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Adenovirus: An emerging factor in red squirrel *Sciurus vulgaris* conservation

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ABSTRACT

1. Adenovirus is an emerging threat to red squirrel *Sciurus vulgaris* conservation, but confirming clinically-significant adenovirus infections in red squirrels is challenging. Rapid intestinal autolysis after death in wild animals frequently obscures pathology characteristic of the disease in animals found dead.
2. We review the available literature to determine current understanding of both sub-clinical and clinically significant adenovirus infections in free-living wild and captive red squirrel populations.
3. Benefits of scientific testing for adenovirus incorporating both transmission electron microscopy (TEM) and polymerase chain reaction (PCR) technologies are compared and contrasted. We favour viral particle detection using TEM in animals exhibiting enteropathy at post mortem and the use of PCR to detect sub-clinical cases where no enteric abnormalities are observed.
4. Adenoviral infections associated with re-introduction studies are evaluated by examination of sporadic cases in wild populations and of data from captive collections used to service such studies.
5. The paucity of data available on adenovirus infection in grey squirrel *Sciurus carolinensis* populations is documented and we highlight that although sub-clinical virus presence is recorded in several locations in

Britain and Italy, no clinically-significant disease cases have been detected in the species thus far.

6. Current speculation for potential inter-specific infection between sciurids and other woodland rodents such as wood mice *Apodemus sylvaticus* is examined. Where sub-clinical adenovirus presence has been detected in sympatric populations occupying the same point food sources, husbandry methods may be used to diminish the potential for cross-infection.
7. Our findings highlight the importance of controlling disease in red squirrel populations by using clearly defined scientific methods. In addition, we propose hypothetical conservation benefits of restricting contact rates between red squirrels and sympatric grey squirrels and of limiting competition from other woodland rodent species.

KEY WORDS: adenovirus infection, conservation, disease, grey squirrel, red squirrel

INTRODUCTION

Historically, disease was not recognized as a mechanism by which red squirrels *Sciurus vulgaris* were replaced by grey squirrels *S. carolinensis* in a landscape. Indeed, it was unclear initially whether the larger grey squirrel was directly involved at all, was taking advantage of space vacated by natural fluctuations in red squirrel population, or ultimately was better adapted to a larger range of habitats (Middleton, 1931). Disease enzootics that were recorded in red squirrel populations were notable for encompassing areas where grey squirrels were absent (Shorten, 1954). Gurnell, (1987) noted “no evidence that grey squirrels brought with them a disease which is causing the downfall of the red”.

By the 1990s, research had focussed heavily upon resource competition (Gurnell and Pepper, 1993), including inter-specific differences in the relative efficiency with which some tree seed is digested (Kenward and Holm, 1993). Inter-specific resource competition, (Wauters *et al*. 2005), negative impacts on red squirrel juvenile recruitment rates (Gurnell *et al.* 2004) and the effects of seed cache piracy (Wauters *et al*. 2002) are today recognised as major contributors to red squirrel extinction in sympatric populations.

However, progressive advances in viral research subsequently established that grey squirrels carry the Squirrel pox virus (SQPV) as a sub-clinical infection, and

90 that inter-specific infection in sympatric red squirrels leads to epizootic disease
91 which is a significant factor in regional population declines in the UK (Rushton *et*
92 *al*. 2006; Sainsbury *et al*. 2008; Carroll *et al*. 2009; Bruemmer *et al*. 2010). Even
93 more recently, adenovirus infection has been identified increasingly as a cause of
94 mortality in free-living wild and captive red squirrel populations. An expanding
95 geographic distribution of cases has been revealed, affecting not only wild
96 populations, but increasingly being associated with high mortalities in captive
97 collections used as both breeding stock and for use in wild population re-
98 enforcement programmes. Additionally, grey squirrels have now been identified
99 as a sub-clinical carrier of the adenovirus among sympatric populations (Everest
100 *et al*. 2009; Romeo *et al* 2014).
101
102 Blood analyses, using enzyme linked immuno sorbent assay (ELISA) and tissue
103 polymerase chain reaction (PCR) techniques, are routinely applied to determine
104 SQPV infection in both squirrel species. Parallel transmission electron
105 microscopy (TEM) screening of skin lesion material can be used to confirm the
106 presence of pox viral particles in typical advanced red squirrel cases. However, in
107 contrast to the detection of SQPV, the detection of adenovirus-associated or
108 clinically-significant adenovirus cases among red squirrels is challenging, due to
109 an absence or non-specificity of external clinical signs of disease. Until relatively
110 recently little was known about this infection in either squirrel species, or its
111 significance in red squirrel declines. Due to increasing scientific activity, both as a
112 retrospective exercise and proactive surveillance, a wider picture is gradually

