular amyloidosis. *Vet Parasitol* 2010;168:338–41.
50. Everest DJ, Butler H, Blackett T, *et al*. Adenovirus infection in red squirrels in areas free from grey squirrels. *Vet Rec* 2013;173:199–200.
51. Everest DJ, Shuttleworth CM, Grierson SS, *et al*. The implications of significant adenovirus infection in UK captive red squirrel (*Sciurus vulgaris*) collections: How histological screening can aid applied conservation management. *Mamm Biol* 2018;88:123–9.
52. Martínez-Jiménez D, Graham D, Couper D, *et al*. Epizootiology and pathologic findings associated with a newly described adenovirus in the red squirrel, *Sciurus vulgaris*. *J Wildl Dis* 2011;47:442–54.
53. Peters M, Vidovszky MZ, Harrach B, *et al*. Squirrel adenovirus type 1 in red squirrels (*Sciurus vulgaris*) in Germany. *Vet Rec* 2011;169:182.
54. Gurnell J, Blackett T, Butler H, *et al*. British red squirrel strongholds: challenges for conservation. In: Shuttleworth CM, Lurz PWW, Hayward MW, eds. Red squirrels – ecology, conservation and management in Europe, 2015:211–33.
55. McInnes CJ, Coulter L, Dagleish MP, *et al*. First cases of squirrelpox in red squirrels (*Sciurus vulgaris*) in Scotland. *Vet Rec* 2009;164:528–31.
56. Mcinnes CJ, Coulter L, Dagleish MP, *et al*. The emergence of squirrelpox in Ireland. Animal Conservation, 2012:16: 51–9.

Figure legends:

Figure 1: An adult female Jersey red squirrel with pronounced exudative dermatitis lesions consistent with fatal exudative dermatitis on the muzzle and nose. Gram-positive bacterial colonies were observed at histology, mostly in the surface of the exudate. Photo: TA Blackett.

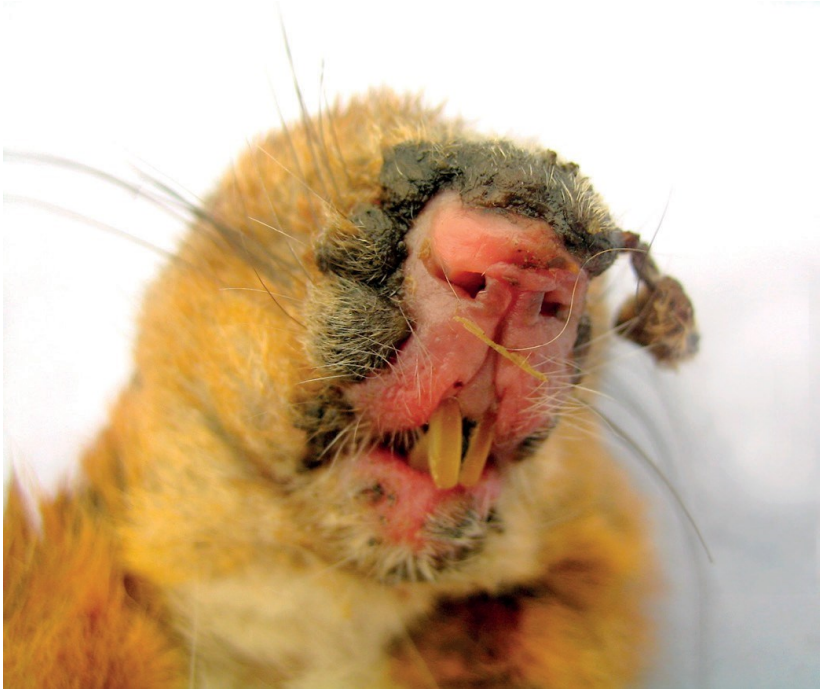

Figure 2: Histological section of the kidney of an adult female Jersey red squirrel with renal amyloidosis. Congo red stain. The staining highlights amyloid deposition both within a glomerulus and also multifocally in the interstitium. Bar=200 µm. Photo: VR Simpson.

