

TWO NOVEL ADENOVIRUSES IN FREE-LIVING BRITISH LIZARDS

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Abstract

In this study, we describe two novel adenoviruses isolated from (i) a common lizard (*Zootoca vivipara*) found dead and examined post-mortem, and (ii) pooled samples from free-living sand lizards (*Lacerta agilis agilis*). Sequencing indicated the two were closely related atadenovirus strains which were distinct from previously recorded adenoviruses in lizards. Adenoviruses are not always associated with disease in squamates but morbidity and mortality have been reported. These are the first known cases of adenovirus infection in free-living native British lizards and further monitoring will be necessary to elucidate the implications of these possible pathogens for vulnerable populations of native reptiles.

The sand lizard (*Lacerta agilis agilis*) has disappeared from much of its former range in the UK with habitat loss and fragmentation cited as the main factors in the species' decline (Edgar and Bird, 2006).

Although listed on the IUCN Red List as a species of least concern internationally (Agasyan 2010), it is one of the UK's rarest reptiles, found only in limited distribution in protected heathland and dune sites (Edgar et al. 2010). As part of various Sand Lizard Species Action Plans (SAP) and recovery plans, extant colonies of sand lizards have been carefully monitored for 50 years in England and Wales, aimed at safeguarding these native populations. In addition, between 1969 and 2019, 76 translocations of approximately 10,000 captive-bred and wild-caught sand lizards have taken place into areas of former range (J Foster 2020, pers. comm.) involving Amphibian and Reptile Conservation (ARC), Natural England (NE), Natural Resources Wales (NRW) and the British Herpetological Society (BHS).

The Disease Risk Analysis and Health Surveillance (DRAHS) programme, a collaboration between Natural England and the Zoological Society of London, works with other wildlife organisations, including ARC, advising on the risks from disease associated with animal translocations for conservation purposes and recommending appropriate mitigation strategies. As part of this programme, DRAHS has been monitoring the health of extant and reintroduced colonies of sand lizards and sympatric reptiles in the UK through clinical and pathological examinations. Free-living animals are captured, clinically examined by a specialist wildlife veterinarian and screened for parasites (viruses, bacteria, protozoa, fungi, helminths and ectoparasites) identified by disease risk analysis as high risk to the conservation of these populations. In addition, comprehensive post-mortem examination is conducted on any reptile found dead in sand lizard habitats to detect disease threats to sand lizard and sympatric reptile populations arising as a result of reintroduction.

Adenoviruses are non-enveloped DNA viruses usually associated with respiratory or enteric disease found in many, and probably all, vertebrate species (Machlachlan and Dubovi (Eds.) 2016). Sequence comparisons suggest adenoviruses are generally species-specific with a low likelihood of inter-species transmission (Peters et al. 2011). However, inter-species transmission of squamate adenoviruses has occurred: Hyndman and Shilton (2011) and Benge et al. (2019) have reported identical/near-identical adenovirus sequences from deeply divergent reptile species in Australia and the USA. Adenovirus

infections have been reported in both healthy free-living and captive lizards (Kubiak 2013; Hyndman et al. 2019; Szivovics et al. 2016) but have also been associated with morbidity and mortality in captive lizards: Seven wild-caught bark anoles (*Anolis distichus*), comprising five *A. d. ravitergum* and two *A. d. ignigularis* from the Dominican Republic, infected with an adenovirus, died in captivity with clinical signs of gastroenteritis consistent with acute adenovirus infection (Ascher et al. 2013) and a captive central netted dragon (*Ctenophorus nuchalis*) was found dead with an adenovirus infection in association with acute necrotising hepatitis (Hyndman and Shilton 2011). Adenoviruses have been detected in healthy captive bearded dragons (*Pogona vitticeps*) in the UK (Kubiak 2013) but have not been reported in free-living lizards in the UK.

[Insert figure 1 here]

Figure 1: Bayesian phylogenetic tree of predicted amino acid sequences of adenoviral DNA polymerase sequences. Bayesian posterior probabilities are provided at each node. The common and sand lizard adenoviruses are bolded and underlined. Brackets indicate genera. GenBank accession numbers are provided in parentheses.

In August 2017, an adult male common lizard (*Zootoca vivipara*) was found dead at East Weavers Down in Hampshire (Grid ref: SU 82187 3135), a sand lizard reintroduction site, and examined post-mortem using the method described by Jaffe and Sainsbury (2018). It was partially decomposed and no gross abnormalities were noted on examination. It was in normal body condition although no fat bodies were noted and the gastrointestinal tract was empty suggesting that it had not eaten for days before death. Although detection of parasites associated with pathological changes may be unrewarding in decomposed cadavers due to contamination and post-mortem overgrowth, a sample

of the gastrointestinal tract was submitted for bacterial culture and a wet preparation of the intestinal contents was examined under light microscopy for parasites. Confluent mixed *Escherichia coli* and *Citrobacter freundii*, probable commensal enterobacteria, were cultured. Samples of the liver, heart, trachea, testis and tongue were fixed in formalin and submitted for histopathology. Decomposition hindered interpretation but no abnormalities were detected in the samples examined. Samples from the liver, brain, skin, one testis and intestine, frozen at -80°C, and dry swabs from the oral cavity and cloaca were examined by PCR, and an adenovirus detected using a pan-adenoviral PCR as described by Wellehan et al. (2004). The obtained PCR product was sequenced using Sanger sequencing methods as described by Salzmann et al (2021) and subjected to BLAST analysis online (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Results showed distant identity to previously described adenoviruses over a portion of the analysed 256 bp sequence.

