

## First Report of *Micrococcus luteus* in the Buccal Cavity of Bearded Dragon (*Pogona vitticeps*) in Namibia

Paidamoyo N. Mataranyika  and Percy M. Chimwamurombe\* 

Natural and Applied Sciences, Namibia University of Science and Technology, Namibia, P. Bag 13388, Windhoek, Namibia.

### Abstract

Central bearded dragons, *Pogona vitticeps*, are ectothermic heliotherms native to dry and arid regions and are often kept as exotic pets in colder less arid countries. They do however, often fall sick resulting in sudden death. This is now known to be caused by bacterial infections such as *Listeria monocytogenes* and *Porphyromonas pogonae* sp. This study therefore, aimed to identify microorganisms present in the buccal cavity of the central bearded dragon (*P. vitticeps*) in Namibia. In order to achieve this, oral swabs were taken from a bearded dragon found in a garden in Windhoek, Namibia and grown in nutrient agar. Colony morphology was described before Gram's staining to differentiate the bacteria. The automated VITEK®2 system was used to identify the bacteria. On nutrient agar, opaque yellow circular colonies were observed after 24hrs incubation at 37°C. A gram-positive coccus shaped bacteria was observed. VITEK®2 analysis identified the bacteria as *Micrococcus luteus*. There has not been a study on the microbiota of the buccal cavity of the central bearded dragon in Namibia.

**Keywords:** *Pogona vitticeps*; *Micrococcus luteus*, buccal microbiota; invasive diseases.

\*Correspondence: pchimwamurombe@nust.na

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## INTRODUCTION

Central bearded dragons, *Pogona vitticeps*, are ectothermic heliotherms native to dry and arid regions. They are largely herbivorous but may consume insects. When kept as pets, bearded dragons require an optimum diet and living conditions. These include regular water supply and calcium fortified insects<sup>1,2</sup>. Despite optimum conditions, bearded dragons have been known to suffer from anorexia, lethargy and weight loss with symptoms including dehydration and limited reaction<sup>3</sup>. When untreated these cases usually lead to death however, sudden death has occurred in some cases soon after the emergence of signs and symptoms of ill health<sup>4</sup>. Tests done during illness or after death have often revealed gastrointestinal bacterial infections. *Porphyromonas pogonae* sp. (*Porphyromonas* spp sometimes known to infect humans as well) and *Listeria monocytogenes* are some of the common bacterial species found to infect bearded dragons (Fig. 1)<sup>2,5</sup>.

There is also a growing concern in the increase in fungal infections among reptiles. Bearded dragons are most likely to be infected by opportunistic fungi, however dermatomycoses have been noted to be on the rise<sup>6</sup>. On the other hand, adenoviral infections are relatively common among reptiles with the greatest number of cases being reported in bearded dragons<sup>7</sup>. Though the relationship between the bearded dragon and microorganisms is evident not much literature is present on bacterial relations particularly from the oral cavity. To the best of our knowledge, no study has been done to evaluate the oral microbiota of bearded dragons. Therefore, this study aimed



**Fig. 1.** Central Bearded Dragon (*Pogona vitticeps*)

to identify buccal cavity bacteria of the central bearded dragon.

## MATERIALS AND METHODS

### Bacterial Isolation

A central bearded dragon (Fig. 1) found in a garden in Windhoek, Namibia was safely captured and transported in a brown bag to the Namibia University of Science and Technology Natural and Applied Sciences laboratory following the institutional rules for the use of animals in research. Oral swabs were taken from the roof, cheek and tongue oral cavity of the bearded dragon. Swabs were immediately inoculated onto nutrient agar plates and incubated at 37°C for 24 hours. The bearded dragon was subsequently released back into the garden. Six selected colonies were sub-cultured onto nutrient agar following a modified description by Kikillus, *et al.*<sup>8</sup>.

### Identification

Descriptive colony morphology<sup>5</sup> was done before differential staining using Gram's staining<sup>9</sup>. In order to identify the isolated bacteria, automated biochemical tests were done using VITEK<sup>®</sup>2 (GP) cards were used depending on the stain result.

### VITEK<sup>®</sup>2 Automated Analysis

Isolated bacteria samples were re-cultured on nutrient agar plates at 37°C for 24hrs before VITEK analysis. Thereafter, a few colonies from each sample were suspended in 0.45% saline solution. An optic density between 0.55 and 0.63 for analysis was preferred. The automated VITEK<sup>®</sup>2 system performed 43 biochemical tests (Table 1) over a period of 10hrs using gram-positive VITEK<sup>®</sup>2 cards.

## RESULTS AND DISCUSSION

The study aimed to identify microorganisms present in the buccal cavity of *P. vitticeps*. The selected colony was observed to be yellow with opaque circular colonies. The colonies had entire edges and raised elevations. Gram-staining showed the cells to be gram positive and coccus shaped, with a few tetrads clustered cells. VITEK<sup>®</sup>2 GP analysis identified the bacteria as *Micrococcus luteus*. Figure 2 shows the colonies on nutrient agar while Table 1 shows a summary of the results.

