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Evidence-based practice

Prevalence of *Devriesea agamarum* in the lizard collection of The Royal Zoological Society of Antwerp

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Abstract
In recent times, *Devriesea agamarum* has been diagnosed more frequently as the causative organism of dermatitis in lizards, particularly in spiny-tailed lizards (*Uromastyx* sp.). Other lizard species such as bearded dragons (*Pogona vitticeps*) are known to be asymptomatic carriers, posing a potential threat to the healthy animals in a collection. This study reports on the isolation and phenotypic identification of *D. agamarum* from six healthy jewelled curly-tailed lizards (*Leiocephalus personatus*) in the reptile collection of the Royal Zoological Society of Antwerp. Two clinical cases were presented in the same study period: a frilled lizard (*Chlamydosaurus kingii*) and a Philippine sailfin lizard (*Hydrosaurus pustulatus*). These animals had abscesses in the oral cavity from which a rich growth of *D. agamarum* was obtained. It was concluded from this study that a quarantine programme for newly acquired animals is important to detect healthy carriers and prevent the spread of infection.

Introduction

The agamid lizard pathogen *Devriesea agamarum*, a coryneform bacterium belonging to the class Actinobacteria, was first described by Martel et al. in 2008 at the Faculty of Veterinary Medicine, Ghent University, Belgium. Since then, the diagnostics, epidemiology and pathology of this novel bacterium have been thoroughly studied by the same department. *Devriesea agamarum* is currently recognised as a common cause of dermatitis and septicaemia in lizards, particularly in spiny-tailed lizards (*Uromastyx* spp.) (Martel et al. 2008; Hellebuyck et al. 2009a). Other lizard species can be asymptomatically infected, especially the bearded dragon (*Pogona vitticeps*), in which *D. agamarum* is part of the normal mouth flora (Hellebuyck et al. 2009a). These healthy carriers are a significant health problem in captive colonies (Devloo et al. 2011). Once the disease is introduced into a lizard collection, it can become a chronic problem and persist for several years (Hellebuyck et al. 2010). Recently, in Croatia, Lukac et al. (2013) were the first outside Belgium to report on skin lesions associated with *D. agamarum* in captive spiny-tailed lizards (*Uromastyx* sp.). The purpose of this study was to investigate the prevalence of *D. agamarum* in the lizard collection of the Royal Zoological Society of Antwerp (RZSA).

Methods

From February to June 2013, 95 apparently healthy lizards representing 26 different species exhibited in the RZSA were examined by swabbing the oral cavity and peri-cloacal area (Table 1). In addition, two clinical cases were presented for examination: a frilled lizard (*Chlamydosaurus kingii*) with suppurative inflammation of a mandible fracture, and a Philippine sailfin lizard (*Hydrosaurus pustulatus*) with abscesses in the oral cavity after a freshwater turtle bite.

The animals were housed in 31 cages. Most cages were environmentally enriched enclosures, harbouring several lizard species. During the study, four newly acquired female jewelled curly-tailed lizards were quarantined together for six weeks. The lizards appeared healthy and skin lesions were not observed. After the quarantine period, two females were housed together with a male lizard from the RZSA collection, forming two new groups. All the animals were tested once, except for the jewelled curly-tailed lizards, which were tested during quarantine, before, and after the new groups were formed. The two clinical cases were retested after the lesions were cured. To examine possible environmental spread of infection, substrate samples consisting of sand or wood chips were collected, and swabs were taken from the surfaces of the cages. Faecal samples were collected if present. In addition, the

Table 1. Lizard species and their environment examined for the presence of *Devriesea agamarum.*

