The implications of adenovirus infection for management of United Kingdom captive red squirrel (*Sciurus vulgaris*) collections

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Adenovirus – captive red squirrel  *Bar*=100nm
Collaborators

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University of Newcastle (Aileen Mill)

University of Liverpool (Julian Chantrey)

Acknowledgments

Thanks to the Red Squirrels Survival Trust (RSST), the East Anglian Red Squirrel Group and the Welsh Mountain Zoo who co-ordinate the UK captive red squirrel breeding scheme.

To the Curators and zoological collection Managers and private veterinary surgeons for submission of varying samples for analysis.

Defra for funding the APHA DoWS, now the GB Wildlife Disease Surveillance Partnership.
The first 3 recorded adenovirus cases in UK captive red squirrels
Collection B : October 2007

Go away and find the evidence!

UK red squirrel group meeting Edinburgh November 2007
Study sample protocol
(Initiated 2010 after detection of more captive cases)

RSST organised a questionnaire on captive red squirrel collection mortalities

Mortalities submitted to study as fresh carcasses for autopsy or archived material

Range of samples sourced (faecal matter, spleen, liver, paraffin embedded tissue blocks) and collected between 2002 and 2016

Clinical findings noted and cause of death established where possible. Transmission electron microscopy (TEM) and polymerase chain reaction (PCR) assays to determine ADV presence

Movement of red squirrels from donor to recipient collections noted wherever possible

26 anonymous UK captive red squirrel collections studied
22 PERMANENT sites and 4 collections related to re-introduction programmes
Negative contrast stain TEM procedure
PCR procedure

Sample nucleic acid extracted by using a kit under manufacturer’s protocols

Extracted nucleic acid was tested using a conventional squirrel adenovirus PCR

Gel prepared and sample amplicons injected into gel and run in electrophoresis tanks

Gels visualised under UV illumination and visible bands of expected size compared against a DNA ladder and positive control
### Cause of death for 101 of 129 red squirrel Post Mortem Examination cases

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Cases</th>
<th>Cause of death</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteropathy (cause unknown)</td>
<td>75</td>
<td>Drowning</td>
<td>1</td>
</tr>
<tr>
<td>Starvation/Malnutrition</td>
<td>8</td>
<td>Fungal necrotizing enteritis&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td><strong>Other viral causes&lt;sup&gt;a&lt;/sup&gt;</strong></td>
<td>4</td>
<td>Ivermectin toxicity</td>
<td>1</td>
</tr>
<tr>
<td>Neoplasia&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
<td>Myocarditis</td>
<td>1</td>
</tr>
<tr>
<td>ADV enteritis</td>
<td>2</td>
<td>Nematodiasis&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial Infection&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2</td>
<td>Renal disease</td>
<td>1</td>
</tr>
<tr>
<td>Coccidiosis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cause of death precluded in the remaining 28/129 animals due to excessive autolysis (11), no observable lesions (10) or no recorded PME data (7).

<sup>a</sup> Recorded as death caused by viral infections, with particles detected by TEM. SQPV (1), Rotavirus (1), Enterovirus-like agent (1), Reovirus/Rotavirus (1)

<sup>b</sup> Lymphosarcoma (2), Strangulated lipoma (1)

<sup>c</sup> *Streptococcus spp.* (1) *Staphylococcus sciuri* (1)

<sup>d</sup> Mucor/Absidia-like organism

<sup>e</sup> *Oxyurid spp.*
Virus identification by Size, Shape and surface Morphology

Adenovirus particles from a captive red squirrel (collection H), detected in intestinal contents by negative stain TEM

Adenovirus is a ds DNA virus

Isometric particle, with Icosahedral shell (capsid) and 20 triangular facets

Often seen as hexagonal particles with triangular cores by TEM

Typically 70-90nm diameter
TEM ultra-thin sectioning (for clinically-significant cases)

Adenovirus in red squirrel enterocytes

(Bar = 500nm)
Nucleotide sequences from first (and second) round products shared 100% identity with a 2007 UK free-living wild red squirrel ADV isolate (JN205244.1)

Amplification of a 407 base pair (bp) fragment (first round product) of the polymerase gene of adenovirus, from five squirrel tissue samples.
Abbreviations: ntc= no template control, M=100 bp DNA molecular weight marker (Promega).
Squirrelpox virus (collection G)

Enterovirus-like agent (collection A)

Rotavirus (collection O)

Reovirus and Rotavirus (collection A)

Rotavirus
ADV-associated deaths and tests for each assay platform utilised for the 129 examined carcasses across the study at the 26 UK collection sites

