

REPORT OF MICROORGANISMS OF SANITARY IMPORTANCE AND WITH ZOONOTIC POTENTIAL (*MUCOR* SPP. AND *AEROMONAS* SPP.) IN BULLFROG (*LITHOBATES CATESBEIANUS*) CULTURE IN THE CENTRAL AREA OF MEXICO.

Reyes-Rodríguez NE¹, Aguilar-Mendoza LF², García-Reyna PB¹, Gómez-de-Anda RF¹, Zepeda-Velázquez AP¹, Acosta-Pérez VJ¹, Vega-Sánchez V¹

¹Área Académica de Medicina Veterinaria y Zootecnia, Instituto de Ciencias Agropecuarias, 43600 Tulancingo de Bravo, Hgo. México.

²Deceased, Vega-Sánchez Vicente. Área Académica de Medicina Veterinaria y Zootecnia, Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo. 43600 Tulancingo de Bravo, Hgo. México

*Corresponding author: Vega-Sánchez Vicente, E-mail: vicente_vega11156@uaeh.edu.mx

ABSTRACT

Bullfrog production is an important source of protein and by-products obtained from the cultivation of different frog species, including the bullfrog (*Lithobates catesbeianus*). However, the species is considered invasive, so its ecological role is of high impact with respect to the pathogens present in the crop. Therefore, the objective of the present study was to report the occurrence of microorganisms of health importance in farmed frogs from the State of Hidalgo. 16 samples were processed by conventional methods for the isolation of *Mucor* spp. and *Aeromonas* spp.. The samples showed the presence of *Mucor* spp. with a 25% [IC 95% 24.78-25.21%] of prevalence, fungi were isolated from ulcerative skin lesions in the 4 *Mucor* spp. positive frogs., while 75% [IC 95% 74.78-75.21%] for *Aeromonas* spp. isolates were obtained as an opportunistic secondary infection. The findings indicate a concomitant infection of pathogens affecting the *L. catesbeianus* population that have human pathogenic potential. Therefore, it's monitoring and management throughout the production chain is imperative to obtain quality and safe products.

KEYWORDS: fungal disease, opportunistic infection, gastrointestinal infection, ulcers and dermatitis.

Introduction

INTRODUCTION:

Bullfrog production is a type of aquaculture based on the production of species of the family Ranidae. Among them, the bullfrog or *Lithobates catesbeianus* stands out as the most relevant worldwide. Nearly 40 countries participate in the production of this species, including Mexico, which occupies the 5th place in world production (García Ayala et al., 2020). *L. catesbeianus* is an excellent organism for production due to biological attributes such as muscle mass development and short production times ranging from three to four months to reach commercial size. It is also important to highlight the obtaining of by-products from its skin, liver and intestines (Borges et al., 2012; Montel Mendoza et al., 2012). In Mexico, its production is supported by a cultivation area of 60 hectares corresponding to 35 registered farms, which produce *L. catesbeianus* in systems ranging from extensive in water reservoirs to intensive using pond systems (Islas-ojeda et al., 2021). Unfortunately, *L. catesbeianus* is considered an exotic species in Mexico with a high invasive potential, which indicates that the species plays an important ecological role (García Ayala et al., 2020; Ibáñez et al., 2011). In this situation, the diseases carried by these frogs are highly relevant for both aquaculture and human health (Urbina et al., 2018). In aquaculture health, the production of amphibians such as *L. catesbeianus* is of particular interest in the monitoring of Ranavirus and the fungi *Batrachochytrium dendrobatidis* and *B. salamandrivorans* (DOF, 2018). Of these pathogens, *L. catesbeianus* and *B. dendrobatidis* are the most prevalent and have been previously described as an infection that generates important economic losses in the sector (Gervasi et al., 2013; Urbina et al., 2018). Likewise, *Achlya* spp. infection has been reported in *L. catesbeianus* (Ruthig & Provost-Javier, 2012). Although in general the most common fungal infections in amphibians are fungal dermatopathies caused by *Basidiobolus ranarum*, *Mucor* spp., *Fusarium* spp. and aquatic molds such as *Saprolegnia* spp. (Pessier & Mendelson, 2017). Among these, mucormycosis is a fungal infection of low host specificity, it has been reported in wild anurans (Berger & Speare, 1997), in platypuses (Connolly, 2015), and even in humans, where genera such as *Rhizopus* spp., *Lichtheimia* spp. and *Mucor* spp., are causative agents of mucormycosis, mainly in immunocompromised patients (Alqarihi et al., 2023). On the other hand, it should be noted that fungal diseases in bullfrogs can give rise to secondary opportunistic infections, including bacterial infections (Paetow et al., 2013). In addition, the bullfrog has been reported as an important reservoir of bacteria of public health importance, where species of *Aeromonas* spp. stand out with the occurrence of *A. media*, *A. veronii*, *A. caviae* and *A. hydrophila* (Zepeda-Velazquez et al., 2023). This bacterial genus has been reported as an important pathogen in the culture

