Bearded dragons (*Pogona vitticeps*) asymptomatically infected with *Devriesea agamarum* are a source of persistent clinical infection in captive colonies of dab lizards (*Uromastyx* sp.)

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Abstract

Devriesea agamarum causes dermatitis and septicaemia in a variety of lizards, notably those belonging to the genus *Uromastyx*, whereas other species such as bearded dragons (*Pogona vitticeps*) seem to be asymptomatic carriers. Using amplified fragment length polymorphism (AFLP), the relatedness between 69 *D. agamarum* isolates was examined. The isolates derived from 44 diseased lizards, of which 31 belonged to the genus *Uromastyx*, and from 25 healthy lizards, of which 21 were bearded dragons. Eight AFLP genotypes were obtained, four of which comprised 93% of the isolates. These four genotypes were each present in 2, 2, 8 and 13 different captive colonies. Up to three genotypes were isolated from a single infected colony simultaneously. On two occasions, the same genotype was found in healthy bearded dragons and diseased *Uromastyx* lizards from the same colony, confirming the role of the former as an asymptomatic source of infection for the latter. Two genotypes, comprising 12 isolates, were exclusively associated with diseased *Uromastyx* lizards, suggesting strain dependent host adaptation. Finally, *D. agamarum* was shown to be able to persist for at least seven years in a lizard colony, persistently causing severe disease in several lizard species.

Key words

Devriesea agamarum, reptile, AFLP, animal pathogens, bacterial infections, bacterial typing

Introduction

Dermatitis and septicaemia caused by *Devriesea agamarum* poses a significant health problem in captive lizards (Hellebuyck et al., 2009a). Most often, *Devriesea* associated disease in a collection of lizards is a chronic problem, which, if left untreated, persists for several years and compromises captive maintenance, especially of dab lizard species (genus *Uromastyx*). Whether the occurrence and persistence of this disease in a collection is caused by a single endemic strain or by multiple strains is not known. Besides, whereas certain saurian taxa such as dab lizards (*Uromastyx* sp.) seem predisposed to severe clinical infection, the bacterium is frequently isolated from the oral cavity of healthy bearded dragons (*Pogona vitticeps*) (Hellebuyck et al., 2009a). This may be due to a difference in host sensitivity or to a difference in bacterial strain virulence.

The aim of this study was to determine the relatedness between *D. agamarum* strains from healthy and diseased lizards in captivity using amplified fragment length polymorphism (AFLP).

Materials and methods

Devriesea agamarum *strains*

Sixty nine *D. agamarum* isolates were obtained frompatients showing dermatitis, cheilitis and/or septicaemia presented at the Faculty of Veterinary Medicine, Ghent University, Belgium and from convenience sampling of captive collections. The 69 lizards, belonging to the genera *Uromastyx* (31), *Pogona* (21), *Crotaphytus* (8), *Agama* (4), *Laudakia* (4) and *Eublepharis* (1), representing 9 saurian species and 23 different collections (Table 1). A collection is defined as one or more lizards belonging to the same owner and kept at the same

locality. Samples derived from lesions from 44 diseased lizards, of which 31 belonged to the genus *Uromastyx*, and from the oral cavity of 25 healthy lizards of which 21 were bearded dragons (*Pogona viticeps*). *D. agamarum* was isolated on sheep blood agar containing colistin and nalidixic acid after 1-3 days of incubation at 30°C. Identification to species level was done as described in Martel et al.(Martel et al., 2008).

*AFLP and PCR*DNA of *D. agamarum* isolates was extracted using Easy DNA kit (Invitrogen).

Restriction endonuclease digestion and adapter ligation for AFLP and PCR amplification were performed using the methods of Ceelen et al.(Ceelen et al., 2006). For the restriction PstI was used. The sequences of the adapter oligonucleotides and the primers for the PCR were described in the article of De Zoysa and Efdtratio (De Zoysa and Efstratiou, 2000).

Gel electrophoresis

The amplified products were separated on 1.5% agarose gel by unidirectional electrophoresis using 0.5X Tris-burate-EDTA buffer, stained with gel red and visualized on a UV transilluminator. Fragment size was determined by comparison with 100 bp DNA ladder (Fermentas).

Analysis of AFLP profiles

The pictures of the gels were imported as TIFF-files into Bionumerics version 4.6 (Applied Maths, Sint-Martens-Latem, Belgium) and a similarity matrix was calculated according to the DICE algorithm using optimization settings and tolerance level of 1.06. A UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram was constructed based on the average similarity matrices of two replicates. The strains were classified in the same

AFLP type if the relatedness was higher than 70%. A three dimensional visualisation was created using the multidimensional scaling tool based on the average similarity matrix.