Species	Number of lizards in a cage	Oral cavity	Peri-cloaca	Faecal sample	Environmental sample	Positive sample
Beaded lizard (*Heloderma horridum*)	3	3			1	
Black iguana (*Ctenosaura similis*)	1	1	1		1	
Black-lined plated lizard (*Gerrhosaurus nigrolineatus*)	4	4	4			
Central bearded dragon (*Pogona vitticeps*)	2	2	2	1	1	
Central bearded dragon (*Pogona vitticeps*)	7	7	7		2	
Central bearded dragon (*Pogona vitticeps*)	1	1	1		1	
Central bearded dragon (*Pogona vitticeps*)	3	3	3			
Chinese crocodile lizard (*Shinisaurus crocodilurus*)	6	6	6		1	
Common blue-tongue skink (*Tiliqua scincoides*)	1	1	1		1	
Common blue-tongue skink (*Tiliqua scincoides*)	4	4	4			
Common green iguana (*Iguana iguana*)	2	2	2		1	
Common leopard gecko (*Eublepharis macularius*)	6	6	6		1	
Common leopard gecko (*Eublepharis macularius*)	1	1	1		1	
Common leopard gecko (*Eublepharis macularius*)	1	1	1		1	
Cuban iguana (*Cyclura nubile*)	4	4			1	
Eastern water dragon (*Physignathus lesueurii*)	1	1	1		2	
Egyptian spiny-tailed lizard (*Uromastyx aegyptia*)	1	1	1		1	
Egyptian spiny-tailed lizard (*Uromastyx aegyptia*)	3	3	3		2	
Fat-tailed gecko (*Hemitheconyx caudicinctus*)	4	4	4	1	1	
Frilled lizard (*Chlamydosaurus kingii*)°	4	4	4	1	1	1oc
Gila monster (*Heloderma suspectum*)	3	3	3			
Green water dragon (*Physignathus cocincinus*)	1	1	1		1	
Jewelled curly-tailed lizard (*Leiocephalus personatus*)*	2	2	2		1	2pc
Jewelled curly-tailed lizard (*Leiocephalus personatus*)**	4	4	4		1	1pc/1oc
Jewelled curly-tailed lizard (*Leiocephalus personatus*)***	3	3	3			3pc/2oc
Jewelled curly-tailed lizard (*Leiocephalus personatus*)***	3	3	3		1	3pc/2oc
Komodo dragon (*Varanus komodoensis*)	2	2				
Northern chuckwalla (*Sauromalus ater*)	3	3	3		2	
Ocellated skink (*Chalcides ocellatus*)	1	1	1		1	
Philippine sailfin lizard (*Hydrosaurus pustulatus*)°	1	1	1		1	1oc
Rhinoceros iguana (*Cyclura cornuta*)	1	1			1	
Ridge-tail monitor (*Varanus acanthurus*)	3	3	3		1	
Rough-scaled plated lizard (*Gerrhosaurus major*)	2	2	2			
Blue spiny lizard (*Sceloporus serrifer*)	2	2	2			
Blue spiny lizard (*Sceloporus serrifer*)	3	3	3		1	
Blue spiny lizard (*Sceloporus serrifer*)	5	5	5		1	
Seychelles giant day gecko (*Phelsuma sundbergi*)	1	1	1			
Solomon Island skink (*Corucia zebrata*)	2	2	2			
Tokay gecko (*Gekko gecko*)	2	2	2		1	

pc: peri-cloaca, oc: oral cavity; *2 ♂ lizards in the collection; ** 4 newly acquired ♀ lizards; *** 1 ♂(*) + 2 ♀(**) lizards caged together; °1 clinical case.

temperature and relative humidity in the cages were measured with a thermo-hygrometer (HI 9565, HANNA instruments® Inc., Woonsocket, Rhode Island, USA). Swabs and substrate samples were immediately processed in the laboratory. Moistened swabs from the lizards and cages were streaked onto CNA agar, a selective medium made of Columbia agar base (LabM Limited, Heywood, Lancashire, UK) supplemented with 5% horse blood, 10 mg colistin and 10 mg nalidixic acid (Sigma-Aldrich Corp., St Louis, Missouri, USA) per litre agar base (Ellner et al. 1966). Substrate samples were soaked in sterile physiological water before inoculation onto CNA