of aquaculture organisms, for example, in cichlid fish such as *Oreochromis* spp. (Sherif & Kassab, 2023), in the cultivation of crustaceans such as *Litopenaeus vannamei* (Zhou et al., 2019), and of course in frog farming where it acts as a causal agent of red leg disease (Rigney et al., 1978) disease that has also been reported in species such as *Aquarana catesbeiana*, *Rana dybowskii* and *Rana catesbeiana* causing ulcerative skin lesions and important economic losses in aquaculture production (Lin et al., 2024; Wei et al., 2023; Zhao et al., 2024). *Aeromonas* spp. is a bacterial genus that also causes gastrointestinal diseases in human populations, in addition to wound and soft tissue infections and even the development of septicemia (Pessoa et al., 2022). The microorganisms associated with frog farming, together with the invasive potential of the species (*L. catesbeianus*), denote the importance of implementing improvements in the production systems, as well as the continuous monitoring of the sanitary status of the organisms (Urbina et al., 2018). Therefore, the objective of this study is to report pathogens of sanitary and public health interest associated with the culture of *L. catesbeianus*, in order to improve the sanitary management of this species of high relevance in aquaculture.

Materials and methods

Study area

The study was conducted in the production of bullfrogs, the samples were provided by a farm located in the municipality of Tezontepec de Aldama, Hidalgo (Figure 1). It is a municipality with a long tradition in aquaculture production, which can be sustained by the availability of water resources, and where mainly cyprinids are produced (Ibáñez et al., 2011; Ponce-Palafox et al., 2010). The municipality has an average temperature of 15-17°C, an annual rainfall of 500 to 600 mm and a semi-dry temperate climate, while its hydrography corresponds to the Pánuco hydrological basin (Instituto Nacional de Estadística y Geografía (INEGI), 2010).

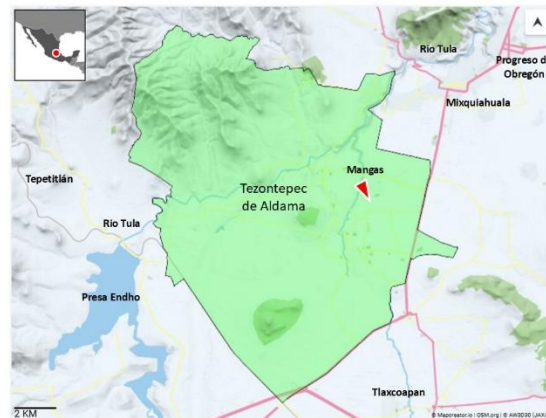


Figure 1. The upper map on the left indicates the position of the State of Hidalgo, corresponding to the center of Mexico. In the enlargement, the municipality of Tezontepec de Aldama is seen in green and with the red arrow, the sampling point in the town of Mangas.

Sample collection

Sixteen specimens of bullfrogs (*L. catebeianus*) were provided for diagnosis, where the inclusion criterion was through the identification of specimens that presented lesions, the frogs came from a fattening production lot where there were 390 specimens in adult stage with a commercial weight between 300-500 g. The specimens provided were deposited in beds of moist sawdust at room temperature (25 ± 2 °C), to avoid physical damage during transport, the frogs were transferred for processing to the Genetics and Genomics Research Laboratory of the Institute of Agricultural Sciences-UAEH.

Euthanasia and necropsy of organisms

The organisms were slaughtered according to the specifications of the current and applicable regulations in Mexico in accordance with NOM-033-SAG/ZOO-2014 (DOF, 2015), where it is specified that euthanasia in amphibians is applied by injecting an overdose (360 mg/mL) of pentobarbital for intracoelomic route. Subsequently, the necropsy of the specimens was developed, with the external visual inspection followed by dissection, the organisms were placed in dorsal decubitus, and the review of muscle tissue was reviewed by cutting with a scalpel from the jaw to the groin. Subsequently, a cut was made through the linea alba for visualization and revision of internal organs such as liver, spleen, lungs, digestive system and kidney (Pessier & Mendelson, 2017). Determination of the fungus *Mucor* spp.

From each of the 16 frogs, 4 fragments of skin associated with lesions suggestive of fungal infection by *Mucor* spp. were aseptically dissected. The fragments were placed and incubated in TSA agar (Trypticasein Soy Agar) added with 300 ppm of Chloramphenicol, at 28°C for 7 days (Christenson et al., 2018), 3 mm² of fungus were then taken from the samples that showed growth and transferred to boxes with Sabouraud agar for incubation at 28°C for 7 days, identification was developed by macroscopic and microscopic evaluation where the isolated

fungus was processed by staining with lactophenol blue for observation by brightfield microscopy (Al-Ameri & Agar-Nasser, 2023; Raposo et al., 2023).

Aeromonas spp. determination

For isolation of *Aeromonas* spp. swab samples were seeded on TSA agar at 25°C for 24 hours. The selected colonies presented size between 4-6 mm and creamy consistency, these colonies were transferred to the selective medium ADA (Ampicillin Dextrin Agar) where the inoculated plates were incubated at 25°C for 24 hours. Subsequently, colonies with morphology suggestive of *Aeromonas* spp. were identified, which are yellow in color, due to their ability to ferment dextrin (Zepeda-Velazquez et al., 2023).

