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1 ***Devriesea agamarum* causes dermatitis in bearded dragons**

2 ***(Pogona vitticeps)***

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Abstract – *Devriesea agamarum* is frequently isolated from dermatitis in lizards, notably from cheilitis in spiny tailed lizards (genus *Uromastyx*). It was the aim of the present study to assess the role of this bacterium as a causative agent of dermatitis by fulfilling Koch's postulates. First, its association with diseased lizards was demonstrated. The bacterium was isolated from several, mainly desert dwelling squamate species showing symptoms of dermatitis and / or septicaemia. The affected lizards mainly belonged to the family of the Agamidae (genera *Pogona*, *Uromastyx*, *Agama*) and in one case to the Iguanidae (genus *Crotaphytus*). Secondly, the occurrence of *Devriesea agamarum* in 66 clinically healthy bearded dragons, 21 clinically healthy *Uromastyx* species and 40 squamate eggshells was studied. The bacterium was isolated from the oral cavity of 10 bearded dragons but from none of the healthy *Uromastyx* species. Hence *Devriesea agamarum* was found to be part of the oral microbiota in *Pogona vitticeps*. Finally, bearded dragons (*Pogona vitticeps*) were experimentally inoculated with *Devriesea agamarum* by direct application of a bacterial suspension on intact and abraded skin. At the scarified skin of all inoculated lizards, dermatitis was induced from which *Devriesea agamarum* was reisolated. In conclusion, *Devriesea agamarum* is a facultative pathogenic bacterium, able to cause dermatitis in agamid lizards when the integrity of the skin is breached.

lizard / *Devriesea agamarum* / dermatitis / bacterial / hyperkeratosis

1. INTRODUCTION

Dermatitis is one of the most frequently occurring diseases in captive reptiles and is often associated with bacteria (Chineme and Addo, 1980; Pasmans et al., 2007), fungi (Frank, 1976; Jacobson et al., 2000) and viruses (Raynaud and Adrian, 1976; Herbst et al., 1999).

Although a variety of bacteria have been associated with dermal disease in captive lizards, their role as primary etiological agents in the onset of dermatitis is questionable (Jacobson, 1992). Predisposing factors such as environmental mismanagement (humidity, temperature, social stress) or other diseases (gastrointestinal, respiratory, ectoparasites) are thought to be of major importance for the development of dermatitis. An unknown member of the phylum Actinobacterium has been isolated from a number of dermatitis cases, mainly in agamid lizards. It has been particularly associated with chronic hyperkeratosis presented as lip and skin fold dermatitis in spiny tailed lizards (*Uromastyx* sp.) (Koplos et al., 2000; Pasmans et al., 2004).

The aim of the present study was to determine if *D. agamarum* is a cause of dermatitis in captive lizards. Additionally, we determined whether *D. agamarum* is a part of the cloaca, skin and/or mouth microbiota of clinically healthy lizards, freshly hatched lizards and squamate eggshells.

2. MATERIALS AND METHODS

2.1. Association of *Devriesea agamarum* with clinical cases of dermatitis in lizards

Over a 3 year period, 28 lizards with dermatitis, occasionally associated with septicaemia, were sampled for the presence of *D. agamarum*. Either swabs (Copan innovation, Italy) from dermal lesions or samples from internal organs and bone marrow were cultured during 24 to 48 hours on colistin nalidixic acid (CNA, Oxoid GmbH, Wesel, Germany) agar at 37°C and 5% CO₂. All isolates that formed smooth, mucoid, whitish small colonies and produced small alpha haemolysis as reported by Martel et al. (in press) for *D. agamarum*, were analysed using API[®] Coryne, API[®] 20 STREP, API[®] 50 CH (bioMérieux, Marcy l'etoile, France) and 16S rRNA gene sequencing, as previously described (Martel et al., in press).

