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# Adenoviruses and Encephalitozoon pogonae in Samples from Bearded Dragons (Pogona spp.) in Europe

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| **Abstract:** | Adenoviruses (AdVs) and the microsporidium Encephalitozoon pogonae have been repeatedly detected in bearded dragons (Pogona spp.). The majority of adenoviruses found in these animals have been characterized as agamid atadenovirus 1 (AgAdV1) in the species Lizard atadenovirus B, although other adenoviruses have also been described sporadically. Infection with AgAdV1 has been hypothesized to increase susceptibility to other pathogens, and co-infections with microsporidia and AdVs have been described in individual cases. In a retrospective study, samples from bearded dragons submitted to a commercial laboratory (Laboklin GmbH & Co. KG, Bad Kissingen, Germany) for the detection of AdVs by PCR, were also screened for the presence of E. pogonae. Samples from 144 animals, mostly cloacal swabs as well as feces and tissue samples, were included in the study. The AdV PCR was positive for 35 (24.3%) samples, the E. pogonae PCR was positive for 28 (19.4%). Sequencing of the products from the AdV PCRs showed that 30 of the viruses detected (85.7%) were identifiable as AgAdV1, and 3 (8.6%) belonged to the species Lizard atadenovirus A. The remaining 2 (5.7%) AdV PCR products were not sequenced. Evaluation of co-infections showed no correlation between infection with an AdV and E. pogonae when all AdV PCR positive samples were included in the analysis (P = 0.1168), and the correlation remained insignificant when only AgAdV1-like viruses were included (P = 0.0557). Adenoviruses and microsporidia are commonly found in swabs and feces of captive bearded dragons in Europe. Understanding their clinical significance for these animals requires further study. |

# Adenoviruses and *Encephalitozoon pogonae* in Samples from Bearded Dragons (*Pogona* spp.) in Europe

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**Abstract:** Adenoviruses (AdVs) and the microsporidium *Encephalitozoon pogonae* have been repeatedly detected in bearded dragons (*Pogona* spp.). The majority of adenoviruses found in these animals have been characterized as agamid atadenovirus 1 (AgAdV1) in the species *Lizard atadenovirus B*, although other adenoviruses have also been described sporadically. Infection with AgAdV1 has been hypothesized to increase susceptibility to other pathogens, and co-infections with microsporidia and AdVs have been described in individual cases. In a retrospective study, samples from bearded dragons submitted to a commercial laboratory (Laboklin GmbH & Co. KG, Bad Kissingen, Germany) for the detection of AdVs by PCR, were also screened for the presence of *E. pogonae*. Samples from 144 animals, mostly cloacal swabs as well as feces and tissue samples, were included in the study. The AdV PCR was positive for 35 (24.3%) samples, the *E. pogonae* PCR was positive for 28 (19.4%). Sequencing of the products from the AdV PCRs showed that 30 of the viruses detected (85.7%) were identifiable as AgAdV1, and 3 (8.6%) belonged to the species *Lizard atadenovirus A*. The remaining 2 (5.7%) AdV PCR products were not sequenced. Evaluation of co-infections showed no correlation between infection with an AdV and *E. pogonae* when all AdV PCR positive samples were included in the analysis ($P = 0.1168$), and the correlation remained insignificant when only AgAdV1-like viruses were included ($P = 0.0557$). Adenoviruses and microsporidia are commonly found in swabs and feces of captive bearded dragons in Europe. Understanding their clinical significance for these animals requires further study.

**Key Words:** Adenovirus, bearded dragon, *Encephalitozoon pogonae*, microsporidia, *Pogona*

## Introduction

The family *Adenoviridae* contains non-enveloped double-stranded DNA viruses currently divided into the genera *Atadenovirus*, *Aviadenovirus*, *Ichtadenovirus*, *Mastadenovirus*, *Siadenovirus*, and *Testadenovirus*. Adenoviruses (AdVs) are commonly found in squamate reptiles and the viruses found in these animals have overwhelmingly been atadenoviruses (Marschang *et al*., 2021). Among squamate reptiles, AdVs appear to be particularly common in bearded dragons (*Pogona* spp.). The AdVs most often found in these lizards belong to the species *Lizard atadenovirus B*, strain agamid atadenovirus 1 (AgAdV1). This virus has been detected in bearded dragons in human care in North America, Europe, Asia, and Australia (Akabane *et al*., 2020; Marschang *et al*., 2021) and has also been detected in free-ranging bearded dragons in Australia (Hyndman *et al*., 2019). The virus strain lizard atadenovirus 2 (LiAdV2) (also referred to as helodermatid adenovirus 2) in the species *Lizard atadenovirus A* was originally detected and isolated from Mexican beaded lizards (*Heloderma horridum*) in Europe (Papp *et al*., 2009). It has since also been detected in bearded dragons in human care in North America (Crossland *et al*., 2018; Benge *et al*., 2019) as well as in a free-ranging central bearded dragon (*Pogona vitticeps*) from western New South Wales, Australia (Hyndman *et al*., 2019). Although AdVs are generally considered one of the most common pathogens found in bearded dragons, there are limited studies evaluating prevalence of these viruses in managed populations. Detection rates of between 4.5 and >50% have been reported in various countries and groups of animals (Kim *et al*.; 2002, Abbas *et al*., 2012; Kubiak, 2013; Akabane *et al*., 2020). In a study screening free-ranging bearded dragons in Australia, 18.75% (9/48) of the animals were positive for AdVs (Hyndman *et al*., 2019).

