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Chapter

Frog Virology: Biosafety in an Experimental Farm

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Abstract

Understanding and detecting diseases of amphibians has become vitally important in conservation and ecological studies and prevent and biosecurity a determinant priority in experimental farms, mainly when related with academic and research activities. Ranavirus belongs to the family Iridoviridae, and causes an emergent infectious disease that affects different species, especially fish, reptiles and amphibians, with a significant contribution to the decline of the population. In amphibian systems, Ranaviruses transmission can occur between vertebrate classes through direct contact, by scavenging or through virus particles persisting in the environment. Subclinical infected individuals may serve as reservoirs in the most susceptible anura species. Humans play a significant role in this emergent disease and biosecurity measures are determinant to prevent the introduction of these viruses, either in commercial or experimental farms. A Biosafety Plan is a fundamental tool in the Ranaviruses prevention and include educational and training programs, relevant to the mission of a Higher Education Institution.

Keywords: amphibians, biosafety plan, infectious diseases, Ranavirus, surveillance

1. Introduction

Emerging infectious diseases are currently a threat to the conservation of global biodiversity [1]. Amphibian diseases linked to declining of amphibian populations, are a constant threat to endangered species, and are frequently a hazard in ranaculture facilities [2]. Many factors have been implicated in these declines in the wild, including introduced predators, increased ultraviolet-B radiation, chemical contaminants, habitat destruction and degradation, and emerging diseases [3]. Amphibians are susceptible to a variety of pathogens, including internal and external parasites, bacteria, fungi, and viruses. Understanding and detecting diseases of amphibians has become vitally important in conservation and ecological studies [2].

Changes in environmental conditions can be a potential driver of emerging infectious diseases [4]. Environmental influence affects the population susceptibility, with seasonal variation in response to climate (temperature) alterations, moisture availability, and their interactions' in amphibian behavior [1]. In fact, pathogens are favored for warmer ambient temperatures, that provide ideal conditions for propagation [4, 5].

The causes of the population decline are complex, but it is clear that infectious agents, either directly, or following environmentally-induced immune suppression,

play an important role in this process [6, 7]. Each of the three major life stages of amphibians (embryos, larvae, and adults) has distinct diseases [2] and at least six groups of viruses have been reported to affect amphibians, including iridoviruses, herpesviruses, and arboviruses.

Some infectious diseases of amphibians share similar pathological signs; thus, their detection, recognition, and correct diagnosis can be a challenge [2, 8]. A group of viruses belonging to the genus Ranavirus are amphibian pathogens, globally distributed, with higher morbidity and mass mortality [2, 8–11]. Ranaviruses infect at least 175 species across 52 families of ectothermic vertebrates, as fish, amphibians, and reptiles, and cause systemic diseases, compromising multiple internal organs [4–6, 12–14]. They are the second most common infectious cause of mortality in amphibians worldwide, after the fungus Batrachochytrium dendrobatidis [5], with a relevant impact in the population decline. As indicative of the ranaviruses host range and their potentially negative effects, ranaviral disease was listed by the World Organization for Animal Health (OIE) as an internationally notifiable disease [2, 8, 9, 15, 16]. Ranavirus is associated with amphibian die-offs, like many other diseases it generally does not lead to the extinction of the host [17].

Ranaviruses may function as a novel or endemic pathogen, associated with the movement of infected amphibians by humans. The infectious process involves genetics, environmental factors (pollution, temperature and other stressors) and inherent biological characteristics of the host (age, life stage, physiological aspects) that directly affect immune competence. Anthropogenic stressors also may facilitate emergence, compromising the imune system [2, 18]. Additionally, subclinical infected hosts may serve as reservoirs for more susceptible amphibian species [18].

Several authors have noted that commercial exchange of live amphibians for food, pets, and laboratory animals may be adversely influencing wild populations by direct harvesting or through the spread of disease [19, 20]. To supplement the higher demand for frogs, and to counteract the effects of over-harvesting, some countries have introduced frog farming.

Dissemination is facilitated by contact with infected individuals or contaminated water as well as inherent behaviors of amphibians such as necrophagy and cannibalism [21]. Measures that prevent or minimize the possibility of introducing potentially pathogenic infectious agents, either wild or captive amphibians, are crucial [22]. Managing ranaviral disease in captive facilities is more straightforward than in natural populations. Isolation of positive individuals and disinfection of animal enclosures are important initial steps, but similar to wild populations, it is essential to minimize possible stressors and maintain proper biosafety procedures to prevent cross contamination [23].