agar. After incubation under aerobic conditions for 24 to 48 hrs at 37° C, the plates were examined for circular, convex, cream-white colonies with an entire margin and a narrow zone of haemolysis. Suspected colonies were examined by gram staining and catalase test, subcultured onto Tryptone soya agar (LabM Limited) with 5% horse blood, and screened with Diatabs® (A/S ROSCO, Taastrup, DK) rapid microbial tests. The Diatabs® used were: alpha galactosidase, hippurate hydrolysis, Voges-Proskauer, leucine aminopeptidase and arginine dihydrolase. Mueller-Hinton Agar (LabM Limited) was used for the amylase test (Lee 1976). Isolates with a typical reaction profile were further characterised with the Api® Coryne test (bioMérieux® SA, Marcy l'Etoile, France). To confirm the phenotypic identification, the isolated *D. agamarum* strains were sent to the Faculty of Veterinary Medicine, Ghent University, Belgium, for 16S rRNA gene sequencing.

Antimicrobial susceptibility of the isolates to ceftiofur 30 µg, clindamycin 2 µg, enrofloxacin 10 µg, erythromycin 78 µg, florfenicol 30 µg, gentamycin 40 µg, penicillin 5 µg, trimethoprim + sulfa 1.25 + 23.7 µg, tetracycline 30 µg and tylosin 150 µg (A/S ROSCO), was determined on Mueller-Hinton Agar (LabM Limited) by the Kirby-Bauer disk diffusion test (Bauer et al. 1966) according to the CLSI standards (2013).

Results

Devriesea agamarum was isolated from six healthy jewelled curly-tailed lizards (*Leiocephalus personatus*) in the reptile collection of the RZSA. The remaining 89 apparently healthy lizards examined in this study cultured negative. At the start of the study, two male jewelled curly-tailed lizards resided in the zoo and were housed together in a cage. Samples taken from the peri-cloacal region of both animals were positive on two sampling occasions. A month later, four newly acquired female animals entered the quarantine facility. In one of the female lizards, *D. agamarum* was isolated from the oral cavity. A second animal cultured positive from the peri-cloacal region. After the quarantine period, two female lizards, chosen at random, were housed together with one of the male lizards. A month later, the animals were sampled and *D. agamarum* was isolated from the peri-cloacal region of all six lizards and from the oral cavity of four individuals (Table 1). Cultures from faecal samples, cage swabs and substrate samples were overgrown with background colonies. As a result, no positive isolations were obtained from these samples. The relative humidity in the cages of the jewelled curly-tailed lizards ranged between 64.5 and 68.5%, the temperature between 27.4 and 29.8° C. Furthermore, rich cultures of *D. agamarum* were obtained from lesions in the oral cavity in the two clinical cases presented: a frilled lizard (*Chlamydosaurus kingii*) and a Philippine sailfin lizard (*Hydrosaurus pustulatus*). The reaction pattern of the strains isolated from the jewelled curly-tailed lizards and the two clinical cases was compared with that of the type strain IMP2 (Martel et al. 2008). All the isolates were gram positive, demonstrating morphology similar to that of corynebacteria. In the rapid microbial tests, positive reactions were obtained for catalase, alpha galactosidase, Voges-Proskauer test, arginine dihydrolase and leucine aminopeptidase. Hippurate hydrolysis and amylase production were not detected. With the Api® Coryne test (bioMérieux SA) all suspected isolates gave the same numerical pattern (3473125) as the type strain. Nitrates were reduced. Pyrazinamidase, beta-galactosidase, alpha-glucosidase, N-acetyl-beta-glucosaminidase, beta-glucosidase (esculine), urease and gelatinase were produced. Glucose, maltose and saccharose were fermented. Pyrrolidonyl arylamidase, alkaline phosphatase and beta-glucuronidase were not produced. No fermentation of ribose, xylose, mannitol, lactose or glycogen was noticed.

The 16S rRNA gene sequencing of the isolates showed 99–100% similarity with *D. agamarum* (IMP2). The isolated strains were in vitro susceptible to the antimicrobial agents tested.