The pathogenicity of AdVs in bearded dragons is not well understood. Infected animals have presented with pneumonia, gastrointestinal disease, and CNS signs, but AdVs have also been detected in clinically healthy animals (Marschang *et al*.,

2021). Clinical signs of disease appear to be more frequent in juvenile bearded dragons than in adults (Jacobson *et al*., 1996; Moormann *et al*., 2009; Doneley *et al*., 2014).

Microsporidia are obligate intracellular fungi. They are a genetically diverse group of microorganisms. Infection is often subclinical in immunocompetent mammals and birds, while they can cause serious disease in immunocompromised hosts (Paré and Conley, 2021). *Encephalitozoon pogonae* was recognized as a pathogen in bearded dragons by Sokolova *et al*. (2016), although previous descriptions of microsporidiosis in bearded dragons are available (Richter *et al*., 2013). *E. pogonae* is closely related to *E. cuniculi*. It has been detected in association with granulomas and necrosis in various tissues (Shibasaki *et al*., 2017; Wünschmann *et al*., 2019; Llinas *et al*., 2021). It has been suggested that it may be associated with aneurysms in bearded dragons in some cases, as it has been shown to cause vascular damage (Wünschmann *et al*., 2019; Kaiser *et al*., 2021).

Co-infections with multiple pathogens have been frequently reported in reptiles in human care, and the effects of individual pathogens, stress, inadequate husbandry, and other factors in infection prevalence and disease development are generally not well understood. Adenoviruses are often detected together with other potential pathogens in infected bearded dragons. These include dependoviruses (family *Parvoviridae*) (Jacobson *et al*., 1996; Kim *et al*., 2002; Pénzes *et al*., 2015), coccidia (Kim *et al*., 2002; Schilliger *et al*., 2016), mycoplasma (Crossland *et al*., 2018), ferlaviruses (Abbas *et al*., 2012), invertebrate iridoviruses (Papp *et al*., 2009), and ranaviruses (Stöhr *et al*., 2013). There are two reports of co-infections with AdVs and *E. pogonae* in bearded dragons (Schilliger *et al*., 2016; Llinas *et al*., 2021). In a report from Europe, a juvenile bearded dragon was co-infected with an atadenovirus, coccidia, and *E. pogonae* (Schilliger *et al*., 2016). In a report on captive bearded dragons in Australia, *E. pogonae* and a virus in the species *Lizard atadenovirus B* were detected in several animals in a collection experiencing granulomatous disease (Llinas *et al*., 2021).

The aim of this study was to screen samples submitted to a veterinary diagnostic laboratory for both AdVs and *E. pogonae*. We hypothesized that all of the AdVs detected would belong to the species *Lizard atadenovirus B* and that there would be a correlation between infection with AdVs and *E. pogonae*.

## Materials and Methods

Samples submitted to Laboklin GmbH & Co. KG (Bad Kissingen, Germany) from veterinarians for routine testing for AdVs by PCR were included in the study. Only samples for which the host species was specified to have been a bearded dragon and sample types that included cloacal swabs, feces, and/or tissue were included. No clinical information on the animals tested was provided. DNA was prepared from samples within 24 h of arrival in the laboratory. Prior to nucleic acid extraction, tissue and fecal samples were placed in MagNA Lyser Green Bead-tubes (Roche Diagnostics, Mannheim, Germany). Tissue samples were then incubated in a tissue lysis buffer (Roche Diagnostics) with proteinase K (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). Fecal samples were incubated in STAR Buffer (Roche Diagnostics). Swabs were incubated in tissue lysis buffer (Roche Diagnostics) and proteinase K (Carl Roth GmbH & Co. KG). DNA was prepared using the Roche MagNA Pure 96 system with the MagNA Pure 96 DNA and Viral RNA Small Volume Kit (Roche Diagnostics) according to the manufacturer's instructions. Adenoviruses were detected using a pan-AdV PCR targeting the DNA polymerase gene (Wellehan *et al*., 2004). *E. pogonae* was detected using a PCR targeting the 18S ribosomal RNA gene (Forward primer: 5' - GTG AGA CCC TTT GAC GGT GT - 3'; Reverse primer: 5' - GCT TCG TCA GCC GCT ATT AC - 3'). Both PCRs used 5 µl of prepared DNA as template. All PCR products of the expected size from both PCRs were sequenced using Sanger sequencing as described previously (Salzmann *et al*., 2021). Sequences were all analyzed by BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Correlations between AdV and *E. pogonae* detection were evaluated using a Chi-squared test. Significance level was set at $P < 0.05$.