emerging of the evolving impact that this virus is having with respect to both sporadic disease cases in free-living wild squirrels across Great Britain and in red squirrel re-introduction and captive breeding programmes. However, the mere presence of adenovirus in the body does not signify disease. The virus may indeed be present as a clinically significant infection, causing the death of the animal; in this case viral particles can be detected by TEM in faecal material or viral DNA can be amplified from tissue material such as the spleen. Adenovirus can also be present as an asymptomatic infection or transience presence, causing no apparent disease signs or indications of ill health, and the animal may be outwardly healthy. Subsequent death due to an unrelated problem could then show the presence of the amplified viral DNA by PCR analysis, whereas TEM would fail to detect any viral particles.

Adenovirus infection damages the villi in the red squirrel intestinal mucosa, but autolysis within hours of death typically confounds histological examination, by precluding detection of characteristic adenovirus inclusion bodies (Erdélyi and Duff, 2012), as seen in Fig. 1. By TEM on ultra-thin sections, these inclusions have been shown to contain abundant viral particles (Fig. 2, arrowed). The findings of enteropathy or diarrhoea are non-specific and are associated with several other diseases (Everest *et al*. 2010a). While it is difficult to obtain histologically-adequate gut wall samples prior to autolysis, experience shows that gross pathological changes indicative of enteropathy, such as liquid intestinal content, correlate strongly with gut viral particle detection by TEM (Fig. 3). The

136 presence of viral particles is therefore considered strongly suggestive of
137 clinically-significant infection (Everest *et al.* 2012b). Nonetheless, in the most
138 autolysed wild squirrel cases, pathologists may assume intestinal material to be
139 of such limited value that it is not retained, even though archival samples of other
140 tissues such as liver or spleen may be. Our understanding of the temporal and
141 spatial scope of clinical adenovirus infection (Fig. 4) has recently been improved
142 through more frequent proactive and reactive post mortem screening, in
143 particular with TEM application.
144
145 We review the current understanding of infection and disease in red squirrels,
146 grey squirrels and other small rodents such as wood mice *Apodemus sylvaticus*,
147 with particular reference to the UK, and highlight key areas for future adenovirus
148 infection research that have particular relevance to the applied conservation of
149 the red squirrel.
150
151 **RED SQUIRRELS**
152
153 **The geographical distribution of adenovirus infection**
154
155 The first reports in the literature of adenovirus in free-living wild red squirrels from
156 Great Britain were recorded from Suffolk (Sainsbury et al. 2001) and Cumbria,
157 England (Duff *et al*. 2007), then from Wales (Everest *et al*. 2008), Scotland
158 (Everest *et al*. 2010a) and finally from Northern Ireland (Everest *et al*. 2012a),

159 demonstrating a wide geographical distribution (Fig. 4; Table 1). Retrospective
160 national surveillance of red squirrel mortalities across Great Britain, reported by
161 Martínez-Jiménez *et al*., (2011) revealed that 60 (12%) of 493 cases showed
162 enteric signs. Of these 60, 13 animals, all of which were exhibiting diarrhoea,
163 were selected for analysis by TEM. Of these 13, two animals (15%; Table 1), one
164 from Cumbria, the other from Lancashire, England were confirmed as adenovirus
165 cases by the TEM detection of viral particles. In another retrospective study,
166 adenovirus particles were identified by TEM in 10 (14%) of 70 free-living wild red
167 squirrels where enteropathy was suspected, from Cumbria, Lancashire and
168 Northumberland, England and Anglesey, Wales (Everest *et al*. 2010b; Table 1).
169 However, given the opportunistic sampling of post mortem cases and the paucity
170 of data from living animals, it is difficult to interpret the importance of adenovirus
171 as an overall contributor to mortality from these studies alone.
172
173 Sainsbury *et al*., (2001) and Martínez-Jiménez *et al*., (2011) both reported on a
174 population re-enforcement study at Thetford Chase (Suffolk, England) in the late
175 1990s, with animals that had been trans-located from Cumbria and had
176 contracted the infection and died in 1997 (Table 1). These animals may have
177 been under stress that could have influenced the course of the disease.
178 Diarrhoea was associated with each of 10 adenovirus cases recorded in red
179 squirrels and intestinal haemorrhage or inflammation was observed in seven
180 cases. The extant Thetford Chase wild red squirrel population at that time was
181 judged to consist of 10 to 20 individuals (no more than 40, Gurnell *et al*. 1997),