2.2. Occurrence of *Devriesea agamarum* in clinically healthy lizards and squamate eggshells

Thirty-eight *Pogona vitticeps* and 21 *Uromastyx* (12 *U. acanthinura*, 7 *U. geyri*, 1 *U. ocellata* and 1 *U. dispar*) without dermal lesions were examined for the presence of *D. agamarum* by dermal sampling using a sterile cotton-tip

applicator at 3 places: 1) the paramedian midbody area of dorsolateral skin 2) the edge of the upper lip and 3) the pericloacal region. Two of the sampled *P. vitticeps* were housed together with an *U. acanthinura* showing marked cheilitis and dermatitis. All remaining *P. vitticeps* were reared in segregation from other lizard species. Additionally, 28 neonatal *P. vitticeps* and their eggshells and 12 eggshells of *U. geyri* were sampled to detect the presence of *D. agamarum*. For this purpose, the eggshells were rinsed in 2 ml of sterile phosphate buffered saline (PBS), followed by culturing swabs, drenched into this suspension, on CNA during 24 to 48 hours at 37 °C and 5% CO₂. All colonies, morphologically similar to *D. agamarum* were further analysed as described above.

2.3. Experimental inoculation of *Pogona vitticeps* with *Devriesea agamarum*

D. agamarum strain IMP 2, isolated from the liver of a dead *Agama impalearis*, was incubated for 24 hours at 37 °C and 5% CO₂. Ten colonies were harvested and transferred in 5 ml of brain heart infusion (BHI) broth. Again, this suspension was incubated for 24 hours at 37 °C and 5% CO₂. The inoculum was diluted with PBS to an optic density of 1.015, which equalled 10⁸ cfu/ml.

Twelve captive bred *Pogona vitticeps* were used in this study, which was approved by the ethical committee of the Faculty of Veterinary Medicine, Ghent University. All animals were found to be clinically healthy and free of intestinal

parasites. The lizards were divided in two groups: one group was inoculated with *D. agamarum* and the other served as a negative control group. All experimental animals were 6 weeks old, weighed 4 to 10 gram and were randomly assigned to one of both groups. The lizards were housed in a room where temperature reached an average of 28-30 °C during 12 hours a day. Self ballasted bulbs (Powersun®, Zoomed) were installed in the enclosures to provide the necessary ultra-violet light and to create a local hot spot.

After local disinfection with ethanol, the following lesions were inflicted in 3 places at the right side in each animal using a 26 gauge needle (Terume Europe N.V., Leuven, Belgium): 1) 3 scratches in the outside border of the upper lip 2) 1 scratch at the medial side of the right knee and 3) 3 parallel dermal perforations in the dorsolateral skin over a distance of 0.5 cm. The bacterial suspension was applied onto the lesions of the lip and the knee, using a swab drenched into the inoculum. A 27 gauge needle (Terume Europe N.V., Leuven, Belgium) was used to infiltrate a total of 200 µl of the bacterial suspension, containing 2×10^7 cfu, into the lesions of the dorsolateral skin. At the left side of each lizard intact skin was inoculated with the bacterial suspension at the 3 corresponding sites. The lizards of the negative control group were inflicted similar lesions but sterile PBS was applied instead of the inoculum.

Seventeen and 24 days post inoculation (P.I.), swabs were collected from all inoculated sites and examined for the presence of *D. agamarum* as described above. In all of the challenged and negative control animals, full thickness skin biopsies were taken at 17 days P.I. from the inoculated areas of intact and

139 abraded dorsolateral skin. Tissues were collected in formalin, embedded in
140 paraffin followed by haematoxylin eosin and Gram staining after sectioning.

142 3. RESULTS

143 144 **3.1. *Devriesea agamarum* is associated with dermatitis, cheilitis and** 145 **septicaemia in captive lizards**

146
147 During a 3 year period 16 cases of *D. agamarum* related dermatitis and/or
148 septicaemia in lizards were demonstrated (Table 1). *D. agamarum* was isolated
149 from proliferative, hyperkeratotic dermal lesions in 1 *Agama impalearis*, 2
150 *Crotaphytus collaris* and 2 *Uromastix acanthinura*. Other isolates were
151 recovered from cheilitis lesions in 1 *C. collaris*, 1 *Pogona vitticeps*, 5 *U.*
152 *acanthinura* (Fig. 1) and 2 *U. geyri*. One strain was isolated from the liver of a
153 dead *A. impalearis* (IMP 2) and 1 strain from the bone marrow of a dead *U.*
154 *geyri*.

155 156 157 **3.2. *Devriesea agamarum* is part of the oral microbiota in healthy bearded** 158 **dragons (*Pogona vitticeps*)**

159
160 *D. agamarum* was isolated from the border of the oral cavity in 8 clinically
161 healthy *P. vitticeps* reared separately from other lizard species. Moreover, the

bacterium was demonstrated in the oral cavity of 2 healthy bearded dragons cohabiting for several years with an *U. acanthinura* showing severe dermal lesions from which *D. agamarum* was isolated. *D. agamarum* could not be detected in any of the 21 clinically healthy lizards of the genus *Uromastix*, 28 neonatal bearded dragons or 40 eggshells.