<table>
<thead>
<tr>
<th>Sample collection</th>
<th>ADV Positive/Tested</th>
<th>Sample collection</th>
<th>ADV Positive/Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 2002 to 2016</td>
<td>24/36 ¹ (23/35) 68% ² (1/20) 5%</td>
<td>N 2011</td>
<td>1/1 ¹ (1/1) 100% ² (0/1) 0%</td>
</tr>
<tr>
<td>B 2004 to 2007</td>
<td>11/12 ¹ (8/9) 89% ² (3/3) 100%</td>
<td>O 2012 to 2013</td>
<td>0/2 ¹ (0/2) 0% ² (0/2) 0%</td>
</tr>
<tr>
<td>C 2004</td>
<td>1/1 ¹ (1/1) 100%</td>
<td>P 2012</td>
<td>1/1 ¹ (1/1) 100% ² (0/1) 0%</td>
</tr>
<tr>
<td>D 2005</td>
<td>1/1 ¹ (1/1) 100%</td>
<td>Q 2012</td>
<td>3/3 ¹ (3/3) 100% ² (0/3) 0%</td>
</tr>
<tr>
<td>E 2006</td>
<td>1/1 ¹ (1/1) 100%</td>
<td>R 2012 to 2014</td>
<td>3/3 ¹ (3/3) 100% ² (0/3) 0%</td>
</tr>
<tr>
<td>F 2007 to 2009</td>
<td>1/2 ² (1/2) 50%</td>
<td>S 2013 to 2014</td>
<td>2/5 ¹ (2/5) 40% ² (0/2) 0%</td>
</tr>
<tr>
<td>G 2007</td>
<td>1/1 ¹ (1/1) 100% ² (0/1) 0%</td>
<td>T 2013</td>
<td>1/2 ¹ (1/2) 50% ² (0/2) 0%</td>
</tr>
<tr>
<td>H 2009 to 2016</td>
<td>11/17 ¹ (5/11) 46% ² (6/13) 46%</td>
<td>U 2013</td>
<td>1/1 ¹ (1/1) 100% ² (0/1) 0%</td>
</tr>
<tr>
<td>I 2010 to 2015</td>
<td>12/14 ¹ (7/9) 78% ² (5/13) 39%</td>
<td>V 2013 to 2016</td>
<td>3/3 ¹ (3/3) 100% ² (0/3) 0%</td>
</tr>
<tr>
<td>J 2010 to 2013</td>
<td>1/3 ¹ (0/2) 0% ² (1/3) 33%</td>
<td>W 2014 to 2016</td>
<td>1/5 ¹ (1/5) 20%</td>
</tr>
<tr>
<td>K 2011 to 2016</td>
<td>3/3 ¹ (1/1) 100% ² (2/3) 67%</td>
<td>X 2015 to 2016</td>
<td>4/5 ¹ (4/5) 80% ² (0/5) 0%</td>
</tr>
<tr>
<td>L 2011</td>
<td>2/2 ¹ (1/1) 100% ² (1/2) 50%</td>
<td>Y 2015</td>
<td>0/1 ¹ (0/1) 0% ² (0/1) 0%</td>
</tr>
<tr>
<td>M 2011</td>
<td>0/1 ¹ (0/1) 0% ² (0/1) 0%</td>
<td>Z 2016</td>
<td>3/3 ¹ (2/2) 100% ² (1/3) 33%</td>
</tr>
</tbody>
</table>

A to Z : ADV positives 92/129 animals (72%): ¹ (71/106), ² (21/86) samples

¹ PCR analyses, ² TEM analyses
Geographic locations of adenovirus infection tested for in the 26 UK captive red squirrel study collections

Bar=100nm
Adenovirus infection associated with donated stock

**Collection A:** hosting quarantine red squirrels for re-introduction programmes, donated by collection Q. Infection started **ALL 11** squirrels lost

**Collection B:** received red squirrels for re-introduction programmes, from collection A, all donated by collection Q. Infection started **ALL 8** squirrels lost

**Collection H:** received red squirrels for breeding and re-introduction programmes, donated by collection R. Infection started **ALL 4 donated and 2 resident** squirrels lost

Adenovirus infection associated with only home bred stock

**Collection I:** no recent donations of squirrels, only home bred stock present. **In total 10 deaths involving adenovirus**

Seven study collections received donated stock from collection I after their adenovirus outbreak. **SIX study collections recorded adenovirus deaths in squirrels after receipt of stock**
ADV-associated cases detected at recipient collections following receipt of donated stock from other captive red squirrel study collections

<table>
<thead>
<tr>
<th>Collection</th>
<th>Donor collection</th>
<th>ADV infection</th>
<th>Stock receipt</th>
<th>ADV year</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Q</td>
<td>Yes</td>
<td>2005</td>
<td>2005</td>
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<tr>
<td>B</td>
<td>Q</td>
<td>Yes</td>
<td>2005</td>
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<tr>
<td>X</td>
<td>I</td>
<td>Yes</td>
<td>2015</td>
<td>2015</td>
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<tr>
<td>Z</td>
<td>I</td>
<td>Yes</td>
<td>2015</td>
<td>2016</td>
</tr>
</tbody>
</table>
Summary of findings

181 animals received 129 (71%) suitable for post mortem examination

26/129 animals (20%) had a confirmed cause of death

92/129 animals (72%) adenovirus positive (14% detected in previous TEM free ranging study)

2/129 (2%) adenovirus encephalitis cases histologically confirmed

Adenovirus more geographically widespread than previously recorded studies in both captive and free ranging collections with 23/26 collections (89%) positive

Difficulty in determining clinical cases when squirrels housed in groups, due to time spent in nest boxes and similar body size and pelage characteristics

Evidence of unreported mass mortalities in collections not resulting in disease investigation

Evidence of animal movement introducing adenovirus infection to recipient collections after stock receipt from donor collections. Insufficient pre-movement testing and bio-security measures documented. Adenovirus also in collections with no known recent stock introduction
Study recommendations

Standard protocols to mitigate for infection spread among collections should be provided

Protocols should incorporate robust quarantine and effective hygiene measures. Rodent control (WOOD MICE) should be incorporated until their possible role in ADV transmission to red squirrels is determined

Pre-movement testing involving TEM and PCR on faecal material as a minimum should be prioritised

These measures will have a financial impact but should help reduce mortality and enhance red squirrel stock welfare in captive breeding collections, improving the chances of successful captive breeding and re-introduction programmes
The implications of adenovirus infection for management of United Kingdom captive red squirrel (Sciurus vulgaris) collections

2007: Go away and find the evidence

2017: WE DID AND WE HAVE
Thank you for listening. Any questions?