Vertebrate iridoviruses, specifically members of the genus Ranavirus, have become a significant cause of disease in ectothermic animals, and that from a virological, commercial and ecological point of view deserve additional study [6]. The aim of this chapter is to introduce common amphibian diseases outlining value biosafety measures in a frog farm, with production, experimentation, and research purposes, as well as academic activities, inserted in a Higher Education Institution.

2. Amphibian viruses

2.1 Ranaviruses

Amphibian ranaviruses are enveloped icosahedral DNA viruses, in the family Iridoviridae, with variable size ranging, depending on the species [5, 7, 13]. Isolates causing disease have been found in wild and cultured amphibians in Australia, the

Americas, Asia and Europe [8–10, 15]. These include Frog virus 3, Tadpole edema virus, *Rana catesbeiana* virus Z, *Bohle iridovirus*, and UK ranavirus. Other ranavirus-like were found in captive frogs (*Rana esculenta*) in Croatia, causing lethargy, edema, hemorrhages, and skin necrosis, and also in wild-caught frogs (*Bufo marinus* and *Hopodactylus* sp.) in Venezuela. In this case infected animals had no external lesions or internal symptoms [8].

The trade of amphibians for food, research [13] and as pets contributed to the dissemination of pathogens such as ranaviruses, within and among continents [8, 9]. In North America, ranaviruses are responsible for massive mortality in amphibian larvae and recent metamorphs, while die-offs rarely occur in adults. These events often occur during summer and involve hundreds to thousands of moribund and dead larvae within a few days [8].

Ranavirus epidemics seem to occur in late spring and in summer, what can be explained by the seasonal amphibian's vulnerability to ranaviral infection when the larvae of many species begin to metamorphose. In fact, many components of the amphibian immune system are down-regulated just prior to metamorphosis [8].

Infections occur mostly in amphibians that breed in standing-water habitats [3, 24], and frog farms are associated with permanent water which may increase the exposure to the pathogen, considering that water is an effective transmission route. Animals can be sublethally infected and contain the virus over a period of at least 1 year [7]. Ranaviruses can cause asymptomatic infections in resistant animals, facilitating the spread of disease with the movement of infected animals, and contributing to the prevalence of the infection in the population [8, 10].

Frog virus 3 (Ranavirus type I), was first isolated from aclinically infected leopard frogs (*Rana pipiens*) collected in the United States in 1962 [7, 8, 10, 22]. Since then, FV3-like viruses, such as Tadpole edema virus (TEV), *Rana catesbeiana* virus Z (RCVZ), and UK Ranaviruses have been study.

In laboratory, Ranavirus was shown to cause edema, necrosis, hemorrhage, and death in embryos, tadpoles, and recent metamorphs. During experimental infections, metamorphic toads developed hemorrhages and edema in the ventral skeletal musculature, stomach, and intestines [8, 10, 24]. Mortality in embryos can occur 3 to 12 days post-exposure and clinical signs include depigmentation, skin sloughing, and spinal curvature [8, 25]. Generally, the lesions caused by FV3 appear to be milder than those caused by TEV [8].

Tadpole edema virus (Ranavirus type III) is the first acutely fatal viral infection of wild tadpoles, such as the bullfrog, *Rana catesbeiana*, bufonids (*Bufo americanus*, *Bufo woodhousei fowleri*), and pelobatids (*Spea intermontana*) [22, 25]. Present gross lesions include marked edema, erythema and hemorrhages of the skin and subcutis of the body and proximal hind limbs, hydro coelom, and petechial hemorrhages in the stomach, intestines and skeletal muscles [8, 25].

Rana catesbeiana virus Z was isolated from cultured *R. catesbeiana* tadpoles in the USA. RCVZ appears to be much more pathogenic than FV3, causing massive mortality of exposed tadpoles. Similar to other ranaviruses, symptoms included edema in the abdomen, hemorrhaging in ventral regions, and lethargy [8].

