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## ORAL MICROBIOTA OF PATAGONIAN LIZARDS OF GENUS *DIPLOLAEMUS* (LEIOSAURIDAE): FABLE TO FACTS

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**R E S U M E N.** — Existe la creencia popular de que los lagartos del género *Diplolaemus* (Leiosauridae) de Patagonia, Argentina, son capaces de conferir mordeduras venenosas que pueden causar inflamación e incluso la muerte de animales de granja o salvajes. No obstante, no existe evidencia de la existencia de glándulas de veneno en estos lagartos. La inflamación y absceso provocado como resultado de las heridas de mordedura podría ser causado por agentes infecciosos. Este es el primer estudio acerca de la microbiota oral de los lagartos del género *Diplolaemus* de Argentina. Para este trabajo se analizó un espécimen de *D. darwini* y tres individuos de *Diplolaemus* «forma alto patagónica». Las muestras de hisopados de boca de los lagartos fueron incubados aeróbica y anaeróticamente, algunos de ellos fueron subcultivados en agar de sangre ovina al 5% e incubados nuevamente. Todas las colonias representativas observadas fueron subcultivadas para su purificación y sujetas a tests bioquímicos. La bacteria *Clostridium perfringens* fue determinada por medio de la técnica de reacción en cadena de la Polimerasa. Se identificaron cuatro especies de bacterias: *Staphylococcus warneri*, *Clostridium bifermentans*, *Clostridium perfringens* y *Stomatococcus muscilaginosus*. Estas especies son conocidas como agentes etiológicos de numerosas infecciones de humanos y animales. Si bien estas bacterias representan sólo un pequeño número de las posibles bacterias aisladas de la cavidad oral de *Diplolaemus*, están indicando la presencia de patógenos humanos y animales.

Palabras clave: *Diplolaemus*, lagartijas, microbiología, enfermedades.

**A B S T R A C T.** — Lizards of genus *Diplolaemus* (Leiosauridae) from Patagonia, Argentina are believed to have «poisonous» bites that can cause inflammation and death of farm and wild animals. However there is no evidence of poisonous glands in these lizards. It is proposed that the inflammation and abscesses resulting from these bite wounds are caused by infectious agent(s). This is the first study of the oral microbiota of *Diplolaemus* lizards from Argentina. One specimen of *D. darwini* and three individuals of *Diplolaemus* «forma alto patagónica» were used for the present work. Swabs were taken from the mouths of the lizards, subcultured onto 5% sheep blood agar plates, incubated at 37°C for 48 hours aerobically and anaerobically. All representative colonies observed were subcultured for purity and subjected to conventional biochemical tests. Isolates identified as *Clostridium perfringens* were typed by a polymerase chain reaction technique. Four species of bacteria were identified as *Staphylococcus warneri*, *Clostridium perfringens*, *Clostridium bifermentans* and *Stomatococcus muscilaginosus*. These species are known etiologic agents of a number of human and animal infections. While these bacteria represent only a small number of possible isolates from the oral cavity of *Diplolaemus* species nevertheless it indicates that human and animal pathogens are present in their oral cavity.

Key words: *Diplolaemus*, lizards, microbiology, diseases.

The *Diplolaemus* lizards (matuastos) are distributed throughout Patagonia, Argentina and Chile's southernmost region. The genus *Diplolaemus* (Leiosauridae) consists of three species (*Diplolaemus darwini*, *Diplolaemus bibroni* and *Diplolaemus leopardinus*) and two forms, not yet recognized as species, which are considered as hybrids of *D. darwini* and *D. bibroni*. These two forms are called *Diplolaemus* «forma alto-patagónica» and *Diplolaemus* «forma sudmendocina», respectively (Cei, 1986, 1993). They are very powerful and aggressive lizards with a large head and strong wide jaws. They are up to 12 cm snout-vent length (Cei, 1986).

At present, there is a general consensus that the way local people perceive and use their natural environment plays an important role in the conservation of resources and its consequent sustainable development. The Convention of Biological Diversity recognizes that biodiversity will not be conserved without understanding how humans interact with the natural environment (Ladio and Lozada, 2000). Individuals have the strongest affinities with family members and select the cultural variants provided by them thus generating a vertical transmission from parents to children. Under these circumstances, most ecological constraints are intimately associated with social factors, both contributing to an adaptive solution (Ladio and Lozada, 2000). There are historical records that show there was a belief at the end of XIX century among local people in Patagonia that «matuastos» were poisonous (Musters, 1997) and this belief still continues to the present day. Credence for this is that the lizards' bite produce abscesses with resulting massive inflammation, sometimes resulting in the death of dogs and sheep (Musters, 1997). However, no poisonous glands have been found in any of the «matuastos» (Cei, 1986; 1993). Therefore to test the hypothesis that the abscesses and inflammation are

caused by infectious agent(s) present in their mouths and transmitted to their prey by the bite of these lizards we performed the present study.