and consequently adenovirus infection was a notable factor in the study. Subsequent research, (Everest *et al*. 2012b; Table 1) has revealed adenovirus infection to be associated with a high proportion of deaths in squirrels housed in captive collections in Wales, indicating that viral epizootics can be locally significant.

Of 13 captive deaths sampled from the re-introductions on the island of Anglesey, situated off the North Wales coast, 12 (92%) were confirmed as positive for the virus (three detecting viral particles by TEM and nine amplifying viral DNA by PCR). Samples from 16 captive deaths at the Welsh Mountain Zoo, Colwyn Bay, Wales (TWMZ) revealed viral DNA amplified by PCR in 14 (88%) cases (Everest *et al.* 2012b). In a further 24 captive deaths originating from zoological collections in England, for which tissue, faecal or intestinal content samples were available, 20 (83%), were observed to be positive for adenovirus (Everest et al., unpublished; Table1).

Analyses performed on 31 free-living wild red squirrels found dead on Anglesey revealed that 13 (42%) were positive for the virus. Of these positive cases, seven (54%), originated from within Newborough Forest and from these, five (71%), were identified as positive by PCR analyses, three of which also tested negative by TEM. One (14%), contained viral particles when analysed by TEM only, and one case was confirmed by both tests. The remaining six cases were from other Anglesey coniferous and broad-leaved woodlands; all were detected as viral

DNA carriers by PCR, but negative for viral particles by TEM (Everest *et al.* 2012b).

In the latest published report of adenovirus in red squirrels from Great Britain, Everest *et al*. (2013) record that nine (45%) of 20 animals were identified as positive for the virus through amplification of viral DNA by PCR. These animals derived from locations on the Isle of Wight, Jersey and Brownsea Island, all islands off Great Britain without grey squirrels. Intestinal content samples from 12 of these 20 animals were originally examined by TEM and found to be negative for virus particles (Everest *et al*. 2010b). This shows the benefit of using parallel TEM and PCR screening to determine sub-clinical virus presence, which can easily go undetected.

There are very limited reports of adenovirus outbreaks in red squirrels from outside the UK. One, involving three deaths, was from a captive collection in Germany (Peters *et al*. 2011); in the other, 77 road traffic accident carcasses from Italy were examined by a combination of TEM and PCR analyses(Romeo *et al*. 2014). Twelve (16%) were positive for amplified viral DNA by PCR (Table 1). As with the outbreak in Germany (Peters *et al*. 2011), and unlike the situation in most of Great Britain, viral presence was detected in red squirrel populations from areas where the grey squirrel was not known to be present in the immediate landscape.

228 **Adenovirus presence determined in deaths by other causes**

229

230 Traumatic deaths, such as drowning and road traffic accidents, have revealed
231 animals positive for amplification of viral DNA by PCR, but negative for faecal
232 viral particle detection by TEM. These cases occur in animals which lack enteric
233 abnormalities at post mortem examination. These findings suggest that sub-
234 clinical infections are present and may be widespread within wild British
235 populations of red squirrels (Everest *et al*. 2012b; Table 1).

236

237 **Adenovirus strain speciation**

238

239 Phylogenetic analysis demonstrates that adenovirus sequences from squirrel
240 samples cluster with mastadenoviruses but are distinct from other adenoviruses
241 within the genus (Sainsbury *et al*. 2001, Peters *et al.* 2011), although squirrel
242 adenovirus has not yet been approved as a species (King et al. 2011).
243 Sequencing has revealed a lack of adenovirus strain variability. The identity of
244 the adenovirus in a partial fragment of the hexon gene from the German outbreak
245 (GU735084) described by Peters *et al*., (2011) was identical to the putative
246 Suffolk strain (Sainsbury *et al*. 2001). In contrast, in those cases described by
247 Everest *et al.* (2012b), sequences were detected which were identical to those
248 found in Cumbrian cases from 2007 (JN205244.1). Everest *et al.* (2012b) used a
249 partial fragment of the polymerase gene, which in turn identified cases that were
250 genetically identical to the grey squirrel cases detected on Anglesey (Everest *et*

al. 2009). This is remarkable, as the cases were separated both spatially and temporally. It has been suggested, therefore, that the viruses involved in each of these cases are very closely related, or perhaps identical (Peters *et al.* 2011).