Results

During external inspection, lesions were found in bullfrogs (*Lithobates catesbeianus*) (Figure 2 and 3). Mainly reddening of the skin was identified, mostly ulcers were found on hind haunches. However, some of these had already healed.



Figure 2. Change of skin color. a. Bright red skin on the lateral portion suggesting active hyperemia. b. Hind limbs appear enlarged and bright red in color suggesting edema and active hyperemia. c. Skin of the ventral area and presents bright red color change due to acute inflammation.

Characterization of *Mucor* spp. in frog samples

The fungus *Mucor* spp. was isolated from lesions present in 4 of 16 bullfrogs sampled, with a prevalence of 25% [IC 95% 24.78-25.21%]. The total number of positive samples corresponded to asylum recovered from skin ulcers, where it was also observed that 100% of the frogs with *Mucor* spp. were positive for *Aeromonas* spp. (Table 1), denoting the presence of both microorganisms in 25% [IC 95% 24.78-25.21%] of the bullfrogs under analysis.

Table 1. Characteristics of infection by *Mucor* spp. in the frog samples, as well as the association with *Aeromonas* spp.

Frog sample	Anatomical ubiquity	<i>Mucor</i> spp.	<i>Mucor</i> spp. and <i>Aeromonas</i> spp.
RN-9	Skin ulcer	+	+/+
RN-10	Skin ulcer	+	+/+
RN-11	Skin ulcer	+	+/+
RN-16	Skin ulcer	+	+/+
Total		4/16	4/16
Prevalence		25% [IC 95% 24.78-25.21%]	25% [IC 95% 24.78-25.21%]

Positive samples for *Mucor* spp. showed the growth of the fungus by the development of a whitish cottony mycelium. The stained samples showed structures of the fungus (Figure 3), where it was possible to recognize the presence of hyphae and reproductive structures such as sporangiophores where hyphae and sporangia, spherical

sporangia, as well as sporangia that showed opening and release of spores.

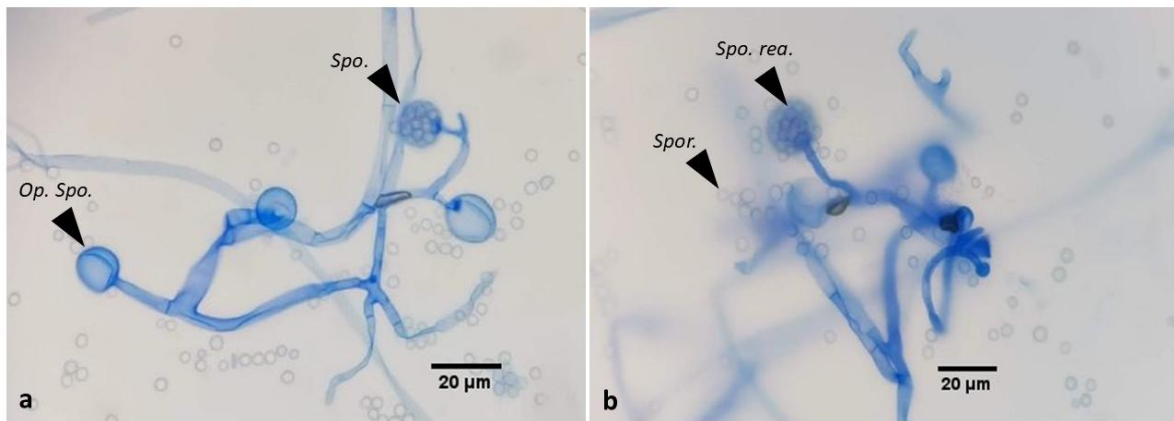


Figure 3. *Mucor* spp. identification; In a we see *Spo.*= Globose sporangium, *Op. Spo.*= Opening of the sporangial wall. In is observed *Spo. rea.*= release of spores and *Spor.*= spores. The structures are indicated with the black arrows.

The four bullfrogs tested positive for the presence of *Mucor* spp. presented lesions (Figure 4) focused in the region of the hind limbs, in general the frogs presented edema and active hyperemia associated with the lesions. On anatomical inspection, skin ulcerations were observed (Figure 4 a, c, d and f) at the level of the phalanges and the humero-tibiofibular joint. Nodular formations were also observed (Figure 4 b and e), in tarsal and carpal regions. Ulcerative dermatitis was observed in chronic condition, where organisms presented different levels of ulcer healing.