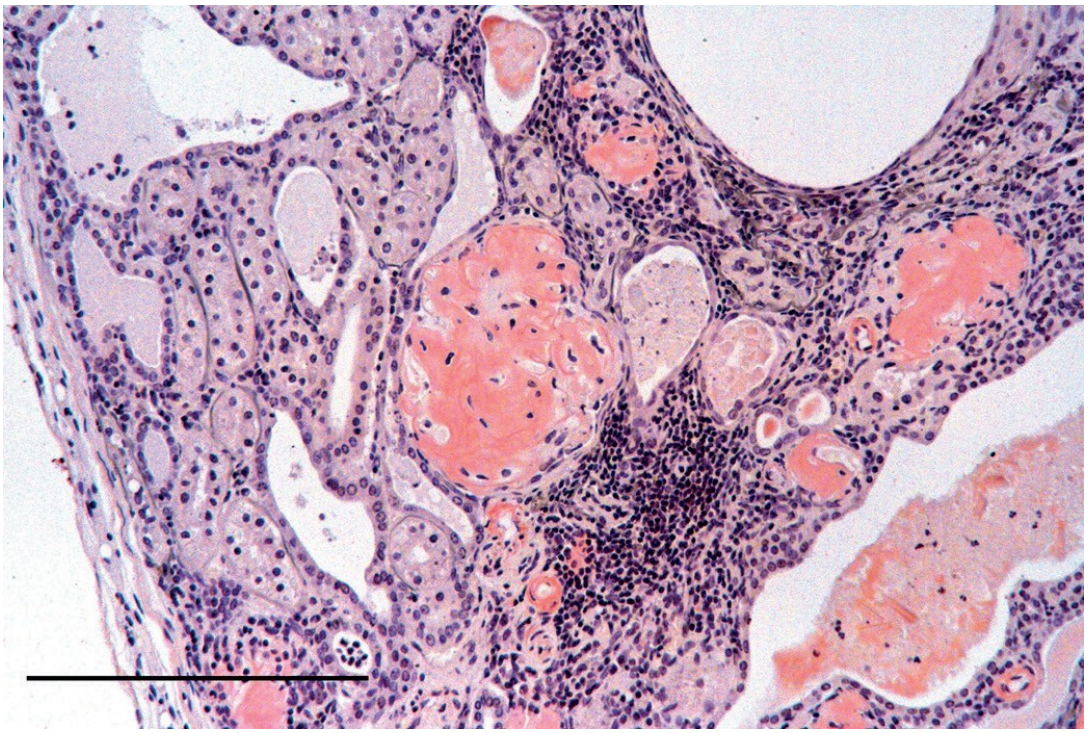

Table 1: Number, sex and age group of the 337 examined squirrels and allocated predominant cause of mortality.

Age	Allocated primary cause of death					Proportion of examined squirrels (total number)
	RTA	Clinical disease	Predation	Trauma, other than trauma associated with RTAs	Suspected poisoning (eg, anticoagulant rodenticide)	
Adults	152 M=78; F=74	80 M=48; F=32	6 M=2; F=4	12 M=5; F=7	1 M=1	74.5% (251) M=134; F=117
Subadults	14 M=8; F=6	20 M=12; F=8	9 M=5; F=4	7 M=4; F=3	3 M=3	15.7% (53) M=32; F=21
Juveniles	5 M=2; F=3	16 M=10; F=6	9 M=5; F=4	3 F=3	0	9.8% (33) M=17; F=16
Proportion of examined squirrels (total number)	50.7% (171) M=88; F=83	34.4% (116) M=70; F=46	7.1% (24) M=12; F=12	6.5% (22) M=9; F=13	1.2% (4) M=4	100% (337) M=183; F=154

F, females; M, males; RTA, road traffic accident.

Table 2: The prevalence of identified pathologies, gross necropsy observations and histological findings identified in 337 squirrels examined between 2007 and 2014