In general, the samples described above have not been randomly sourced and case selection was influenced by carcass suitability and value in terms of post mortem examination. This is particularly true for captive collections, where the prevailing close confinement within enclosures would have allowed for easy spread of the virus between individual animals, thus accounting for the apparently high incidence of infection in such collections.

GREY SQUIRRELS

Given the role that grey squirrels play in SQPV infection in red squirrel populations, it is natural to investigate whether sympatric grey squirrel populations are also a source of inter-specific adenovirus infection. Romeo *et al*., (2014) found PCR amplified adenoviral DNA in only two (1%) of 232 grey squirrels from Italy. Screening of tissues from wild adult grey squirrels (n=18) trapped and euthanased at the Welsh Mountain Zoo in 2011 failed to reveal viral particles in the gut by TEM (which would have suggested clinically-significant infection), yet 10 of these 18 animals (56%) were positive by PCR analyses on spleen tissue, (Everest *et al*. unpublished) and were hence

274 determined as adenovirus carriers. Although the numbers of animals were small
275 in this study, the PCR figure is very similar to the 60% sero-prevalence reported
276 by Greenwood and Sanchez, (2002) using murine adenovirus ELISA tests for
277 antibodies in grey squirrels from the same zoo; a location where dead captive red
278 squirrels have been found with enteric symptoms and viral particles in the
279 intestinal tract.
280
281 At the Newborough Forest re-introduction site on Anglesey, adenovirus DNA was
282 detected by PCR analysis from two grey squirrels caught in 2006 (Everest *et al*.
283 2009). Wider PCR screening of archived and proactively-sourced blood
284 sampling, involving over 200 samples and thus forming a study larger than that
285 reported by Romeo *et al.* (2014), was subsequently undertaken and reported by
286 Everest *et al*. (2012b, Table 2) for both Anglesey locations and woodland in
287 Gwynedd, North Wales, within a few kilometres of the Menai Straits. Spleen
288 tissue collected from the Gwynedd site was examined in 2012 (Everest *et al*.
289 unpublished), and amplification of DNA revealed a much higher percentage of
290 positives (54%) than in blood (7%, see Table 2).
291
292 The 2012 Gwynedd result (Everest *et al*. unpublished) was further confirmed,
293 when both spleen and blood were available for analysis from each of 14 adult
294 grey squirrels trapped at the Welsh mountain Zoo. Adenovirus DNA was detected
295 from spleen tissue in eight cases (57%), but there were no positive results from
296 the 14 blood samples from the same animals (Everest *et al*. unpublished), thus

297 demonstrating that source tissue type is an important consideration in adenovirus
298 screening.
299
300 Historically, assessing infection in grey squirrels is challenging, as previously
301 reported blood based testing was serologically based. Thus exposure to the virus
302 could result in potentially long-lasting sero-conversion, although this may wane
303 with age. In contrast, an animal may be viraemic (and therefore PCR-positive) for
304 only a short period, meaning PCR analyses have only a small time window to be
305 effective for viral diagnosis. In this context, serologically- based ELISA analyses
306 may be more sensitive in nature than PCR techniques.
307
308 Although evidence of infection has been found, no clinically-significant cases of
309 adenovirus have been identified to date in grey squirrels and viral particles have
310 been absent from intestinal content examined by TEM studies of grey squirrels
311 from Cumbria (*n*=36), Wales (*n*=58, Everest *et al*. unpublished), Thetford Chase
312 study (*n*=10, Martínez-Jiménez *et al*. 2011) and Italy (*n*=3, Romeo *et al*. 2014).
313
314
315 **SMALL RODENTS**
316
317 Peters *et al.,* (2011), documented adenovirus infection by TEM in a captive red
318 squirrel collection from Germany, and red squirrel infections have been recorded
319 on both the Isle of Wight and Jersey (Everest *et al.* 2013), all of which are regions

where the grey squirrel is absent. Additionally, Romeo *et a*l., (2014) documented infections in red squirrels in areas where the grey squirrel was not known to be present. This means that alongside intra-specific and potential grey squirrel to red squirrel infections, inter-specific infection from other small rodents such as wood mice is possible.