Discussion

Devrieseasis is a chronic problem that can persist for several years in a lizard collection (Devloo et al. 2011). Recent studies have shown that spiny-tailed lizards (*Uromastyx* spp.), desert-dwelling agamid lizards inhabiting the North African region and the Middle East, are more susceptible to the development of *D. agamarum*-associated dermatitis (Hellebuyck et al. 2009a, Lukac et al. 2013; Martel et al. 2008), whereas bearded dragons (*Pogona* spp.), from semi-desert regions and open woodlands of Australia, in which *D. agamarum* is part of the normal mouth flora, may act as asymptomatic carriers (Hellebuyck et al. 2009a; Devloo et al. 2011). Both lizard species are kept in the RZSA, but *D. agamarum* could not be isolated from four spiny-tailed lizards and 13 bearded dragons during the present study. On the other hand, positive isolations were obtained from the male jewelled curly-tailed lizards (*Leiocephalus personatus*) in the collection. Two of four newly acquired female jewelled tailed curly-lizards tested positive during the quarantine period. In spite of the positive results observed in both the male and female lizards, it was decided to form two groups with a male from the collection and two of the quarantined female lizards. A month later, *D. agamarum* could be isolated from all six animals (Table 1.). As these animals remained in good clinical health, no treatment was applied. The jewelled curly-tailed lizards were considered as asymptomatic carriers. Consequently, care was taken to avoid contact with other species. Unlike bearded dragons, jewelled curly-tailed lizards are not desert dwellers, preferring sandy coastal areas, but they can also be found near shrubs or planting in urbanised areas. The high humidity levels measured in the cages of the curly-tailed lizards are beneficial to the survival of *D. agamarum*, which can remain viable for more than 5 months in a moist environment (Hellebuyck et al. 2010). Regular cleaning, removing of faecal material and quarterly disinfection with sodium hypochlorite may reduce the infection risk. During this study, no isolations could be made from faecal samples and the captive environment because of overgrowth by contaminants (Table 1). Hence, the development of a selective enrichment broth is required for the recovery of *D. agamarum* from mixed bacterial samples. On the other hand, primary culture was sufficient for the isolation of *D. agamarum* from skin lesions, or even from asymptomatic carriers, as rich growth of the organism was obtained on CNA agar.

It has been demonstrated that devrieseasis in lizards cannot be induced by inoculating intact skin. Skin abrasions appear to be necessary and bite-related lesions inflicted by cage mates are considered as important causes (Hellebuyck et al. 2009a). Therefore, *D. agamarum* is regarded as a secondary invader.

In the clinical cases described here, the affected piece of bone was removed from the mandible of the frilled lizard (*Chlamydosaurus kingii*), the abscesses in the oral cavity of the Philippine sailfin lizard (*Hydrosaurus pustulatus*) were removed with a curette, and the wounds of both animals were cleaned mechanically. Clinical improvement and elimination of *D. agamarum* was observed without the application of an antiseptic or antibacterial therapy. In agreement with other reports, the isolated strains were very susceptible to the antibiotics tested. Ceftiofur is the drug of choice and has proved to be highly successful for treatment of *D. agamarum* associated dermatitis in lizards (Hellebuyck et al. 2009b).

Due to the heterogeneity within the group of coryneform bacteria, their identification is often difficult (Funke and Bernard

2011). The results of the rapid microbial tests selected were identical for all suspected isolates and the biochemical reactions obtained with the Api Coryne system generated the same numerical profile. *Devriesea agamarum* is not added to the analytical profile index; consequently, the profile obtained is listed as doubtful. Nevertheless, we may conclude that *D. agamarum* can be successfully identified with a small range of easy-to-perform conventional tests. The accuracy of the phenotypic identification was confirmed by 16S rRNA gene sequencing.

The present study demonstrates the occurrence of *D. agamarum* in the lizard collection of the RZSA and adds to the growing evidence that *D. agamarum* is a potential pathogen in lizard collections. In conclusion, newly acquired lizards should be kept in quarantine and examined to prevent the introduction of asymptomatic carriers. Proper housing and protective measures to avoid skin abrasions and bite-related lesions are advisable.

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