Results

Results are summarized in Table 1 and Figures 1 and 2. Eight distinct AFLP types (A-H) were obtained. The types obtained from two independent experiments showed high similarity. The four AFLP types A, B, C and D, represented by 28, 24, 9 and 3 isolates respectively, comprised 93% of all isolates and derived from 13, 8, 2 and 2 collections respectively. Up to three different AFLP types were isolated from diseased animals from a single collection (collection B). AFLP types A and B were isolated both from diseased and healthy animals in 3 and 1 collections respectively. From one animal there is an isolate of the oral cavity (isolate 48) and cheilitis (isolate 50.1). In this case, the same AFLP type A was found. Strains belonging to the closely related AFLP types C and D were isolated exclusively from diseased *Uromastyx* lizards.

Exchange of animals could be demonstrated between following collections: A, D, H, I and R received animals that were previously kept in collection B. The AFLP types found in collection B (type A, B and C) were also present in these other collections. It is possible that the animals of these collections were infected by the animals from collection B.

Discussion

The 8 AFLP types obtained in this study show pronounced variation between *D. agamarum* strains isolated from lizards. Up to three strains were isolated from a single captive colony

simultaneously. The majority of the isolates belonged to two AFLP types (A and B), that are widespread over different collections of lizards. Interestingly, both types were isolated from diseased and healthy animals. Since most of the lizards of the genera *Uromastyx, Laudakia, Agama* and *Crotaphytus* that were infected with one of both AFLP types showed clinical signs of dermatitis and/or septicaemia, types A and B seem to clinically affect a wide variety of lizards. However, these AFLP types were also highly prevalent in the oral cavity of asymptomatic bearded dragons. Disease associated with this bacterium is very rare in this species. In contrast, *D. agamarum* is usually absent from the oral cavity of healthy *Uromastyx* but highly prevalent in diseased animals from this genus (Hellebuyck et al., 2009a). Our findings thus suggest that AFLP types A and B are highly prevalent as part of the normal oral microbiota of bearded dragons, which thus constitute an important source of clinical infection for several other lizard species. Accordingly, the difference in occurrence of *D. agamarum* associated disease in different saurian taxa can be largely attributed to a different host susceptibility.

In contrast with the previous finding, the two closely related AFLP types C and D, comprising 12 isolates, were isolated exclusively from diseased *Uromastyx* lizards. Although one of the collections in which *Uromastyx* lizards were infected by type C also harbored bearded dragons, this AFLP type could not be isolated from the latter. The source of infection for these types is thus not clear. Possibly, both types represent genotypes, adapted to dab lizards. Persistence of a single AFLP type during a prolonged period of time is demonstrated in collection A, from which AFLP type A was isolated over a period of 7 years from diseased lizards, belonging to three genera and four species. Besides cheilitis and dermatitis, disease in this colony was characterized by high mortality (Hellebuyck et al., 2011). This finding suggests that a single clone of *D. agamarum* may become endemic in a captive colony of lizards, persistently causing severe disease. Persistence of the bacterium may be promoted by

the presence of asymptomatic carriers. Moreover, *D. agamarum* is highly resistant in the environment, especially under humid conditions (Hellebuyck et al., 2011). Eliminating *D. agamarum* from lizard collections thus poses a difficult challenge and should include treating all clinically infected animals and thorough environmental decontamination (Hellebuyck et al., 2011). Since bearded dragons represent a major reservoir of infection with *D. agamarum* for dab lizards, we advise against housing these species together. Elimination of *D. agamarum* from infected animals using long term antimicrobial treatment has indeed been described (Hellebuyck et al., 2009b). The only preventive measure at present to avoid *D. agamarum* associated disease in captive lizards consists of quarantine and entry control of newly acquired animals (Pasmans et al., 2008).

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Table 1: Strains of *Devriesea agamarum* used in this study, their origin, year of isolation and AFLP type. Isolates obtained from diseased animals are shaded. A collection is defined as all lizards belonging to the same owner and kept at the same locality.

Figure 1. Dendrogram of the obtained AFLP fragments of *Devriesea agamarum* isolates. Cluster analysis was performed with UPGMA using the DICE algorithm and a tolerance and optimization level of 1.06%. The strains were classified in the same AFLP type if the relatedness was higher than 70%.

Figure 2. Three dimensional plot of the multidimensional scaling analysis of *Devriesea agamarum* isolates marked according to disease status (a) and genus (b) of the host. Black dots represent diseased animals and white dots healthy animals in (a). Black dots represent *Uromastyx sp.*, white dots *Pogona sp.* and grey dots the other lizard species in (b).