In order to investigate this potential infection route, Everest *et al*., (2013) examined the spleens of wood mice trapped on the Island of Anglesey for the presence of adenovirus by PCR analyses. Adenoviral DNA was amplified from three of 15 mice (20%), trapped at two woodland sites which had red squirrel feeding stations and where cases of clinically-significant adenovirus infection of wild red squirrels had been recorded. Two of 24 (8%) mice trapped in north Wales within woodland enclosures housing captive red squirrels also tested positive for adenovirus by PCR. Our results therefore demonstrate the potential for adenovirus infection in sympatric communities of grey squirrel, red squirrel and wood mice.

The PCR primers used to test the wood mice samples had been designed based on a sequence of the adenoviral DNA polymerase gene from squirrel samples (JN205244.1; Everest *et al.* 2012). However, further investigations into whether these primers would detect other adenoviruses were not undertaken.
It is therefore unclear at present whether the strain detected in mice is identical to that detected in squirrels, and so further molecular sequencing is required.

343 Greenwood and Sanchez (2002) used an ELISA derived for the serological
344 detection of murine adenoviruses to detect adenovirus in grey squirrels.
345 Therefore, it is possible that either cross reactivity exists between species-
346 specific viruses from the two species, or an identical virus infects both.
347
348
349 **DISCUSSION**
350
351 Retrospective study of archived tissue and blood samples (Everest *et al*. 2010;
352 2012b; Martínez-Jiménez *et al.* 2011) has advanced our understanding of both
353 the temporal and spatial distribution of adenovirus infection within red squirrel
354 populations. Recent examination of trauma deaths has also revealed sub-clinical
355 infections in wild individuals at the time of death, namely negative TEM results for
356 adenovirus particles in faecal and intestinal samples but positive results for viral
357 DNA from tissues by PCR analysis (Everest *et al*. 2012b; Romeo *et al*. 2014).
358 Much however, remains unclear about the epizootiology, in particular, the roles of
359 squirrel population density and stress. Currently, much of our understanding is
360 based upon captive collections and there is therefore also a pressing research
361 need to investigate the distribution and effects of the infection among wild red
362 and grey squirrel populations. Opportunities for application of a qPCR technique
363 to quantitate virus load in faeces, tissues and blood in order to partition
364 pathological from asymptomatic infections would also be beneficial.
365

366 Research in North Wales (Everest *et al*. 2009; 2012b; Greenwood and Sanchez,
367 2002), and in Italy by Romeo *et al.*, (2014) has demonstrated adenovirus
368 infection or exposure in grey squirrels, but whether this has any clinical
369 significance in these populations remains unknown. To this end, a controlled
370 challenge experiment in grey and red squirrels using the same virus isolate would
371 also help to advance our understanding.
372
373 There is also a paucity of data on adenovirus prevalence within regional grey
374 squirrel populations in the UK. An annual survey combining PCR and TEM
375 analyses was limited solely to squirrel populations in North Wales. Additional
376 regional studies of this type would therefore be useful
377
378 Given that grey squirrels appear to be infected with both adenovirus and SQPV,
379 the accepted management practice of removing grey squirrel populations to
380 control SQPV infections would also mitigate the potential for adenovirus infection.
381 Additionally, conservation managers could potentially evolve protocols to combat
382 potential infection pathways involving other woodland rodents such as wood
383 mice, although there may be a significant cost implication to this approach. On
384 Anglesey, adenovirus infection risk was highlighted as a major difficulty faced
385 during the re-introduction of red squirrels to Newborough Forest (Shuttleworth *et*
386 *al.* 2008). Release protocols have been modified with animals now housed for
387 only a few weeks, during which faecal and blood screening is undertaken for
388 adenovirus (Shuttleworth, 2010). More widely, it has been recommended that