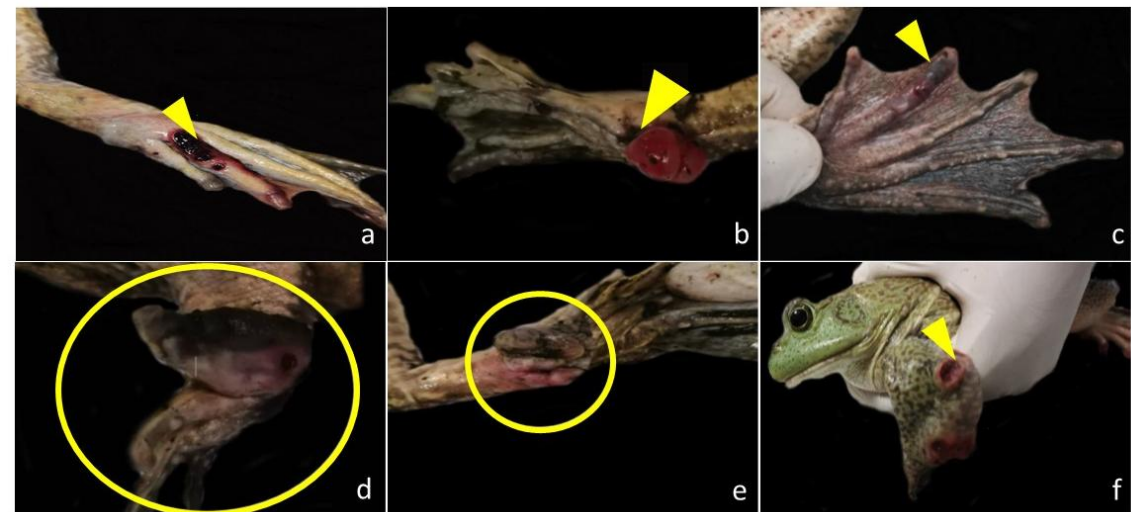


Figure 4. Lesions in *L. catesbeianus* related to *Mucor* spp.. The signology is indicated with yellow marks; In a, severe acute ulcerative dermatitis is observed in forelimb phalanx. In b, acute nodulation is observed in tarsi area. In c, focal acute ulcerative dermatitis is observed in phalanges folds. In d ulcerative dermatitis in forelimb phalanges. In e chronic nodular lesions in carpus and in f severe ulcerative dermatitis in humero-tibiofibular joint.

3.2. *Aeromonas* spp. determination in frog samples

Bullfrog samples evidenced a prevalence of 75% [95% CI 74.78-75.21%] for *Aeromonas* spp. where 12 of 16 frogs were positive, the recovery of isolates was associated with ulcerative lesions in 6 (50%) frogs tested, evidencing its presence as an opportunistic secondary infection. However, 6 frogs (50%) showed *Aeromonas* spp. from sampling of internal organs such as intestines, kidney and liver, the findings are suggestive of the development of infection at the systemic level.

Table 2.- Recovery of *Aromonas* spp. In the frog samples, the anatomical regions related to this pathogen are listed.

Frog sample	Anatomical ubiquity of isolate	<i>Aeromonas</i> spp.
RN-1	Paw injury	+
RN-2	Intestine	+
RN-3	Kidney	+
RN-4	Intestine	+
RN-6	Paw ulcer	+
RN-7	Paw ulcer	+

RN-9	Skin ulcer and intestine	+			
RN-10	Skin ulcer and liver	+			
RN-11	Intestine and liver	+			
RN-12	Haunch injury and liver	+			
RN-13	Skin ulcer	+			
RN-16	Skin ulcer	+			
	Total	12/16			
	Prevalence	75%	[IC	95%	74.78-75.21%]