Gross necropsy/histology findings (n=number of squirrels from which representative tissue samples were histologically examined or ectoparasites were found)	Percentage (number) of affected squirrels
Skin: (80)	
Fatal exudative dermatitis	61.3 (49)
Hyperkeratosis (muzzle or pinnae)	11.3 (9)
Chronic muzzle dermatitis with epidermal hyperplasia	7.5 (6)
Muzzle epidermal hyperplasia	2.5 (2)
Hyperkeratosis between the nasal apertures and upper lips in association with a hypodermal foreign body	1.3 (1)
Ectoparasites: (337)	
Fleas (species not determined)	35.6 (120)
Dermacarus sciurinus mites	16.3 (55)
Metalistrophorus pagenstecheri mites	2.1 (7)
Neotrombicula autumnalis larvae	1.5 (5)
Ixodes species ticks	0.9 (3)
Respiratory: (lung 304)	
Hepatozoon species within the alveolar interstitium	16.1 (49)
Pneumonia (ie, bronchopneumonia, bronchointerstitial pneumonia or interstitial pneumonia), no microorganisms demonstrated	2.0 (6)
Interstitial pneumonia associated with *Toxoplasma gondii* infection	1.6 (5)
Bronchointerstitial pneumonia with a heavy Gram-negative *coccobacillus* infection	0.3 (1)
Multifocal necrotic mycotic pneumonia	0.3 (1)
Mycotic pleurisy (confirmed by culture as *Mucor hiemalis)*	0.3 (1)
Alveolar septa amyloid deposition	0.3 (1)
Bronchoalveolar carcinoma	0.3 (1)
Pulmonary atelectasis and focus of metaplastic bone	0.3 (1)
Hepatobiliary: (316)	
Hepatic capillariasis (including the four cases involving only *Capillaria hepatica* adults)	33.5 (106)
Mononuclear/non-specific hepatitis, no microorganisms demonstrated	5.1 (16)
Multifocal hepatic necrosis due to *T gondii* infection	1.9 (6)
Hepatic necrosis, no microorganisms demonstrated	1.9 (6)
Granulomatous hepatitis, no evidence of parasitic infection	1.3 (4)
Hepatic sinusoid amyloid deposition	1.3 (4)
Hepatic lipidosis	1.3 (4)
Hepatobiliary cysts	0.9 (3)
Hepatic fibrosis	0.6 (2)
Chronic hepatitis with fibrosis and cholestasis of undetermined cause	0.3 (1)
Cholangiohepatitis	0.3 (1)
Renal: (kidney samples 325; adrenal gland samples 168)	
Renal amyloid deposition	16.6 (54)
Pyelonephritis	5.5 (18)
Renal tubular degeneration and/or necrosis	4.0 (13)
Interstitial nephritis	3.1 (10)
Glomerulonephropathy, no microorganisms demonstrated	0.9 (3)
Adrenal gland amyloid deposition	1.2 (2)
Bacterial nephritis	0.3 (1)
Renal tubular abnormalities*	0.3 (1)
Protein losing nephropathy with minimal light microscopic glomerular changes	0.3 (1)
Renal cortical infarcts with fibrosis	0.3 (1)
Renal tubular and interstitial deposits of oxalate	0.3 (1)
Splenic: (295)	
Splenic amyloid deposition	13.6 (40)
Splenic lymphoid necrosis associated with *T gondii* infection	2.4 (7)

Table 2 Continued

Gross necropsy/histology findings (n=number of squirrels from which representative tissue samples were histologically examined or ectoparasites were found)	Percentage (number) of affected squirrels
Splenic lymphoid necrosis and histiocytosis	0.3 (1)
Focal splenic abscess	0.3 (1)
Pancreatic: (226)	
Pancreatic amyloid deposition within the pancreatic interstitium, acini and islets of Langerhans.	7.1 (16)
Dicrocoelid trematodes in large pancreatic ducts	2.2 (5)
Pancreatic necrosis due to *T gondii* infection	0.4 (1)
Pancreatic nodular hyperplasia	0.4 (1)
Alimentary tract: (dentition 246; gastrointestinal tract 13; mesenteric lymph node 181)	
Incisor malocclusion and overgrowth	2.8 (7)
Mesenteric lymph node necrosis associated with *T gondii* infection	2.2 (4)
Mesenteric lymph node amyloid deposition	1.7 (3)
Necrotic enteritis	15.4 (2)
Maxillary abscess associated with the upper molars	0.8 (2)
Lower incisor abscess	0.4 (1)
Amyloid deposition within the small intestine wall	7.6 (1)
Granulomatous lymphadenitis and lymphangitis	0.6 (1)
Endoparasites: (faecal samples 8)	
Coccidian oocysts	100 (8)
Nematode larvae (species not determined)	12.5 (1)
Reproductive: (uterus 5)	
Foetal mummification	40 (2)
Foetal and placental necrosis with endometritis	20 (1)
Septic metritis	20 (1)
Cardiac: (289)	
Necrotic myocarditis (including four squirrels with toxoplasmosis)	1.7 (5)
Non-specific myocarditis	1.0 (3)
Bacterial myocarditis	0.3 (1)
Vascular amyloidosis	0.3 (1)
Cardiac fibrosis	0.3 (1)
Miscellaneous: (337)	
Dermal abscesses (ie, tail, right forelimb, inguinal, lower jaw region)	1.2 (4)
Mesenteric steatitis of unknown cause, no microorganisms demonstrated	1.2 (4)
Multicentric lymphoma (at histology round cell infiltrates were found in the mesenteric lymph node, spleen, liver, kidney and lung)	0.3 (1)
Intestinal torsion at the root of the mesentery	0.3 (1)

For the number of tissues from each body system sampled, the proportion of affected squirrels with the specified disease is illustrated.
*Suspected primary renal tubular defects (ie, dilation and cyst formation with small primitive tubules).