389 hygiene protocols at supplemental feeding hoppers routinely focus upon limiting
390 adenovirus infection via faecal-oral routes (Everest *et al*. 2012b). Given our
391 recent findings and because of the potential for transmission of other rodent-
392 borne infections, this should encompass mouse control.
393
394 If wood mice act as an infection reservoir, there are obvious implications for
395 scenarios that concentrate their activities at point food sources such as garden
396 bird tables or supplemental feed hoppers also visited by red squirrels. It may
397 therefore be prudent to place red squirrel supplemental feed hoppers on posts
398 with cone shaped baffles near the base to prevent mice from accessing the
399 hopper above, instead of, as is common practice, fixing hoppers to tree trunks,
400 which allows mice easy access. Accumulation of discarded shells and food
401 remains beneath hoppers should be minimised. Trapping protocols should
402 include regular disinfection of all traps, not only those that have contained grey
403 squirrels, so as to limit any potential mouse to red squirrel inter-specific virus
404 transmission.
405
406
407 **CONCLUSIONS**
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409 This review of the available evidence within the published literature, coupled with
410 recent findings, lead us to conclude that adenovirus should be regarded as a
411 serious disease threat to the various red squirrel re-introduction and captive

breeding programmes, and to red squirrel populations in places where grey squirrels, red squirrels and wood mice can interact at point food sources. We also conclude that TEM, while excellent at detecting clinically-significant infection from intestinal samples, is not as sensitive as PCR for detecting sub-clinical adenovirus cases, and that spleen tissue is a better material to screen by PCR than blood. ELISA-based assay on blood samples is the only test available for live animalsl at present. To address practical and potentially also welfare considerations, alternative assay platforms should be investigated for live animal testing. We would also recommend that disease investigations and adenoviral infection surveillance extend to all three rodent species identified in this review, and where possible, include parallel PCR and TEM sample testing of tissue and intestinal content samples, respectively.

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572 **Table 1.** Test results for adenovirus from tissues or intestinal or faecal content

573 material from red squirrels *Sciurus vulgaris*.

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Reference	Study location	Number Tested	Number (%) Positive
Duff *et al*. 2007	Cumbria wild	2	2/2(100%)
Everest *et al.* 2008	Anglesey captive	3	3/3 (100%)
Everest *et al*. 2010a	Scotland wild	1	1 (100%)
Everest *et al*. 2012a	Northern Ireland wild	2	1/2 (50%)
Martínez-Jiménez *et al*. 2011	Great Britain wild	13	2/13 (15%)
Martínez-Jiménez *et al*. 2011	Suffolk captive	10	10/10 (100%)
Everest *et al*. 2010b	Great Britain wild	70	10/70 (14%)
Sainsbury *et al*. 2001	Suffolk captive	6	3/6 (50%)
Everest *et al.* 2012b	Anglesey captive	13	12/13 (92%)
Everest *et al*. 2012b	Zoo captive Wales	16	14/16 (88%)
Everest *et al*. 2012b	Anglesey wild	31	13/31 (42%)
Everest, *et al.* unpublished	England captive	24	20/24 (83%)
Everest *et al.* 2013	Isle of Wight/ Jersey wild	20	9/20 (45%)
Peters *et al*. 2011	Germany captive	3	3/3 (100%)
Romeo *et al.* 2014	Italy wild	77	12/77 (16%)

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578 **Table 2.** Positive PCR test results for adenovirus DNA from blood and spleen

579 tissue from grey squirrels Sciurus carolinensis in North Wales. Total number

580 tested and percentage positive are shown.

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	Anglesey		Gwynedd (Bangor Area)	
	Blood	Spleen	Blood	Spleen
2007 [1]	0% (55)	-	-	-
2010 [1]	23% (26)	-	21% (39)	-
2011 [2]	-	-	10% (48)[3]	-
2012 [2]	25% (4)	-	7% (15)	54% (35)

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583 [1] Everest *et al*. (2012b); [2] Everest *et al*. unpublished.

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585 [3]10% (n=48), adults were 14% (n=28) and juveniles 5% (n=20)