DISCUSSION

The report of outbreaks of *Mucor* spp. in frog populations is scarce in the specialized literature, although it has been reported as an etiological agent of mucormycosis in amphibians (Pessier & Mendelson, 2017). The prevalence observed in *L. catesbeianus* was 25%. Greater than that reported for *Mucor amphibiorum* in the toad *Bufo marinus*, which from a sample of 3,518 organisms recorded a prevalence of 0.71% (Speare et al., 1994). In contrast, this pathogen has also been reported in frogs from the Perth Zoo in Australia, where frogs of the species *Litoria adelensis* and *Litoria infrafrenata*, presented necrotizing skin affections and mortality of up to 80% caused by *M. amphibiorum* (Crepper et al., 1998). The genus *Mucor* has reports in frog species such as *Litoria caerulea* in Queensland, Australia (Berger & Speare, 1997) and a few reports of affection in wild platypus (*Ornithorhynchus anatinus*) (Connolly, 2015; Obendorf et al., 1993), denoting that *Mucor* spp. and in particular the species *M. amphibiorum* is a pathogen of low host specificity and that they can affect in a wide range of prevalence, in this scenario pathogens infecting more than one host becomes relevant in their transmission and affection among ecological communities (Ruthig & Provost-Javier, 2012). *Mucor* spp. is a pathogen that is found as yeast in the form of “spherules” when skin infection develops in frogs, while hyphae development occurs in culture medium or in the environment when favorable conditions are present, so its culture allows the identification of the fungus (Connolly, 2015). The fungi recovered and isolated in this work, presented a rapid growth with development of whitish mycelium and the recognition of sporangiophores and sporangia. It has been reported that *Mucor* spp. is recognized by the presence of sporangia in globose sporangiophores that can be observed singly or in branched, these sporangia have a thin wall that breaks irregularly to release round or ellipsoidal sporangiospores when the fungus matures (Iglesias-Osores & Rodríguez-Perla, 2020). Organisms exhibiting infection denote lethargic behavior, poor body condition (Berger & Speare, 1997). The skin lesions present “spherules” corresponding to *Mucor* spp., the signology described as granulomas in different tissues, including liver, kidney, bladder, lung and skin. (Berger & Speare, 1997; Connolly, 2015; Speare et al., 1994), as well as the development of ulcerative skin lesions (Obendorf et al., 1993), concordant lesions as observed with the specimens of *L. catesbeianus* that were analyzed in this work. It should be noted that the genus *Mucor* is a causal agent of infections in humans, however, it receives little attention in its follow-up due to its low prevalence worldwide, although it has a high mortality rate when it occurs, which ranges between 40 and 100% (Cánovas-Márquez et al., 2023). Rhino-orbito-cerebral, disseminated pulmonary, cutaneous or gastrointestinal symptoms occur in the presence of infection. Immunocompromised patients with comorbidities such as diabetes and recently COVID-19 may exacerbate the symptoms of the infection or give way to mucormycosis as a secondary opportunistic infection (Hamaamin Hussien et al., 2022; Prabhu & Patel, 2004). On the other hand, fungal infections in aquatic organisms have been related to bacterial infections, where *Aeromonas* spp. has been reported as a bacterium that can cause secondary infections in weak populations with other infections (Kusdarwati et al., 2017). The presence of *A. hydrophila*, can develop acute, subacute, chronic or latent infection, in contrast to the lesions in the frogs analyzed in this work, suggesting a chronic type of infection, which presents ulcerative lesions at the skin level (Kusdarwati et al., 2017). In the present study, 100% (4) of bullfrogs that presented *Mucor* spp. were positive for *Aeromonas* spp. and showed ulcerative lesions, although this bacterium could also be isolated in up to 75% of the organisms analyzed. In Mexico there is a report of dermatosepticemia caused by *Aeromona hydrophila* isolated from skin, mouth, liver and kidney (Ortega Santana et al., 2024) in wild leopard frogs (*Rana pipiens*), infection that reflects erythema, hemorrhage and erosion in different anatomical regions at the cutaneous level (Ortega Santana et al., 2024). Likewise *Aeromonas* spp. has been related to health events in production frogs, *Pelophylax ridibundus* was reported with red leg syndrome related to *Aeromona veronii* infection in adults, while mortality related to *A. hydrophila* was identified in the production ova, both cases in Saudi Arabia (Khalifa et al., 2021; Khalifa & Bekhet, 2018). On the other hand, cultured *Rana temporaria* reported infection by *Aeromonas salmonicida* and *A. sobria*, with a mortality of 40% in Italy (Pastorino et al., 2023). In addition, *Aeromonas veronni* isolates have been recovered in bullfrog (*L. catesbeianus*) culture from frogs that presented red leg disease and abdominal distension. Among the isolates, the presence of virulence genes and resistance to vancomycin, sulfamethoxazole-trimethoprim (SMZ-TPM), lincomycin, penicillin and ampicillin was reported, indicating problems of bacterial resistance in the culture of *L. catesbeianus*. (Lin et al., 2024). In this study, bacteria of the genus *Aeromonas* were identified, which have a potential pathogenic potential for humans. Outbreaks are associated with the consumption of contaminated food such as fish (Cortes-Sanchez et al., 2019), so the consumption of this product can be a risk factor. It can cause gastrointestinal diseases, in addition to wound and soft tissue infections and even the development of septicemia, so its relevance in public health is evident (Pessoa et al., 2022). However, previous

reports indicate that concomitant infections between fungi and bacteria, can exacerbate health outbreaks in aquatic organisms and generate significant economic losses (Dincturk et al., 2018; Kusdarwati et al., 2017; Yardımcı & Turgay, 2021). In addition, it should be noted that in bullfrogs, it is difficult to clarify the cases of mortality, so resorting to a multidisciplinary approach can help prevent health events, therefore the care of water quality, monitoring of cyanotoxins and microbiological loads are necessary to safeguard the health of the organisms, throughout the aquaculture production.

CONCLUSION

Bullfrog (*L. catesbeianus*) farming in the State of Hidalgo is an emerging aquaculture activity that is susceptible to infections by opportunistic pathogens, which impact the health status of cultured populations, and even other populations such as wild populations due to the high invasive potential of this species. The presence of *Mucor* spp. denotes the development of ulcerative lesions and skin nodules in the organisms analyzed, which compromises adequate production. However, the presence of *Aeromonas* spp. is evidence that this bacterium is naturally found in the cutaneous microbiome of the bullfrog, however, it was also identified as an opportunistic infection in all the frogs that presented ulcerative lesions positive for *Mucor* spp. Both microorganisms also have a pathogenic potential for human populations, so it is important to continue monitoring these microorganisms present in the bullfrog (*L. catesbeianus*) culture, in addition to implementing good animal husbandry practices in the production of this species in order to reduce the risks associated with the occurrence and spread of *Mucor* spp. and *Aeromonas* spp, in order to obtain quality and safe products.

