SHORT COMMUNICATIONS

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Cutaneous Bacteria of Confiscated *Telmatobius culeus* in Lima, Peru

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ABSTRACT: The Lake Titicaca frog is endangered due to threats such as water pollution, introduced species, and overharversting for markets, where people consume them as frog juice. This study, conducted June to November 2012, aimed to determine the bacteria microflora living on the skin of frogs confiscated from the La Parada market, Lima, Peru, and housed individually in the Laboratory of Wildlife at the Faculty of Veterinary Medicine and Zootechnic of the Universidad Peruana Cayetano Heredia in Lima, Peru. Samples collected with sterile swabs and cultured on blood, tryptic soy, and MacConkey agars were investigated using commercially available test kits, to investigate the commonly encountered bacterial and potentially zoonotic microorganisms associated with their consumption. We found three species of zoonotic concern in the genus *Vibro*: *Vibrio alginolyticus*, *Vibro cholerae*, and *Vibro fluvialis*. Other Gram-negative species cultured included two different colonies of *Aeromonas hydrophila*, or *Aeromonas caviae* or *Aeromonas sobria*; *Pseudomona luteola*; one example of *Weeksella virosa* or *Empedobacter brevis*; and *Citrobacter freundii*. Gram-positive bacteria detected were *Staphylococcus* spp., *Micrococcus* spp., and *Erysipelothrix rhusiopathiae*. We recommend against the consumption of this frog due to the pathogens it may carry that could cause serious illness among consumers and in vendors who handle animals.

Key words: Bacteria, frog's juice, Lake Titicaca frog, skin, *Telmatobius culeus*.

The Lake Titicaca frog (*Telmatobius culeus*) is classified as endangered due to threats from water pollution, predation by introduced species, and poaching (IUCN SSC Amphibian Specialist Group 2020). The species is endemic to Lake Titicaca, which straddles the border between Peru and Bolivia (IUCN SSC Amphibian Specialist Group 2020). On the Peruvian side of the lake, people harvest frogs and transport them to markets in Lima, Cusco, and Arequipa, where they are consumed as frog juice. Vendors claim that the frogs have medicinal properties that are useful in treating various diseases and in increasing mental lucidity and virility (Angulo 2008). In the markets, vendors keep frogs in plastic buckets filled with water (Catenazzi et al. 2010) in unhygienic conditions, such that many animals die or become ill prior to use (R.E. pers. obs.). These unhygienic conditions can pose a health risk for consumers who ingest them raw (Villena et al. 2008; Serrano-Martínez et al. 2017). Vendors kill frogs with a sharp blow to the head and then remove the skin and viscera (Catenazzi et al. 2010) before blending them with other ingredients. Although Peruvian law prohibits use of the species, illegal trade and sales of the extract continue (Ministerio de Agricultura del Perú 2014).

Between June and November 2012, the Forest and Wildlife Technical Administration of Lima seized 14 frogs at the La Parada market, Lima, Peru, and transferred them to the Laboratory of Wildlife of the Faculty of Veterinary Medicine and Zootechnic at the Universidad Peruana Cayetano Heredia. We maintained the animals in glass aquariums, one for each animal, with dechlorinated tap water. We collected samples within 2–4 wk of receiving the frogs. The Institutional Ethics Committee for the Use of Animals of the Universidad Peruana Cayetano Heredia approved this study prior to its implementation.

We removed frogs from aquariums using a small pond net for sampling and processed them in the Laboratory of Microbiology of the same university. We used new sterile and disposable gloves to handle each individual. We placed animals in sterile hermetic ziplock bags (previously irradiated with ultraviolet light for 30 min) containing 20 mL of sterile distilled water for 30 s. We repeated this procedure three times using new, sterile equipment for each rinse. After the three rinses, we used two different swabs to sample each individual (one on the abdomen and one on the dorsum). We extended a swab dorsally from just posterior to the head down to the pelvic region. We then extended a swab ventrally between the forelimbs, posterior to the pelvic area. We placed the swabs in a sterile plastic container (one for each swab) with 20 mL of distilled water (previously irradiated with ultraviolet light for 30 min). We vortexed the swabs in their containers and then removed and discarded the swab (Culp et al. 2007). Subsequently, we immediately divided 300 μL of the liquid sample into three equal amounts that we spread onto three different agar plates using a Drigalski spatula: 100 μL on blood agar, 100 μL on tryptic soy agar, and 100 μL on MacConkey agar. We used the same procedure for each of the dorsal and ventral samples, resulting in a total of six agar plates for each frog. We incubated the plates at room temperature for 6 d and stored those with bacterial growth at a cool temperature (4 C±2 C) for later identification.