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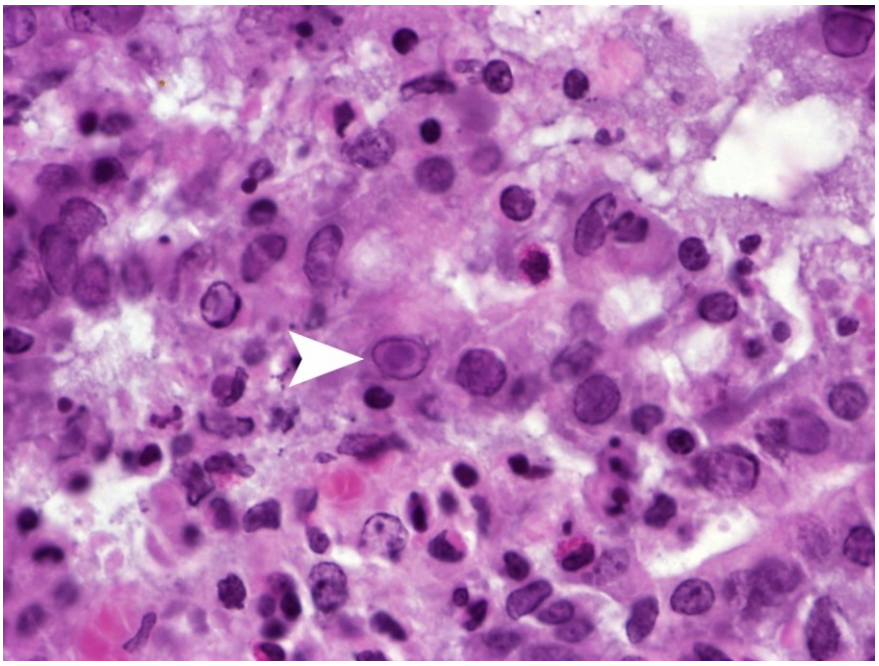
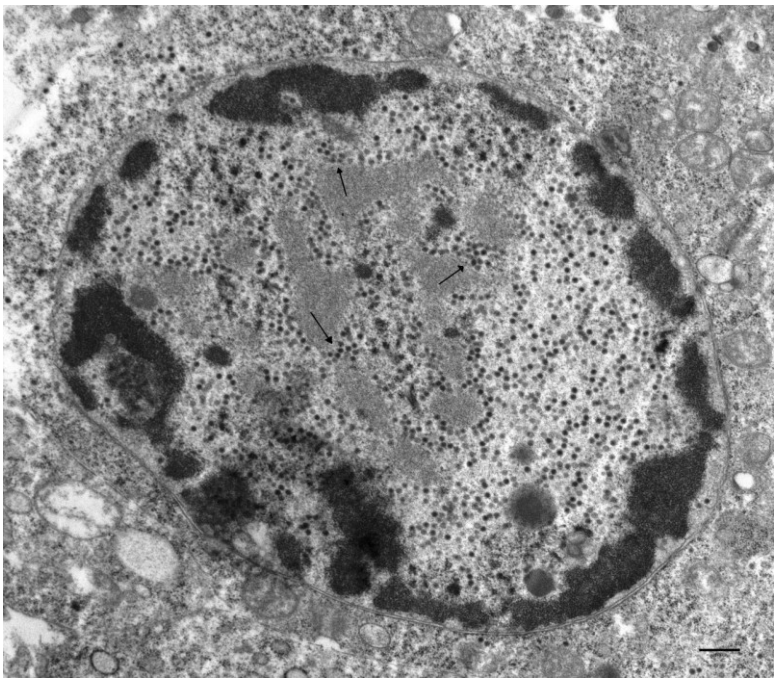
Figure 1. Haematoxylin and Eosin histology image of a section of red squirrel *Sciurus vulgaris* small intestine, showing intra-nuclear virus inclusion bodies (arrowed) and extensive damage to the villi, findings consistent with adenovirus infection. x600 magnification.



599 **Figure 2.** Micrograph of adenovirus particles (arrowed) detected in an ultra-thin
600 section of enterocytes from a red squirrel *Sciurus vulgaris* large intestine. Bar
601 (bottom right) = 500 nm.



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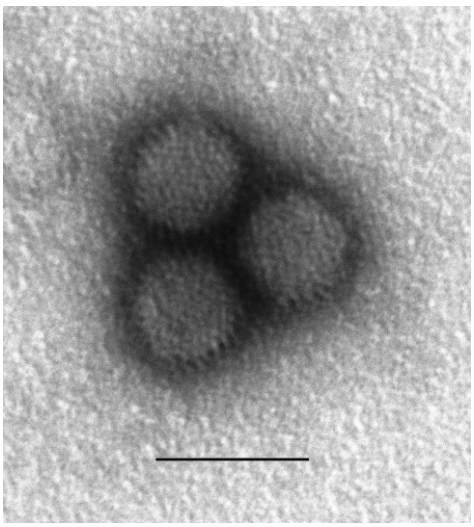
603 **Figure 3.** Micrograph of adenovirus particles detected in contents from the large
604 intestine of a captive red squirrel *Sciurus vulgaris*. *Bar = 100nm.*



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606 **Figure 4.** Location of adenovirus positive free-living wild (●) and captive (□) red

607 squirrel *Sciurus vulgaris* cases from Great Britain, as analysed by PCR and TEM.