The contemporary strains in the United Kingdom, in common frogs (*Rana temporaria*) and in captive-breeding facilities [12, 26] worldwide, may had origin in North America [8]. Four clinical syndromes were associated with ranavirus-like particles, in English populations of the European common frog: "ulcerative syndrome", "hemorrhagic syndrome", "ulcerative and hemorrhagic syndrome" [7, 12, 26], and "reddened skin syndrome" [25]. The ulcerative form of the disease is characterized by ulcers of the skin and the skeletal muscle, and sometimes digits necrosis, while the hemorrhagic form is described with internal hemorrhages, commonly involving the gastrointestinal and reproductive tracts [12].

The second distinct amphibian ranavirus species discovered was *Bohle Iridovirus* (BIV), isolated from metamorphosed ornate burrowing frogs (*Limnodynastes ornatus*) in Australia [8, 22]. Experimentally, BIV is highly pathogenic to tadpoles and metamorphs of *L. ornatus*, and also, to tadpoles, metamorphs and adults of the giant toad, *Bufo marinus*. Lesions produced by BIV are multifocal necroses of the liver, mesonephroid, and lungs [25].

Species within the anuran family Ranidae were generally more susceptible to ranavirus infection than other family's species (Hylidae, Bufonidae, Scaphiopodidae), as shown by phylogenetic comparative methods [5, 24].

2.2 Herpesviruses

Other viral infection of the North American leopard frog is caused by the herpesvirus, and induces a form of renal adenocarcinoma, known as Lucke's renal tumor. The tumor grows during the spring and summer, with the virus being shed in the spring to infect other frogs. Renal failure occurs with weight loss and death. There is no treatment for this disorder [27].

A herpesvirus-like dermatitis with numerous dorsal and lateral epidermal vesicle, was also detected in specimens of the spring frog, *Rana dalmatina*, in a north Italy region [24]. These enveloped viruses tend to be less stable in the environment, and transmission, from one enclosure to another by human vectors, is feasible but could be prevent by good hygiene practices [18].

2.3 Arboviruses

Arboviruses are known to infect hosts by infected arthropods. Amphibians and reptiles have been studied as potential reservoir hosts of Chikungunya virus. The possible role of ectothermic vertebrates as reservoirs or overwintering hosts has been evaluated for several arboviruses, and numerous species of mosquitoes have been described to feed on a variety of reptiles and amphibians, including mosquitoes such as *Aedes aegypti* [28].

Amphibians are infected with virus through physical contact, skin exposure to contaminated water or direct ingestion of viruses [3, 29].

In one study, a frog (*Rana ridibunda*) was found to be viremic and was able to transmit the virus to *Culex pipiens*, a bloodsucker [30]. Therefore, a frog-mosquito-frog cycle also appears to be possible under certain ecological conditions.

Since necrophagy and cannibalism are considered important forms in direct transmission of viruses in amphibians, both in the tadpole and metamorphosed phases, the ingestion of virus-carrying insects can also be a form of infection. Transmission by necrophagy and cannibalism is common in host species such as *Arnbystollla tigrinum*, *Rana sylvatica* and *R. latastei* [29, 31] and infections acquired by these routes appear to be more lethal.

Thus, the problem of transmission of arboviruses between amphibians and, as they may be carriers, must be taken in the spread of this class of viruses to insects and their transmission to other vertebrates (including humans).

3. Transmission

Ranavirus horizontal transmission can occur via direct (necrophagy, cannibalism, [13, 17] touching, biting, [8] scavenging, virus particles persisting in the environment), or indirect routes (fomites, soil, contaminated water) [8, 12, 15]. The potential for human involvement in transmission and spread of diseases, within

and among amphibian populations, is very significant [16]. Three is no evidence of ranavirus vertical transmission [32].

Rate and infection outcome vary with the route of exposure [12, 15]. Due to nutritional and energetic limitations and physiological trade-offs, host life history characteristics such as fast development, short life span, and high fecundity can be associated with increased susceptibility to pathogens [24]. Concerning nonenveloped viruses, like iridoviruses, with a tendence to be stable in the environment, prevent spread presents a greater challenge [33].

Spread of ranaviruses may be due to water movement, via fomites sedimentation, or by sublethally/aclinically infected animals [8]. Supplying several tanks with water from a single source, and allowing the water to run through successive tanks, may contribute to a serious outbreak of diseases [32]. Larvae could become infected with ranavirus when exposed to water that previously housed infected larvae [8]. Habitats, with optimal conditions for the pathogen's persistence, may form "reservoirs" [1].