3.3. *Devriesea agamarum* causes dermal lesions in bearded dragons (*Pogona vitticeps*)

None of the negative control animals developed dermal pathology. The applied lesions healed in a few days time.

At 5 days post infection (P.I.) all 6 inoculated lizards had developed a macroscopic dermatitis in the area of the applied dorsolateral skin lesions. In 2 lizards the development of multiple plaques was noted and in 4 out the 6 inoculated lizards nodular lesions were observed. These nodules had an average, maximal diameter of 3 mm and height of 2.5 mm at the end of the trial. All these dorsolateral lesions showed a discoloured, irregular and scabby superficial aspect at the end of the observation period.

At 9 days P.I., 3 challenged lizards showed clearly distinguishable crusts at the scratches made in the right upper lip. This was accompanied by a discrete but diffuse swelling of the right edge of the oral cavity during the last days of the trial. In one inoculated animal a scabby lesion was noted at the left edge of the mouth where the inoculum was applied onto intact skin.

At the medial surface of the knee, a distinctive crust was observed at 7 days P.I. in 5 inoculated lizards.

From all of the lesions at the right side dorsolateral region *D. agamarum* could be isolated during the last week of the trial. From the inoculated sites at the right side lips and knees, *D. agamarum* could be isolated in 3 and 4 lizards, respectively. The bacterium could not be isolated from inoculation sites without applied lesions, not even from the scabby lesion at the left edge of the mouth that was observed in one inoculated animal.

In all 6 inoculated lizards but in none of the negative control animals, pathological changes were observed in the skin biopsies (Fig. 2 a, b). These consisted of mild to severe epidermal hyperplasia with ortho- and parakeratosis. In one section epidermal spongiosis was noted. Serocellular crust formation, due to exudation of inflammatory proteins and degenerated inflammatory cells was apparent in 4 out of 6 samples. In all of the sections extensive colonisation of the superficial corneal layers by rod-shaped, Gram positive bacteria was observed. Hyperaemia, moderate edema and the perivascular influx of heterophils were present in the dermis.

4. DISCUSSION

D. agamarum was recently designated to a novel genus and species on the basis of morphological, chemotaxonomic and phylogenetic differences from other coryneform bacteria. For this Gram positive, rod shaped and non sporulating bacterium, *Brachybacterium faecium* (95%) and *Dermabacter hominis* (95%) were determined as nearest phylogenetic neighbours based on 16S rRNA gene sequence analysis (Martel et al., in press). The occurrence of dermal disease in reptiles has also been associated with other members of the phylum Actinobacterium, such as *Dermatophilus* and *Mycobacterium* species (Chineme and Addo, 1980; Greer et al., 2003; Wellehan et al., 2004).

D. agamarum was isolated from several clinical cases of naturally infected lizards indicating this bacterium to be involved in chronic dermatitis that can result in septicaemia. Especially desert dwelling species seem to be more susceptible to the development of *D. agamarum* associated dermatitis. Particularly in *Uromastix* lizards, the bacterium was isolated from all cheilitis and dermatitis cases included in this study. In fact, cheilitis in *Uromastix* lizards, often combined with dermatitis, is one of the most frequently occurring diseases in these lizards in captivity.

D. agamarum was isolated from the oral cavity in 8 clinically healthy *P. vitticeps* reared isolated from other lizard species as well as in 2 healthy *P. vitticeps* cohabiting with a *D. agamarum* infected *Uromastix*. Therefore *D. agamarum* can be considered a common constituent of the oral microbiota of

captive *P. vitticeps*. Despite the relatively high occurrence in this species, isolation of *D. agamarum* could only be achieved at one occasion from a *P. vitticeps* with cheilitis. On the other hand, the bacterium was not isolated from any of the clinically healthy *Uromastyx* lizards. Combined with the high occurrence of *D. agamarum* in diseased *Uromastyx*, this finding suggests a species dependent sensitivity to *D. agamarum* associated disease. Moreover, bearded dragons might represent a reservoir of the bacterium for squamate species highly sensitive to *D. agamarum* associated disease.