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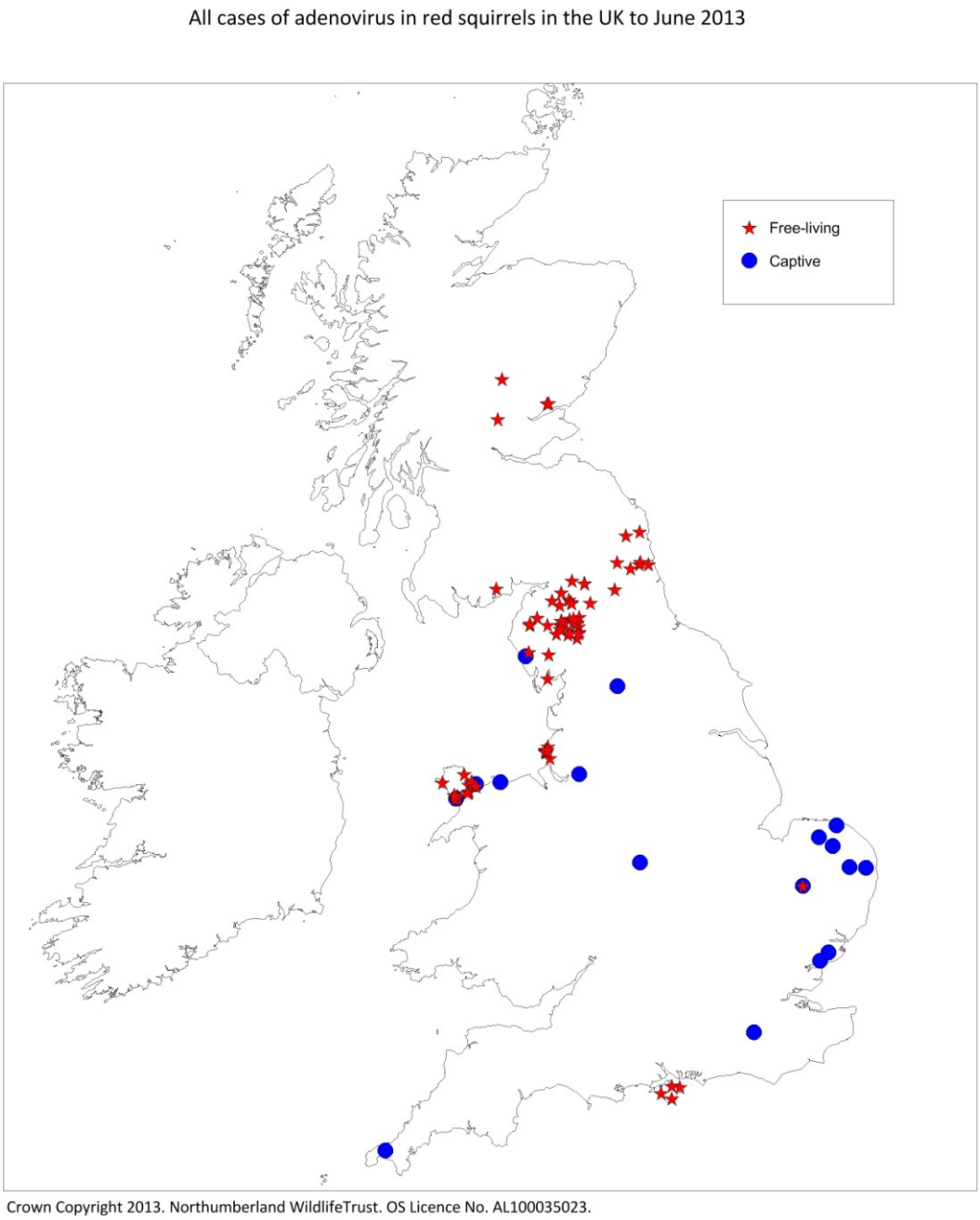

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