Under laboratory conditions, test animals may not be exposed to the normal array of environmental conditions (diel temperature fluctuations, exposure to proper ultraviolet-B radiation), microbial communities, or other environmental elements that could influence transmission [3]. Transmission through indirect routes had been demonstrated in the laboratory [8] and, previous laboratory studies shown that ranaviruses can persist from days to years, depending on the environmental conditions [8, 34].

4. Biosecurity

Ranaviruses are emerging pathogens and a threat to global amphibian populations. Following the guidelines of the World Organization for Animal Health [35], biosafety measures, a set of management and physical measures designed to mitigate the risk of introduction of pathogenic agents into, or spread within, or release from, aquatic animal populations, should be implemented in aquaculture establishments.

Managing ranaviral disease in captive facilities is more straightforward than in natural populations, requiring surveillance, control measures and basic biosecurity conditions, namely for the purpose of international trade [21, 35]. The definition of compartment, one or more aquaculture establishments under a common biosecurity management system containing an aquatic animal population with a distinct health status, should encompass disease-specific epidemiological factors, the aquatic animal species in the compartment, production systems, biosecurity practices, infrastructural factors and surveillance [35].

4.1 Disease surveillance

Amphibian ranaviruses have been found in animals that are traded over international borders for a variety of reasons, including human consumption and the pet trade [14]. The OIE listing provides the impetus for disease surveillance and required testing of amphibians prior to transport among states or between nations [2, 35, 36].

Epidemiological and geographic factors should be taken into consideration in disease surveillance, as the disease status in adjacent areas and in areas epidemiologically linked to the compartment and the location, disease status and biosecurity of the nearest epidemiological units or other epidemiologically relevant premises [35, 36].

Disease transmission can occur between captive and free-ranging populations and a strategy of comprehensive disease surveillance in captive amphibians and

frog farm facilities, should be implemented. Captive breeding population health status must be considered when intended for release [2], and is not recommend wild amphibians, housed for any period of time, returned to their natural population unless been kept in isolation and their captive history consider as disease-free [16]. Disease emergence also may occur through geographical transport of pathogens.

Ranaviruses isolated from frog farm facilities appear to be more virulent than wild strains, emphasizing the importance for disease monitoring at these facilities. In areas with multiple endemic ranaviruses strains or species, slight variations in genetic coding can increase virulence.

Isolation of positive individuals and disinfection of animal enclosures are important steps, but similar to wild populations, it is essential to minimize possible stressors and maintain proper biosafety procedures to prevent cross contamination [23].

Simultaneous infection by multiple pathogens is possible, and some diseases become evident only after the post-metamorphic (Lucke's tumor herpesvirus). Also, the lack of gross signs of disease does not imply healthy populations, as tadpoles with no signs of illness can be infected with ranaviruses [2].

In the event of a die-off in a captive facility, freshly dead animals should be submitted for diagnostic evaluation. Live animals that are infected should be euthanized or treated, if a treatment exists, and facilities decontaminated with disinfectant [2]. To identify the causal factors for outbreaks, ideally host densities and stages of development, water and ambient temperature, and water quality should be measured during surveillance programs [36].

Testing for Ranavirus can be done with lethal and non-lethal samples. Testing liver samples for infection is a common lethal sampling technique to estimate ranavirus prevalence because the pathogen often targets this organ, especially in larval amphibians, and the liver is easy to identify and collect [11].

False negative can result from testing tail clips in [2, 11], and occur when the number of virions circulating in the host's tissues is low, or few virions are shed [11]. Lethal samples (organ tissue) will likely result in greater detection of ranavirus compared to nonlethal samples (swabs, tail-clips) [11, 36]. Non-lethal sampling techniques can be useful for ranavirus surveillance, although the prevalence of infection may be underestimated when compared to results obtained with liver samples [11].

Sample collection may include whole live or dead animals, sections of tissues, swabs of lesions or orifices or habitat samples. To prevent disease transmission between infected and uninfected individuals [17] and protect professionals from zoonotic diseases, is mandatory wearing disposable gloves when handling amphibians and, between animals, change gloves. When handling amphibians, professionals should use disposable vinyl or nitrile gloves, rinsed with distilled or sterilized water [2, 16]. Dipping gloves into disinfectant between processing animals might reduce iatrogenic pathogen transmission, however, these practices may have toxic effects on wild animals [17].