REFERENCE

1. Al-Ameri, K., & Agar-Nasser, A. (2023). Isolation of *Mucor* spp. from patients with otitis media and its sensitivity. *International Journal of Health Care and Biological Science*, 4(2), 57–62.
2. Alqarihi, A., Kontoyiannis, D. P., & Ibrahim, A. S. (2023). Mucormycosis in 2023: an update on pathogenesis and management. *Frontiers in Cellular and Infection Microbiology*, 13(September), 1–15. <https://doi.org/10.3389/fcimb.2023.1254919>
3. Berger, L., & Speare, R. (1997). Mucormycosis in a Free-ranging Animal Australia mortality amphibians. *Journal of Wildlife Diseases*, 33(4).
4. Borges, F. de F., do Amaral, L. A., & de Stéfani, M. V. (2012). Caracterização de efluentes de viveiros de engorda de rã-touro (*Lithobates catesbeianus*, Shaw, 1802). *Acta Limnologica Brasiliensia*, 24(2), 160–166. <https://doi.org/10.1590/S2179-975X2012005000035>
5. Cánovas-Márquez, J. T., Lax, C., Tahiri, G., Navarro, E., Nicolás, F. E., & Garre, V. (2023). Advances in understanding infections caused by the basal fungus *Mucor*. *PLoS Pathogens*, 19(6 June), 1–8. <https://doi.org/10.1371/journal.ppat.1011394>
6. Connolly, J. H. (2015). Mucormycosis in the platypus and amphibians caused by *Mucor amphibiorum*. *Microbiology Australia*, 36(2), 83. <https://doi.org/10.1071/ma15027>
7. Cortes-Sanchez, A. de J., Espinosa-Chaurand, L. D., Garza-Torres, R., Diaz-Ramirez, M., Salgado-Cruz, M. de la P., Sánchez-Minutii, L., & García-Barrientos, R. (2019). Foodborne diseases, fish and the case of *Aeromonas* spp. *African Journal of Agricultural Research*, 14(11), 617–628. <https://doi.org/10.5897/ajar2019.13872>
8. Christenson, J. C., Korgenski, E. K., & Relich, R. F. (2018). Laboratory diagnosis of infection due to bacteria, fungi, parasites, and rickettsiae. In *Principles and practice of pediatric infectious diseases* (pp. 1422-1434). Elsevier.
9. Creeper, J., Berger, L., & Huntress, S. (1998). An outbreak of mucormycosis in slender tree frogs (*Litoria adelensis*) and white-lipped tree frogs (*Litoria infrafrenata*). *Australian Veterinary Journal*, 76(11).
10. Dincturk, E., Tanrikul, T. T., & Culha, S. T. (2018). Fungal and Bacterial Co-Infection of Sea Bass (*Dicentrarchus labrax*, Linnaeus 1758) in a Recirculating Aquaculture System: *Saprolegnia parasitica* and *Aeromonas hydrophila*. *Aquatic Sciences and Engineering*, 33(3), 67–71. <https://doi.org/10.26650/ASE201811>
11. DOF. (2015). NORMA Oficial Mexicana NOM-033-SAG/ZOO-2014, Métodos para dar muerte a los animales domésticos y silvestres. *Diario Oficial de La Federación*.
12. DOF. (2018). ACUERDO mediante el cual se dan a conocer en los Estados Unidos Mexicanos las enfermedades y plagas exóticas y endémicas de notificación obligatoria de los animales terrestres y acuáticos. Secretaría de Gobernación. https://www.dof.gob.mx/nota_detalle.php?codigo=5590745&fecha=30/03/2020
13. García Ayala, B., Fuentes Fariás, A., & Gutiérrez Ospina, G. (2020). Phenotypic variation in American bred under intensive systems in Mexico: A preliminary report. *Veterinaria México OA*, 7(1). <https://doi.org/10.22201/fmvz.24486760e.2020.1.747.http>
14. Gervasi, S. S., Urbina, J., Hua, J., Chestnut, T., Relyea, R. A., & Blaustein, A. R. (2013). Experimental Evidence for American Bullfrog (*Lithobates catesbeianus*) Susceptibility to Chytrid Fungus (*Batrachochytrium dendrobatidis*). *EcoHealth*, 166–171. <https://doi.org/10.1007/s10393-013-0832-8>
15. Hamaamin Hussien, N., Hameed Hasan, A., Jamalis, J., Shakya, S., Chander, S., Kharkwal, H., Murugesan, S., Ajit Bastikar, V., & Pyarelal Gupta, P. (2022). Potential inhibitory activity of phytoconstituents against black fungus: In silico ADMET, molecular docking and MD simulation studies. *Computational Toxicology*, 24(July), 100247. <https://doi.org/10.1016/j.comtox.2022.100247>
16. Ibáñez, a L., Espinosa-Perez, H., & Garcia-Calderón, J. L. (2011). Recent data on the distribution of the exotic

species used in Mexican freshwater fisheries based on fish stocking [Datos recientes de la distribución de la siembra de especies exóticas como base de la producción pesquera en aguas interiores mexicanas]. *Revista Mexicana de Biodiversidad*, 82(3), 904–914. <http://www.scopus.com/inward/record.url?eid=2-s2.0-80155199301&partnerID=40&md5=df0da8ac8f5c3fb686350c8f84507e60>