*Micrococcus luteus* is a coccus shaped gram-positive bacteria. It is an aerobic

**Table 1.** Results summary for *M. luteus*

ID	Morphology	Gram stain	Shape	VITEK result			
G4.2	Yellow, opaque circular colonies with raised elevations and entire edges.	Positive	Coccus	Micrococcus luteus			
Biochemical tests							
Reactions	Result	Acid Production	Result	Growth in 6.5% NaCl	Result	Antibiotic resistance	Result
Phosphati-dylinositol phospholipase C		Amygdalin		Single test	-	Polymyxin B	+
Arginine dihydrolase (two tests)	+	Xylose	+			Optochin	+
$\beta$ -galactosidase	+	$\alpha$ -cyclodextrin	+			Novobiocin	+
$\alpha$ -glucosidase	+	Sorbitol	-			O129	-
Alanine-phenylalanine-proline arylamidase	-	Galactose				Bacitracin	-
L-aspartic acid		Ribose	+				
Aryl-amidase		Lactate					
$\beta$ -galactosidase	+	Lactose	+				
$\alpha$ -mannosidase		N-acetylglucosamine	+				
Alkaline phosphatase		Maltose	+				
L-leucine aryl-amidase		Mannitol	+				
Proline arylamidase		Mannose	+				
$\beta$ -glucuronidase (two tests)		Methyl- $\beta$ -D-glucopyranoside	-				
$\alpha$ -galactosidase	-	Pullulan	-				
L-pyroglutamic acid arylamidase		Raffinose	+				
Alanine arylamidase		Salicin	+				
Tyrosine arylamidase		Sucrose	+				
<i>Andurease</i>		<i>Trehalose</i>	+				

Table 1 also indicates the biochemical test results [10–12].

exopolysaccharide producing bacteria that may be found on the skin, mouth and sometimes in the throat of mammals<sup>13,14</sup>. Oral microbial flora of reptiles includes bacteria from *Proteus*, *Porphyromonas*, *Micrococcus*, *Salmonella* and *Staphylococcus* genera<sup>5,8,15</sup>. Variations, however, may be present among bearded dragons depending on the diet based on whether they are kept as pets (captive) or in the wild<sup>16</sup>. As such, the oral cavity of snakes and other reptiles alike are more often than not infected by aerobic bacteria<sup>17</sup>.

Described species of *Micrococcus* are often responsible for invasive diseases such as

pneumonia, peritonitis and endocarditis among others<sup>18</sup>. *M. luteus* has been isolated from the surface of human skin, water and soil<sup>19</sup>. It has been noted to be an opportunistic bacterium and immunocompromised patients pose the greatest risk of infection<sup>20,21</sup>. Treatment of *M. luteus* infection typically involves the use of antibiotics such as penicillin, vancomycin and gentamicin<sup>22</sup>. Cefoxitin has also been successfully used to treat peritonitis as a result of *M. luteus* infection<sup>23</sup>. The pathogenicity of *M. luteus* is not only of concern to humans. Swabs were collected from crocodiles which were dying suddenly on a crocodile farm.



**Fig. 2.** *Micrococcus luteus* on nutrient agar

Strains including those of *M. luteus* isolated from the crocodiles dying suddenly were identified as being pathogenic. Though another bacterium (*Edwardsiella tarda*) caused death, the presence of *M. luteus* and other species contributed to ill health<sup>24</sup>.

This is of particular concern as domestic reptiles kept as pets harbor pathogenic bacteria which may be transferred to humans. Bacteria from the *Salmonella* species, though intestinal, are the most common pathogens associated with captive reptiles. This runs the risk of infecting other pets within the same household<sup>15</sup>. Bacterial pathogens in the oral cavity of bearded dragons becomes of great concern in the event that a bite occurs. Lizard bites rarely occur as there are limited human-lizard interactions. However, bacterial infections from lizard bites can occur with one such case being fatal as a result of septicemia<sup>25</sup>. The same concern is raised in snakes as venom is not the only challenge presented by venomous snakes. As such bacterial infections are often reported following snake bite incidents in cases where the patient survives the bite<sup>16,17</sup>.

## CONCLUSION

Central bearded dragons harbor aerobic bacteria in their oral cavity, some pathogenic. In this study *M. luteus* was identified in the buccal cavity of *P. vitticeps*. Currently, there are no reported cases on bearded dragon bites on humans. However, they are often kept as pets with close interactions with humans therefore, caution should be exercised when handling them, cleaning their cages and feeding them to prevent the possibility of contracting *M. luteus*. Future work with regards to the *P. vitticeps* could include identifying more microorganisms from the buccal

cavity and gut with special attention to possible pathogenicity.

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## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR'S CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This article does contain studies with animals performed by the authors in a humane way following approved guide lines by our institutional Ethics committee.

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