Table 3: Various concurrent gross and histological findings were observed in 56 squirrels that had amyloid deposits identified within one or more of their sampled tissues

Gross and histology findings (n=total number of squirrels affected)	Proportion of squirrels with the specified pathology that had concurrent amyloidosis	Total number of squirrels with localised amyloidosis (organ within which the amyloid deposits occurred)	Total number of squirrels with generalised amyloidosis
Skin:			
Fatal exudative dermatitis (49)	36.7% (18/49)	8 (renal); 2 (splenic)	8
Muzzle hyperkeratosis (8)	25% (2/8)	0	2
Chronic muzzle dermatitis with epidermal hyperplasia (6)	16.7% (1/6)	1 (renal)	0
Abscess on tail (1)	100% (1/1)	1 (renal)	0
Dermal abscess in inguinal region (1)	100% (1/1)	1 (renal)	0
Respiratory:			
Hepatozoon species within the alveolar interstitium (49)	20.4% (10/49)	2 (splenic)	8
Multifocal necrotic mycotic pneumonia (1)	100% (1/1)	1 (renal)	0
Interstitial pneumonia, no microorganisms seen (4)	25% (1/4)	0	1
Bronchoalveolar carcinoma (1)	100% (1/1)	0	1
Pulmonary atelectasis and focus of metaplastic bone (1)	100% (1/1)	0	1
Hepatobiliary:			
Hepatic capillariasis (including cases involving only *Capillaria hepatica* adults) (106)	24.5% (26/106)	7 (renal); 4 (splenic)	15
Granulomatous hepatitis, no evidence of parasites (4)	25% (1/4)	1 (splenic)	0
Hepatic necrosis, no microorganisms seen (6)	33.3% (2/6)	1 (splenic)	1
Mononuclear hepatitis (16)	6.25% (1/16)	1 (renal)	0
Hepatobiliary cysts (3)	33.3% (1/3)	0	1
Renal:			
Pyelonephritis (18)	72.2% (13/18)	11 (renal)	2
Interstitial nephritis (10)	50% (5/10)	2 (renal)	3
Bacterial nephritis (1)	100% (1/1)	0	1
Renal tubular degeneration and/or necrosis (13)	92.3% (12/13)	10 (renal)	2
Glomerulonephropathy, no microorganisms seen (3)	33.3% (1/3)	1 (renal)	0
Renal tubular abnormalities* (1)	100% (1/1)	0	1
Renal cortical infarcts with fibrosis (1)	100% (1/1)	1 (splenic)	0
Renal tubular and interstitial deposits of oxalate (1)	100% (1/1)	1 (renal)	0
Splenic:			
Focal splenic abscess (1)	100% (1/1)	0	1
Pancreatic:			
Trematode parasites in large pancreatic ducts (5)	40% (2/5)	2 (renal)	0
Pancreatic nodular hyperplasia (1)	100% (1/1)	0	1
Alimentary tract:			
Maxillary abscess associated with the upper molars (2)	100% (2/2)	0	2
Lower incisor abscess	100% (1/1)	0	1
Reproductive:			
Foetal and placental necrosis with endometritis (1)	100% (1/1)	0	1
Septic metritis (1)	100% (1/1)	1 (renal)	0
Foetal mummification (2)	50% (1/2)	0	1
Cardiac:			
Bacterial myocarditis (1)	100% (1/1)	0	1
Necrotic myocarditis, no microorganisms seen (1)	100% (1/1)	1 (splenic)	0

In nine squirrels with amyloidosis (ie, localised renal amyloidosis n=3, generalised amyloidosis n=6), no concurrent gross or histological findings were identified.
*In a single squirrel, extensive tubular abnormalities were observed (ie, dilation and cyst formation with small primitive tubules), which were suspected to reflect primary tubular defects.