## Results

At least one of the two pathogens included in the study were detected in 53 of the 144 (36.8%) samples tested (Table 1). In the AdV PCR, products of the expected size were obtained from 35 of the 144 samples tested (24.3%). Of the AdV PCR positive samples, 32 were swabs, one was a mixed swab and fecal sample, and two

were fecal samples (Table 1). Sequencing identified 30 of the 35 samples (85.7%) as belonging to the species *Lizard atadenovirus B*, three (8.6%) as belonging to the species *Lizard atadenovirus A*, while the sequence data was lost for analysis due to a laboratory error in two cases (5.7%) (Table 1).

Positive results for *E. pogonae* were obtained from 28 of the 144 samples tested (19.4%) (Table 1). Of the *E. pogonae* PCR positive samples, 24 were swabs, two were fecal samples, one was a mixed swab and mucous sample, and one was a tissue sample that was not further identified. All PCR products were identified as *E. pogonae* specific based on sequencing.

Both AdV and *E. pogonae* were detected in 10 animals (6.9%) (Table 1). When all 35 animals that had a positive PCR result for AdVs were included in calculations, there was no significant correlation between AdV and *E. pogonae* detection ($P = 0.1168$). When only those animals in which an AdV in the species *Lizard atadenovirus B* was identified were included, the correlation with *E. pogonae* detection approached significance, but was still above the cut-off ($P = 0.0557$).

## Discussion

While AdV infections are well documented in squamates in general and in bearded dragons in particular, there is little data indicating the prevalence of these viruses in bearded dragons in human care around the world. There is also limited data on the specific AdV species found, although the majority of detections in which the virus has been further characterized have been identified as AgAdV1 in the species *Lizard atadenovirus B*. The identification of the majority of the AdVs found in the present study as belonging to this group was therefore expected. The other AdV found in individual samples, clustering in the species *Lizard atadenovirus A*, has also been found previously in bearded dragons, but only in sporadic cases. A virus with the same sequence was found in a pet bearded dragon in Florida, USA (Benge *et al*., 2019). That animal was reported to have been ill, although no specific clinical signs were reported. The same virus was detected in a clinically healthy free-ranging central bearded dragon in New South Wales, Australia (Hyndman *et al*., 2019). In a study detecting antibodies against various atadenoviruses in a wide range of reptiles, antibodies against AgAdV1 were most commonly found in agamids (mostly bearded

dragons) (33.2% positive, n = 223), followed by antibodies against LiAdV2 (helodermatid adenovirus 2, in the virus species *Lizard atadenovirus A*) (15.2% positive) (Ball *et al*., 2014). In the two cases reported here in which the sequence of the AdV PCR product could not be evaluated, this was due to a laboratory error, so that the specificity of the PCR products and the identity of the viruses in these two cases cannot be confirmed.

Detection of *E. pogonae* in bearded dragons has so far been limited to individual case reports or case series, and no information on screening of larger numbers of animals or prevalence in specific populations has previously been available. Infections with this pathogen have been reported in pet bearded dragons in Australia (Llinas *et al*., 2021), the USA (Sokolova *et al*., 2016; Wünschmann *et al*., 2019), Canada (Kaiser *et al*., 2021), Japan (Shibasaki *et al*., 2017), and France (Schilliger *et al*., 2016). Based on the limited data available, it was somewhat surprising that *E. pogonae* was found in such a relatively high percentage of cases (19.4% of the animals tested).

Disease associated with both AdV and microsporidia in bearded dragons has been hypothesized to be dependent on various factors, including co-infections. Co-infections between AdVs and *E. pogonae* have been described in several cases (Schilliger *et al*., 2016; Llinas *et al*., 2021). However, the interaction between infection, co-infection, and disease is not yet understood for either group of pathogens. In the dataset presented here, there was no correlation between AdV and *E. pogonae* detection. Regarding the ten cases in which both pathogens were detected, the AdV was identified as an AgAdV1 in eight cases, while the virus could not be identified due to a laboratory error in two cases. Looking at only the co-infections between *E. pogonae* and AgAdV1, the correlation did approach significance but did not fall below the specified significance level of $P = 0.05$. Since both pathogens appear to be relatively common among pet bearded dragons in Europe, it is necessary to screen a larger number of animals for both in order to fully understand whether infection with one pathogen influences the probability of infection with the other. It would also be helpful in future studies to determine the health status of the animals in order to evaluate the role of each pathogen separately and together in disease development.