As it could not be ascertained, due to decomposition, if there were lesions associated with the adenovirus infection in the common lizard, health examinations of reptiles at East Weavers Down were undertaken in 2018 to assess the implications of virus presence to the health of the populations. Five sand lizards, 11 slow-worms (*Anguis fragilis*) and a common lizard were captured and screened for adenoviruses by PCR testing of cloacal and pharyngeal swabs but no further infection was detected.

In 2019, DRAHS was asked to report on the health of sand lizards in a newly-discovered coastal colony in Wales following concerns that they had been unofficially released from an unknown captive source rather than representing an existing native population. Funding from the Welsh government enabled an investigation involving detailed clinical examination of 21 male and 7 female adult sand lizards from this population using the method described by Jaffe and Sainsbury (2018). All were in good to above average body condition although 50% (n=14/28) showed signs of recent trauma, including tail autotomy and/or scarring to the head or dorsum, suggestive of predation attacks or inter-species fighting. Sand lizard 210519/03 was at the lower end of the body weight range for the group (8.0g;

mean male bodyweight 9.6g (range 6.0 to 14.0g; n = 21)), harbouring a tick confirmed by the Tick Surveillance Scheme (Public Health England) as an *Ixodes ricinus* nymph, and an unknown gram-negative enteric bacterium classified as API bioMeriueux 6704512 was isolated in mixed culture from a faecal sample from this lizard.

Two pooled cloacal swab samples (each from two male sand lizards, including sand lizard 210519/03) were positive for a novel adenovirus most similar in sequence to that identified in the common lizard at East Weavers Down, with only three nucleotide exchanges between the two sequences. However, as the samples had been pooled it was not known if lizard 210519/03 was the source of the adenovirus identified. BLAST analysis showed some similarity between the detected sequences and corresponding sequences from avian and reptilian atadenoviruses. Phylogenetic analysis of 83 aa showed that the viruses detected in the common and sand lizards were most closely related to one another and clustered in the genus *Atadenovirus*, together with an adenovirus from a long-tailed grass lizard (*Takydromus sexlineatus*) that died in captivity in Hungary (GenBank accession No. AJW67396.1) (Fig. 1). The sequences obtained in this study have been submitted to GenBank and given the accession numbers MW091488 and MW091489.

Although adenoviruses are commonly found in squamates and other reptiles overseas, they are not believed to have previously been reported in free-living, wild lizards in the UK. Knowledge of viruses of free-living reptiles in the UK is limited. It is possible therefore that adenoviruses are a previously unrecognised part of the intestinal flora of free-living lizards in the UK and that the atadenovirus detected might naturally occur in free-living, wild lizards in the UK. However, the Welsh sand lizard population is of unknown origin and sand lizards released at East Weavers Down were originally wild-caught but may have been in contact with exotic reptiles during brief periods in captivity or mixed with dispersing captive-bred sand lizards released into nearby areas. It is therefore also possible, given the similarity of the newly-identified atadenoviruses to adenoviruses found in exotic species, that a novel virus has been introduced with released sand lizards as a result of direct or indirect contact with

exotic reptiles in captivity. Further surveillance work will be required at these and other sites in order to improve knowledge of viruses in free-living reptiles in the UK.

It is not clear whether adenovirus infection in the two lizards described was associated with clinical disease. However, it is probable, given that adenovirus infection has been identified as an occasional cause of morbidity and mortality in other lizard species (Ascher et al. 2013; Hyndman and Shilton 2011; Ball et al. 2014), that, under certain circumstances, adenoviruses may cause disease in native UK lizards. In rodents, disease associated with adenovirus infection is believed to be linked to stress (Martinez-Jimenez et al. 2011) and this may also be the case in reptiles. Elevated corticosterone levels suppress immunocompetence in reptiles and may therefore predispose animals to disease from infections that are normally tolerated (Martinez-Silvestre 2014). Concurrent disease, starvation and translocation have all been cited as stressors with the potential to reduce immunocompetence (Griffiths et al. 2011; Freitag et al. 2000; Dickens et al. 2010).

These findings highlight the importance of a disease risk analysis prior to release of wild animals (Sainsbury and Vaughan-Higgins 2012), and the potential harm from unauthorised releases. In addition, we reiterate recommendations for post-mortem examination by a wildlife veterinarian of any free-living or captive animal found dead.

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