Samples can be frozen in a standard 20 °C freezer if stored for short duration (1 month); otherwise, should be stored in an 80 °C freezer. Samples can also be promptly fixed in 75% ethanol or 10% neutral buffered formalin for histology. Swabs are typically performed in the oral then cloacal regions, and the swab stored, placed on ice and frozen similar to tissues [2].

Lethal infectious diseases of amphibians may response to stressors, whether anthropogenic or natural [2], and some natural factors are host density, species composition, temperature, and host development [36]. Prevention of the spread of endemic diseases to naive populations or species is a high conservation

priority [2], thus is very important to implement appropriate strategies to minimize this risk [16].

No treatment or vaccine are currently available for ranaviruses [9, 16], but the potential for development of a Ranavirus vaccine is promising particularly considering that prior infection with a ranavirus led to enhanced immunity against subsequent exposure [37], particularly valuable in captive populations [21].

Organizations with limited knowledge about ranaviruses, in the region, supplementary efforts and time are required to document the distribution of ranaviruses, identify infection hotspots, and implement disease intervention strategies that thwart the introduction of ranavirus or reduce its prevalence [36].

4.2 Human and animal safety

Commercial exchange of live amphibians for food, pets, and laboratory animals may be adversely influencing wild populations by direct harvesting or through the spread of disease. Ranaviruses can remain viable outside of hosts for a considerable duration, and can be transported on sampling equipment, recreational gear and fomites [21, 25, 34].

Few infectious diseases of amphibians are contagious to humans, even if mandatory the decontamination of surfaces that come in contact with water bodies that contain amphibians to stop the unnecessary spread of the pathogen [21, 34]. Professionals should wear sanitary wear protection, gloves and waterproof footwear, easily disinfected, when monitoring or capturing animals. Disposable gloves should be worn whenever handling amphibians, and hands washed thoroughly after removing gloves [2].

A correct distinction between cleaning, disinfecting, and sterilizing should be considered. Cleaning refers to the action of physically removing organic and inorganic debris. Disinfecting reduces the load of contaminating organisms to a large extent, but not completely. For a well-established amphibian collection, that has had no infectious diseases or new specimens added within a year, there is little need to attempt to sterilize cages and tools. However, if a collection is experiencing disease, and/or is adding new animals, items should be sterilized [38].

Washing and disinfecting equipment is recommended whether in the presence of pathogens or not [2]. Disinfectants must be safe for use with amphibians and must inactivate a significant proportion of Ranavirus to be considered effective [9]. Common disinfectants used are chlorhexidine [2], potassium compounds [9] and sodium hypochlorite (bleach) [9, 16, 19]. Bleach is often preferred because it is cost effective, easily obtained, and effective against most bacteria and many viruses. However, bleach is not very effective at inactivating Ranavirus, requiring at least a 3% concentration [2, 9] for 10 to 15 minutes between animals [9, 16, 19] which can be toxic to amphibians. In contrast, chlorhexidine used at a dosage that is safe for amphibians (0.75% for a 1-minute exposure) can inactivate Ranavirus [2, 9]. For potassium peroxymonosulfate is recommend at 1.0% solution for disinfecting equipment for 10 minutes [9]. After disinfection, equipment may be allowed to air dry or rinsed with clean water [2].

Proper health of any aquaculture operation depends on water quality, proper nutrition, quarantine and sanitation. Warm (e.g., >25 °C) and frequently filtered water, along with low host densities, may be good preventative strategies to minimize ranavirus outbreaks in captivity [23, 39]. Sanitation can be achieved by: avoid accumulation of organic matter; disinfections of nets and other equipment used; and providing clean environment [32, 40].

Morbid animals and carcasses should not be released or discarded at the same or other sites because this may facilitate the spread or persistence of infectious

diseases. Dead amphibians that are not used for testing should be placed in double-layered plastic trash bags and disposed by burial or incineration [2, 22, 41].

4.3 Recommended procedures – biosafety plan

Health examinations to captive anuran and good biosecurity methods need to be employed because, often, little is known about the life cycles of infectious diseases, modes of transmission, and the persistence of the pathogen within and outside the amphibian host. The goal of biosecurity is to prevent mechanical transmission of pathogens and contaminants from one location to another by equipment, supplies and people, involving the safety of the humans and animals and disinfection of facilities and equipment [2].

In many cases, a pathogen will only cause disease in a host if environmental conditions are favorable. Such circumstances cause prevalence of disease in a population and leads to host mortality in frog farm facilities or wild populations. In general, the most important environmental factors affecting pathogen survival are temperature, moisture and solar, although pH, the presence of organic matter and exposure to chemicals can also be important [1].