For the identification of colonies of *Enterobacteriaceae* and other nonfastidious Gram-negative bacteria and identification of Gram-negative non-*Enterobacteriaceae*, we used API-20 E and API-20 NE commercial kits following the manufacturer's specifications (API® bioMérieux, Inc., Durham, North Carolina, USA). The use of API-20 is an acceptable method for the identification of the more commonly occurring members of the family *Vibrionaceae* (Overman et al. 1985).

In six of the 14 frogs, we found the same species of bacteria in the dorsum and abdomen. In three animals, no bacterial growth was obtained.

We found the following microorganisms on Lake Titicaca frogs: from the abdomen, two different colonies of *Aeromonas hydrophila* or *Aeromonas caviae* or *Aeromonas sobria* (the API system used does not distinguish between these species); *Pseudomona luteola*; *Vibrio alginolyticus*; *Vibrio cholerae*; *Vibrio fluvialis*; and *Weeksella virosa* or *Empedobacter brevis* (again, the API system used does not distinguish between these); and from the dorsum, *V. cholerae* and *Citrobacter freundii*. After identifying *V. cholerae*, we placed the colony in thiosulfate citrate bile sucrose agar to grow and validate our results.

We found a predominance of *Aeromonas* spp. and *Vibrio* spp. on Lake Titicaca frogs, including *V. cholerae*, which can cause diarrhea, dehydration, and death in people (Fernandez and Alonso 2009). *Vibrio alginolyticus* usually occurs in marine environments and causes superficial infections, especially on open wounds, media, and external otitis (Reilly et al. 2011), or even necrotizing fasciitis in immunosuppressed individuals (Gomez et al. 2003). The cosmopolitan species *V. fluvialis* occurs in contaminated water worldwide, mostly affecting developing countries (Igbinosa and Okoh 2010); it can cause bloody diarrhea and wound infections, possibly leading to septicemia in immunosuppressed people.

We also identified *Weeksella virosa*, which usually occurs in the urogenital tract of women (Martinez and Ovalle 2011) or in the oral and nasal mucosa of dogs and cats (Nuñez-Tamayo et al. 2003). This opportunistic bacterium lives in water, soil, and wastewater (Hassl et al. 2002), and it can cause sepsis if it enters an open wound (Nuñez-Tamayo et al. 2003). It has also been isolated from other anurans, such as *Hyla crepitans*, causing epidermal cysts (Hassl et al. 2002).

Staphylococcus spp. occur widely worldwide as part of the normal flora of the skin of animals and people, most often found in moist areas of the body, such as the armpits and perineum (Quinn et al. 2011).

Micrococcus spp. also inhabit the skin of a wide variety of mammals, including humans and other primates, rodents (rats and squirrels), raccoons (*Procyon lotor*), horses, and dogs, as well as saltwater and freshwater fish. *Micrococcus* in some fish may result from overcrowding in captive populations or pollution of water by fishing nets (Kocur et al. 2006).

Distributed widely worldwide, *Erysipelothrix rhusiopathiae* comprises part of the flora of various species, such as pigs, sheep, horses, saltwater fish, reptiles, cetaceans, crustaceans, birds, and others. It can live and grow for long periods of time in the mucosal lining of fish. It negatively impacts livestock producers, and in humans, it can cause cutaneous lesions or generalized septicemia associated with endocarditis (Boerner et al. 2004).

Our methods enabled us to determine the genera for the pathogens we found, but not always the species. We recommend conducting additional research in Lake Titicaca to sample both the frogs and the water and using PCR techniques to determine the species and genus of all pathogens found. Our results lead us to recommend against the consumption of Lake Titicaca frogs, due to the potential presence of bacteria pathogenic for humans, which could cause serious illness among consumers or vendors who handle the animals.