Strain IMP 2 of *D. agamarum* isolated from the liver of a dead *A. impalearis* induced dermatitis in all of the 6 inoculated animals during a 24 day observation period and the agent was re-isolated from these lesions. Hence Koch's postulates were fulfilled (Evans, 1976; 1977).

Despite using a strain isolated from the liver of an animal that died due to septicaemia, in none of the challenged lizards signs related to systemic spread of *D. agamarum* were seen. Variation in length of the observation period or the inoculated lizard species could influence the outcome of infection with *D. agamarum*.

D. agamarum associated dermatitis could only be provoked after inoculation of skin lesions. Dermatitis could not be induced by inoculating intact skin. Therefore, skin lesions appear to be necessary to develop *D. agamarum* related dermatitis.

In conclusion, this study demonstrates that *D. agamarum* is part of the oral microbiota of *P. vitticeps* and is able to cause cheilitis, dermatitis and septicaemia in lizards using skin lesions as a portal of entry.

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Figure 1. Spiny tailed lizard (*Uromastyx*
acanthinura) with *Devriesea agamarum*
 associated cheilitis presented as chronic
 hyperkeratosis.

Figure 2. HE stained sections of dorsolateral
 skin collected in bearded dragons (*Pogona*
vitticeps), 17 days after inoculating dermal
 perforations either with PBS (**a**) or *Devriesea*
agamarum strain IMP 2 (**b**).

(**a**) A relatively thin Stratum germinativum
 and Stratum corneum, melanocytes and
 normal dermis with loose connective tissue
 are apparent. Scale bar = 50 µm.

(**b**) Note the epidermal hyperplasia with
 orthokeratosis, serocellular crust formation and
 hyperaemia. Scale bar = 50 µm.

sg, Stratum germinativum; sc, Stratum corneum;
 mc, melanocyt; d, dermis; hp, epidermal
 hyperplasia; ok, orthokeratosis; s, serocellular
 crust; h, hyperaemia.

Table 1. Bacteria and/or fungi isolated as pure and/or abundant cultures from 28 clinical dermatitis cases in lizards.

Wild caught (WC) or captive bred (CB)	Lizard species	Lesions	Bacteriological and/or mycological agent identified
CB	<i>Pogona vitticeps</i>	Dermatitis	No
CB	<i>Pogona vitticeps</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Agama impalearis</i>	Dermatitis	<i>Devriesea agamarum</i>
WC	<i>Agama impalearis</i>	Dermatitis/Septicaemia	<i>Devriesea agamarum</i>
WC	<i>Physignathus concincinus</i>	Dermatitis	<i>Dermatophilus congolensis</i>
CB	<i>Physignathus concincinus</i>	Dermatitis	<i>Staphylococcus aureus</i>
CB	<i>Physignathus concincinus</i>	Dermatitis	<i>Staphylococcus aureus</i>
CB	<i>Physignathus concincinus</i>	Dermatitis	<i>Staphylococcus aureus</i>
WC	<i>Uromastix acanthinura</i>	Dermatitis	<i>Devriesea agamarum</i>
WC	<i>Uromastix acanthinura</i>	Dermatitis	<i>Devriesea agamarum</i>
WC	<i>Uromastix acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastix acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastix acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastix acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastix acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastix geyri</i>	Dermatitis/Septicaemia	<i>Devriesea agamarum</i>
WC	<i>Uromastix geyri</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastix geyri</i>	Cheilitis	<i>Devriesea agamarum</i>
CB	<i>Crotaphytus collaris</i>	Dermatitis	<i>Devriesea agamarum</i>
CB	<i>Crotaphytus collaris</i>	Dermatitis	<i>Devriesea agamarum</i>
CB	<i>Crotaphytus collaris</i>	Cheilitis	<i>Devriesea agamarum</i>
CB	<i>Iguana iguana</i>	Dermatitis	<i>Nannizziopsis vriesii</i>
CB	<i>Iguana iguana</i>	Dermatitis	No
CB	<i>Iguana iguana</i>	Dermatitis	No
CB	<i>Iguana iguana</i>	Dermatitis	No
CB	<i>Iguana iguana</i>	Dermatitis	<i>Staphylococcus aureus</i>
CB	<i>Iguana iguana</i>	Dermatitis	<i>Staphylococcus aureus</i>
WC	<i>Cyclura nubila</i>	Dermatitis	No