Another factor that might influence infection rate, as well as disease development, is the age of the animals. It would be helpful in future studies to also document the age of the animals to determine whether this is a factor that can influence the prevalence of infection with AdVs and microsporidia as well as whether age influences disease development following infection with one or both pathogens.

The majority of samples screened in this study were cloacal swabs. The samples were all submitted to the laboratory for screening for AdVs. Cloacal swabs as sample material are generally recommended for AdV testing in bearded dragons as virus is mostly found in the intestine and liver (Marschang *et al*., 2021). Samples used for the detection of microsporidia in bearded dragons have included mostly tissues from dead animals (Richter *et al*., 2013; Schilliger *et al*., 2016; Sokolova *et al*., 2016; Shibasaki *et al*., 2017; Wünschmann *et al*., 2019; Kaiser *et al.*, 2021; Llinas *et al*., 2021). Only one study reported detection in samples from live bearded dragons, including oral-cloacal swabs and blood (Llinas *et al*., 2021). Based on the detection rate in the present study, cloacal swabs appear to be a useful sample for the detection of *E. pogonae* in bearded dragons, although phase of infection and disease status could influence detection and require further study.

The sample set used for the present study was biased, as samples were submitted by veterinarians to a diagnostic laboratory for testing for AdVs. No information was provided on the reasons for testing, but these likely included both testing to determine possible causes of disease as well as health screenings. Nevertheless, it is interesting to note that both of the pathogens included in this study were found relatively frequently in the animals tested. Based on the results of the present study, screening for both AdVs and microsporidia should be considered in health screening of bearded dragons.

**Disclaimer:** The authors are employed by a commercial veterinary diagnostic laboratory (Laboklin GmbH & Co., KG, Bad Kissingen, Germany).

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306 **Table 1:** Samples from bearded dragons (*Pogona* sp.) that tested positive for
307 adenoviruses (AdVs) and/or *Encephalitozoon pogonae* by PCR. For the AdVs, the
308 viral species to which the identified virus likely belongs is listed. - = PCR negative.

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| No. | Species | Sample type | AdV PCR and sequencing result | *E. pogonae* PCR and sequencing result |
|---|---|---|---|---|
| 1 | *Pogona* sp. | Swab | *Lizard atadenovirus A* | - |
| 2 | *Pogona* sp. | Swab | *Lizard atadenovirus A* | - |
| 3 | *Pogona* sp. | Swab | *Lizard atadenovirus A* | - |
| 4 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 5 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 6 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 7 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 8 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | - |
| 9 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | - |
| 10 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | - |
| 11 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | - |
| 12 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | - |
| 13 | *Pogona vitticeps* | Swab and feces | *Lizard atadenovirus B* | - |
| 14 | *Pogona vitticeps* | Feces | *Lizard atadenovirus B* | - |
| 15 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 16 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 17 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 18 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 19 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 20 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 21 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | - |
| 22 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 23 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 24 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 25 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | - |
| 26 | *Pogona* sp. | Swab | *Lizard atadenovirus B* | *E. pogonae* |
| 27 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | *E. pogonae* |
| 28 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | *E. pogonae* |
| 29 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | *E. pogonae* |
| 30 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | *E. pogonae* |
| 31 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | *E. pogonae* |
| 32 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | *E. pogonae* |
| 33 | *Pogona vitticeps* | Swab | *Lizard atadenovirus B* | *E. pogonae* |
| 34 | *Pogona vitticeps* | Swab | PCR positive, no sequence available | *E. pogonae* |
| 35 | *Pogona* sp. | Feces | PCR positive, no sequence available | *E. pogonae* |
| 36 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 37 | *Pogona vitticeps* | Swab | - | *E. pogonae* |
| 38 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 39 | *Pogona vitticeps* | Tissue | - | *E. pogonae* |
| 40 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 41 | *Pogona* sp. | Swab | - | *E. pogonae* |

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|---|---|---|---|---|
| 42 | *Pogona vitticeps* | Swab | - | *E. pogonae* |
| 43 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 44 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 45 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 46 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 47 | *Pogona vitticeps* | Swab | - | *E. pogonae* |
| 48 | *Pogona* sp. | Feces | - | *E. pogonae* |
| 49 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 50 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 51 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 52 | *Pogona* sp. | Swab | - | *E. pogonae* |
| 53 | *Pogona vitticeps* | Swab and mucous | - | *E. pogonae* |

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