17. Iglesias-Osores, S., & Rodríguez-Perla, H. (2020). Características Microbiológicas de *Mucor* sp. *Revista Experiencia En Medicina Del Hospital Regional Lambayeque*, 6(1), 10–12. <https://doi.org/10.37065/rem.v6i1.403>

18. Instituto Nacional de Estadística y Geografía (INEGI). (2010). *Compendio de información geográfica municipal 2010*. <https://www.inegi.org.mx/app/areasgeograficas/?ag=08020#collapse-Resumen>

19. Islas-ojeda, E., García-Munguía, A., Chávez-González, L., López-Gutiérrez, M., Hernández-Valdivia, E., & García-Munguía, C. (2021). Sustainable production of bullfrogs (*Lithobates catesbeianus*) with reused water from a Biofloc system Producción sostenible de rana toro (*Lithobates catesbeianus*) con agua de reúso de un sistema Biofloc Global fish production has increased steadily ov. *Abanico Veterinario*, December, 1–15.

20. Khalifa, A. Y. Z., AlMalki, M. A., & Bekhet, G. M. (2021). Pathological and mortality findings associated with *Aeromonas hydrophila* from frog eggs in Al-Ahsa region of Saudi Arabia. *Aquaculture Research*, 52(3), 1227–1236. <https://doi.org/10.1111/are.14981>

21. Khalifa, A. Y. Z., & Bekhet, G. (2018). First isolation and characterization of the pathogenic *Aeromonas veronii* bv. *veronii* associated with ulcerative syndrome in the indigenous *Pelophylax ridibundus* of Al-Ahsaa, Saudi Arabia. *Microbial Pathogenesis*, 117, 361–368. <https://doi.org/10.1016/j.micpath.2017.10.019>

22. Kusdarwati, R., Kismiyati, Sudarno, Kurniawan, H., & Prayogi, Y. T. (2017). Isolation and Identification of *Aeromonas hydrophila* and *Saprolegnia* sp. on Catfish (*Clarias gariepinus*) in Floating cages in Bozem Moro Krembangan Surabaya. *IOP Conference Series: Earth and Environmental Science*, 55(1). <https://doi.org/10.1088/1755-1315/55/1/012038>

23. Lin, H., Zeng, G., Yu, Y., Li, H., Chen, K., Qin, Z., Jiang, B., Li, W., Su, Y., Lin, L., & Liu, C. (2024). Acute septicemia and diagnostic evaluation of *Aeromonas veronii* infection in American bullfrogs (*Aquarana catesbeiana*). *Aquaculture*, 580(P2), 740349. <https://doi.org/10.1016/j.aquaculture.2023.740349>

24. Montel Mendoza, G., Pasteris, S. E., Ale, C. E., Otero, M. C., Bühler, M. I., & Nader-Macías, M. E. F. (2012). Cultivable microbiota of *Lithobates catesbeianus* and advances in the selection of lactic acid bacteria as biological control agents in raniculture. *Research in Veterinary Science*, 93(3), 1160–1167. <https://doi.org/10.1016/j.rvsc.2012.05.007>

25. Obendorf, D. L., Peel, B. F., & Munday, B. L. (1993). *Mucor amphibiorum* infection in platypus (*Ornithorhynchus anatinus*) from Tasmania. *Journal of Wildlife Diseases*, 29(3), 485–487. <https://doi.org/10.7589/0090-3558-29.3.485>

26. Ortega Santana, C., Fajardo, R., Escobedo-Bonilla, C. M., Irgang, R., Valladares-Carranza, B., Martínez, S., Saldarriaga-Córdoba, M., & Avendaño-Herrera, R. (2024). Outbreak of *Aeromonas hydrophila* bacterial dermatosepticemia in wild leopard frogs (*Rana pipiens* complex) in Mexico. *Veterinaria Mexico OA*, 11. <https://doi.org/10.22201/fmvz.24486760e.2024.1311>

27. Paetow, L. J., McLaughlin, J. D., Pauli, B. D., & Marcogliese, D. J. (2013). Mortality of American bullfrog tadpoles *Lithobates catesbeianus* infected by *Gyrodactylus jennyae* and experimentally exposed to *Batrachochytrium dendrobatidis*. *Journal of Aquatic Animal Health*, 25(1), 15–26. <https://doi.org/10.1080/08997659.2012.722170>

28. Pastorino, P., Colussi, S., Varello, K., Meletiadis, A., Alberti, S., Di Blasio, A., Tedde, G., Begovoieva, M., Peano, A., Rossi, L., Renzi, M., Acutis, P. L., Barceló, D., & Prearo, M. (2023). Interdisciplinary approach to solve unusual mortalities in the European common frog (*Rana temporaria*) in two high-mountain ponds affected by climate change. *Environmental Research*, 222(February). <https://doi.org/10.1016/j.envres.2023.115411>