Also, the oral microbial flora can be involved in serious infections of several captive lizards such as agamids, chameleons, skinks and iguanas, producing periodontal disease which is an important cause of disease and death of these animals (McCracken and Birch, 1994; McCracken *et al.*, 1999). However, information about the oral microbial flora of lizards in their nature environment is scanty (Ciofi, 1999). This preliminary study was therefore designed to determine if the lizards of the genus *Diplolaemus* carry pathogenic bacteria in their oral cavity.

One specimen of *D. darwini* and three individuals of *Diplolaemus* «forma alto patagonica» were captured in the field and transported alive to the laboratory. The lizards were clinically examined (including the mouth) and a sample was taken from the mouth of each lizard using a swab moistened with sterile saline. The swabs were inoculated onto 5% sheep blood agar plates, and incubated aerobically and anaerobically. Swabs were also inoculated into cooked meat broth (CMB), incubated anaerobically and then subcultured onto 5% sheep blood agar plates and incubated again. All incubations were carried out at 37°C for 72 h and all representative colonies observed on the agar plates were subcultured for purity and subjected to conventional biochemical tests as previously described, including carbohydrate fermentation, hemolysis of sheep erythrocytes and lecithinase activity (Sterne and Batty, 1978). Isolates were identified as *Clostridium perfringens* by a polymerase chain reaction (PCR) technique to amplify the alpha, beta, epsilon and iota toxin genes of this microorganism, as previously reported (Uzal *et al.*, 1997).

All the lizards used in this study were clinically normal when swabs were ob-

tained from their mouths. No gross abnormalities were observed in the mouth of any of the animals investigated.

*Staphylococcus warneri* was isolated from *D. darwini*. No other aerobic or anaerobic microorganisms were cultured from this individual. *Clostridium bifermentans*, *C. perfringens* and *Stomatococcus mucilaginosus* were isolated from the three individuals of *Diplolaemus* «forma alto patagonica». The *C. perfringens* isolated was typed as type A by PCR.

Bacteria isolated from the oral cavity of *Diplolaemus* in this study are etiological agents of animal and human diseases. *S. warneri* has been associated to a number of human and animal infections, including endocarditis (Fabbri *et al.*, 1990), septicemia, osteomyelitis (Bryan *et al.*, 1987) and urinary tract infections (Leighton and Little, 1986). This microorganism is also invasive in cancer patients (Eng *et al.*, 1982) and elicits the release of a factor that strongly activates the human immunodeficiency virus type 1 (HIV1) influencing the progression of HIV1 infection (Klebanoff *et al.*, 1994). *S. mucilaginosus* is a normal inhabitant of the human oral cavity, but it can also produce septic shock, meningitis, acute respiratory distress syndrome, endocarditis and catheter-related infections in humans (Ascher *et al.*, 1991; Gruson *et al.*, 1998). *C. bifermentans* produces necrotizing pneumonia, empyema (Jonsson *et al.*, 1983), endocarditis (Kolander *et al.*, 1989), liver and abdominal abscess (Nachman *et al.*, 1989; Rechtman and Nadler, 1991), septic arthritis (Nolan *et al.*, 1972) and osteomyelitis in human beings (Scanlan *et al.*, 1994). *C. perfringens* type A is commonly found in the soil and intestinal tract of humans and other animals (Stratonova, 1969; Uzal and Marcellino, 2002); it produces gas gangrene and food poisoning in humans (Chakrabarty and Boro, 1981) and it is also responsible for enterotoxaemia and malignant oedema in several animal species (McClane, 1996). Although this microorgan-

ism is ubiquitous and it can be found in soil and feces of clinically normal animals and human beings, is considered an opportunistic pathogen that can proliferate and produce disease under appropriate conditions. The detection of the alpha toxin gene in the isolated strain is an indicator that this was a potential pathogen that carries a virulence factor in its genome.

The oral cavity of the Komodo saurian lizard, *Varanus komodoensis*, supports a large numbers of bacteria and it is thought that bite wounds inflicted by this lizard can result in serious infection and septicemia that may eventually kill the prey (Ciofi, 1999). In one study of the oral cavity of *V. komodoensis*, approximately 50 different bacterial species were identified and at least seven of these were highly pathogenic (Arnett, 1998). Our results, albeit from a reduced number of animals and isolates, show that pathogenic bacteria are found in the mouth of the *Diplolaemus* lizards, as well. Nevertheless, the number of bacteria species found in *Diplolaemus* lizards were much smaller than that found in the Komodo dragon. Differences in the diet (the Komodo dragon is carnivorous while the *Diplolaemus* lizards are insectivorous) and habitat (Komodo Island is a tropical environment with a high rainfall rate, while most of Patagonia is a cold semi-desert) are probably responsible for the difference in oral microflora between these reptiles, among others. The present results indicate that, at least potentially, the bites of the matuastos may result in transmission of infectious agent(s) causing serious infection. More research is currently under way to further test this hypothesis.

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