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LITERATURE CITED

Angulo A. 2008. Consumption of Andean frogs of the genus *Telmatobius* in Cusco, Peru: Recommendations for their conservation. *TRAFFIC Bulletin* 21: 95–97.

Boerner L, Nevis K, Hinckley L, Weber E, Frasca S. 2004. *Erysipelothrix septicemia* in a little blue penguin (*Eudyptula minor*). *J Vet Diagn Invest* 16: 145–149.

Catenazzi A, Vredenburg V, Lehr E. 2010. *Batrachochytrium dendrobatidis* in the live frog trade of Telmatobius (Anura: Ceratophryidae) in the tropical Andes. *Dis Aquat Organ* 92:187–192.

Culp C, Falkinham J III, Belden L. 2007. Identification of the natural bacterial microflora on the skin of eastern newts, bullfrogs' tadpoles and a redback salamanders. *Herpetologica* 63:66–71.

Fernandez S, Alonso G. 2009. Cólera y *Vibrio cholerae*. *Rev Inst Nac Hig* "*Rafael Rangel*" 2:51.

Gomez J, Fajardo R, Patiño J, Arias C. 2003. Necrotizing fasciitis due to *Vibrio alginolyticus* in an immunocompetent patient. *J Clin Microbiol* 41:3427–3429.

Hassl A, Url A, Rebel-Bauder B. 2002. *Weeksella virosa* colonised epidermal cysts in *Hyla crepitans*. *Herpetozoa* 14:127–131.

Igbinosa E, Okoh A. 2010. *Vibrio fluvialis*: An unusual enteric pathogen of increasing public health concern. *Int J Environ Res Public Health* 7:3628–3643.

IUCN (International Union for Conservation of Nature) SSC (Species Survival Commission) Amphibian Specialist Group. 2020. *Telmatobius culeus*. In: *The IUCN red list of threatened species,* 2020 (errata version):e.T57334A178948447. https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T57334A178948447.en. Accessed January 2021.

Kocur M, Kloos W, Schleifer K-H. 2006. The genus *Micrococcus*. In: *The prokaryotes*, 3rd Ed., Dworkin M, Falkow S, Rosenberg, Schleifer K-H, Stackebrandt E, editors. Springer, New York, New York, pp. 961–971.

Martinez M, Ovalle A. 2011. *Weeksella virosa*. *Rev Chil Infect* 28:429–430.

Ministerio de Agricultura del Perú. 2014. *Decreto Supremo que aprueba la actualización de lista de clasificación y categorización de las especies amenazadas de fauna Silvestre legalmente protegidas. 2014*. http://minagri.gob.pe/portal/download/pdf/marcolegal/normaslegales/decretosupremos/2014/ds04-2014-minagri.pdf. Accessed December 2020.

Núñez-Tamayo E, Chamero-Melgarejo S, Ramírez-Martínez N. 2003. Sepsis por *Weeksella virosa* en herida por mordedura animal. Reporte de un caso. *MediSur* 1:136–139.

Overman T, Kessler J, Seabolt J. 1985. Comparison of API 20E, API Rapid E, and API Rapid NFT for identification of members of the family Vibrionaceae. *J Clin Microbiol* 22:778–781.

Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S, Hartigan PJ. 2011. *Staphylococcus* species. In: *Veterinary microbiology and microbial disease*. 2nd Ed. Wiley-Blackwell, Oxford, UK, pp. 179–187.

Reilly GD, Reilly CA, Smith EG, Baker-Austin C. 2011. *Vibrio alginolyticus*–associated wound infection acquired in British waters, Guernsey, July 2011. *Euro Surveill* 16:19994.

Serrano-Martínez E, Quispe M, Plascencia L, Hinostroza E. 2017. Parásitos zoonóticos en ranas destinadas a la elaboración de bebidas para el consume humano en Lima, Perú. *Rev Inv Vet Perú* 28:642–649.

Villena M, Morales S, Enciso M. 2008. Microbiological findings in anurans of human consumption in Peru: Risk to public health. In: *Proceedings of the Association of Reptilian and Amphibian Veterinarians*, ARAV Fifteenth Annual Conference, Los Angeles, California, 12–15 October, p. 52.

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