29. Pessier, A., & Mendelson, J. (2017). NECROPSIA DE ANFIBIOS. In A. Pessier & J. Mendelson (Eds.), *Manual para el control de enfermedades infecciosas en colonias de supervivencia de anfibios y programas de reintroducción* (First Ed., pp. 523–536). <https://doi.org/10.1201/b10848-50>

30. Pessoa, R. B. G., Oliveira, W. F. de, Correia, M. T. dos S., Fontes, A., & Coelho, L. C. B. B. (2022). *Aeromonas* and Human Health Disorders: Clinical Approaches. *Frontiers in Microbiology*, 13(May). <https://doi.org/10.3389/fmicb.2022.868890>

31. Ponce-Palafox, JI2, A.-F., Castillo-Vargasmachuca SG1, R. C. G., Valle, B., al, Ma1, R. D. D., F1, M. C., R1, N. V., Ja1, G. G., & P1., L. L. (2010). the Effect of Chemical and Organic Fertilization on Phytoplankton and Fish Production in Carp (*Cyprinidae*) Polyculture System. *Revista Biociencias*, 1(August), 44–50.

32. Prabhu, R. M., & Patel, R. (2004). Mucormycosis and entomophthoromycosis: A review of the clinical manifestations, diagnosis and treatment. *Clinical Microbiology and Infection*, 10(SUPPL. 1), 31–47. <https://doi.org/10.1111/j.1470-9465.2004.00843.x>

33. Raposo, C., Serrano, I., Cunha, E., Couto, M. P., Lopes, F., Casero, M., Tavares, L., & Oliveira, M. (2023). Phenotypic Characterization of Oral *Mucor* Species from Eurasian Vultures: Pathogenic Potential and Antimicrobial Ability. *Life*, 13(8). <https://doi.org/10.3390/life13081638>

34. Rigney, M. M., Zilinsky, J. W., & Rouf, M. A. (1978). Pathogenicity of *Aeromonas hydrophila* in red leg disease in frogs. *Current Microbiology: An International Journal*, 1(3), 175–179. <https://doi.org/10.1007/BF02601673>

35. Ruthig, G. R., & Provost-Javier, K. N. (2012). Multihost saprobes are facultative pathogens of bullfrog *Lithobates catesbeianus* eggs. *Diseases of Aquatic Organisms*, 101(1), 13–21. <https://doi.org/10.3354/dao02512>
36. Sherif, A. H., & Kassab, A. S. (2023). Multidrug-resistant *Aeromonas* bacteria prevalence in Nile tilapia broodstock. *BMC Microbiology*, 23(1), 1–14. <https://doi.org/10.1186/s12866-023-02827-8>
37. Speare, R., Thomas, A. D., O'shea, P., & Shipton, W. A. (1994). Amphibiorum in the Toad, *Bufo marinus*, in Australia. *Journal of Wildlife Diseases*, 30(3).
38. Urbina, J., Bredeweg, E. M., García, T. S., & Blaustein, A. R. (2018). Host–pathogen dynamics among the invasive American bullfrog (*Lithobates catesbeianus*) and chytrid fungus (*Batrachochytrium dendrobatidis*). *Hydrobiologia*, 817(1), 267–277. <https://doi.org/10.1007/s10750-018-3614-z>
39. Wei, D., Cheng, Y., Liao, W., Yu, Q., Shi, J., Xia, X., Chen, M., Han, S., & Li, P. (2023). Composition and function of the skin microbiota were altered of red leg syndrome in cultured bullfrog (*Rana catesbeiana*). *Aquaculture Reports*, 29(December 2022), 101487. <https://doi.org/10.1016/j.aqrep.2023.101487>
40. Yardımcı, R. E., & Turgay, E. (2021). Diagnosis of *Aeromonas sobria* and *Saprolegnia* sp. co-infection in rainbow trout fry (*Oncorhynchus mykiss*). *Aquatic Research*, 4(1), 65–72. <https://doi.org/10.3153/ar21006>
41. Zepeda-Velazquez, A. P., Gómez-De-Anda, F. R., Aguilar-Mendoza, L. F., Castrejón-Jiménez, N. S., Hernández-González, J. C., Varela-Guerrero, J. A., de-la-Rosa-Arana, J. L., Vega-Sánchez, V., & Reyes-Rodríguez, N. E. (2023). Bullfrogs (*Lithobates catesbeianus*) as a Potential Source of Foodborne Disease. *Journal of Food Protection*, 86(4), 100067. <https://doi.org/10.1016/j.jfp.2023.100067>
42. Zhao, R., Wang, J., Wang, D., Wang, Y., Hu, G., & Li, S. (2024). Isolation, Identification, and Characterisation of a Novel ST2378 *Aeromonas hydrophila* Strain from Naturally Diseased Frogs, *Rana dybowskii*. *Pathogens*, 13(7). <https://doi.org/10.3390/pathogens13070552>
43. Zhou, H., Gai, C., Ye, G., An, J., Liu, K., Xu, L., & Cao, H. (2019). *Aeromonas hydrophila*, an emerging causative agent of freshwater-farmed whiteleg shrimp *litopenaeus vannamei*. *Microorganisms*, 7(10). <https://doi.org/10.3390/microorganisms7100450>