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

2 **(*Pogona vitticeps*)**

3

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

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

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1. INTRODUCTION

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49 Dermatitis is one of the most frequently occurring diseases in captive reptiles
50 and is often associated with bacteria (Chineme and Addo, 1980; Pasmans et al.,
51 2007), fungi (Frank, 1976; Jacobson et al., 2000) and viruses (Raynaud and
52 Adrian, 1976; Herbst et al., 1999).

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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.

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2. MATERIALS AND METHODS

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

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

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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

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

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106 **2.3. Experimental inoculation of *Pogona vitticeps* with *Devriesea agamarum***

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

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

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

123 After local disinfection with ethanol, the following lesions were inflicted in 3
124 places at the right side in each animal using a 26 gauge needle (Terume Europe
125 N.V., Leuven, Belgium): 1) 3 scratches in the outside border of the upper lip 2)
126 1 scratch at the medial side of the right knee and 3) 3 parallel dermal
127 perforations in the dorsolateral skin over a distance of 0.5 cm. The bacterial
128 suspension was applied onto the lesions of the lip and the knee, using a swab
129 drenched into the inoculum. A 27 gauge needle (Terume Europe N.V., Leuven,
130 Belgium) was used to infiltrate a total of 200 µl of the bacterial suspension,
131 containing 2×10^7 cfu, into the lesions of the dorsolateral skin. At the left side of
132 each lizard intact skin was inoculated with the bacterial suspension at the 3
133 corresponding sites. The lizards of the negative control group were inflicted
134 similar lesions but sterile PBS was applied instead of the inoculum.

135 Seventeen and 24 days post inoculation (P.I.), swabs were collected from all
136 inoculated sites and examined for the presence of *D. agamarum* as described
137 above. In all of the challenged and negative control animals, full thickness skin
138 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.

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3. RESULTS

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

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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*.

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

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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

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

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168 **3.3. *Devriesea agamarum* causes dermal lesions in bearded dragons (*Pogona***
169 ***vitticeps*)**

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

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

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

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

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

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

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4. DISCUSSION

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

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

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

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

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

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

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

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

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

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

354 (**a**) A relatively thin Stratum germinativum
355 and Stratum corneum, melanocytes and
356 normal dermis with loose connective tissue
357 are apparent. Scale bar = 50 μ m.

358 (**b**) Note the epidermal hyperplasia with
359 orthokeratosis, serocellular crust formation and
360 hyperaemia. Scale bar = 50 μ m.

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

365

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>Uromastyx acanthinura</i>	Dermatitis	<i>Devriesea agamarum</i>
WC	<i>Uromastyx acanthinura</i>	Dermatitis	<i>Devriesea agamarum</i>
WC	<i>Uromastyx acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastyx acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastyx acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastyx acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastyx acanthinura</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastyx geyri</i>	Dermatitis/Septicaemia	<i>Devriesea agamarum</i>
WC	<i>Uromastyx geyri</i>	Cheilitis	<i>Devriesea agamarum</i>
WC	<i>Uromastyx 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