The biosecurity program of a production unit must use healthy and disease-free breeders [32, 35, 41]; disease testing of all incoming lots [19]; treatment of water to eliminate pathogens [41]; sterilization and maintenance of materials and equipment; use of personal hygiene measures including hand, footwear, and clothing washing [16]; knowledge of potential pathogens, sources of risk and methods for their control and eradication; development and use of batches that are resistant to specific pathogens; and maintaining the environment in optimal conditions within all phases of quarantine [41].

Structural aspects should, then, be considered as the water supply (an effective transmission medium for ranaviruses) warm and frequently filtered; effective means of physical separation and facilities for people entry including access control; vehicle and vessel access [2, 23, 35]. Inadequate transportation prior to arrival at the facility, inappropriate housing and overcrowding are husbandry practices that facilitate infection diseases [18, 32].

Facilities should also consider location (isolation from other facilities); animal management and practices (unloading and loading); facilities for the introduction of material and equipment; infrastructure to store feed and veterinary products, and isolation facilities for introduced aquatic animals (quarantine) [18, 32, 35].

Quarantine is a vital component of a production-level biosecurity program, which includes a set of standard used procedures and is an essential part of good management for research facilities or farms. It is an important risk management measure and is a key activity that should be considered when developing strategies during farm production [41]. Quarantine areas can be relatively rustic for this purpose [22] and enclosures should be easily disinfected [39].

The protocol should include a detailed clinical examination that includes monitoring the animal's weight, physical posture, and changes in appearance. At least one blood smear can provide important information on the animal's health, stress, and immune status. A stool examination should be performed [22] as well as microbial culture of the oropharynx and cloaca. Diligent surveillance of the amphibian is essential for successful quarantine. Feeding time generally stimulates activity and allows to assess the amphibian's vigor and appetite [39].

A minimum length of 30 days is recommended for the quarantine period of any amphibian that arrived with a clean fecal sample, but often 60 days of quarantine is needed to process an amphibian through a prophylactic protocol. Wild-caught amphibians, whether obtained directly or indirectly, should be held for an extended

quarantine of 90 days or more [19, 22, 41]. This is also recommended for amphibians of unknown origin and those that have been exposed to especially stressful conditions during shipment or prior to shipment.

Quarantine tools and cages should be maintained well separated from established amphibians. New and ill amphibians should be serviced after the healthy and established members of a collection. Disposable vinyl or nitrile gloves are recommended [16, 17] when working with the quarantined amphibians [34]. Washing and disinfection procedures; disposal of aquatic animal waste; measures to prevent exposure to fomites or vectors; feed supply/source are hygienic or nutritional measures determent not only in the quarantine facility but in all the farm, integrating a well-designed Biosecurity Plan [21, 35, 36, 41].

The integrity of an experimental farm relies on effective biosecurity, with the implementation and monitorization of a biosecurity plan [21]. Following OIE guidelines, this plan integrate the potential pathways for introduction of identified (aquatic animal movements, wild aquatic animals, potential vectors, vehicles, people, biological products, equipment, fomites, feed, waterways); the critical control points for each pathway; measures to mitigate exposure for each critical control point; standard operating procedures; corrective actions; process verification and documentation; contingency plan; educating and training program (for workers, farmers and students) and a surveillance program [35, 36].

5. Conclusion

Amphibians are declining globally and emerging infectious diseases are one of the causes. Ranaviruses have a significant impact on diverse populations of ectothermic animals.

Interactions of amphibians with pathogenic organisms are extremely complex. Laboratory experiments, conducted on animals that are either captive-bred or have been maintained for extensive periods in captivity, are very important to understand the susceptibility of amphibians to disease.

Poor biosecurity practices can increase pathogen transmission and disease-related mortality in amphibians. Co-housing infected amphibians with uninfected individuals, even at low densities, increased disease-related mortality. Frog farm facilities should consider establishing amphibian disease surveillance programs and biosafety protocols for Ranavirus.

Biosafety measures should be implemented in aquaculture facilities, particularly in experimental/commercial farms, and a comprehensive biosecurity plan must be developed, implemented and monitored. Infrastructural factors, hygiene and disinfection, nutritional management and water supply are determinant to reduce or control risk infection. Education and training should be encourage concerning amphibian diseases and public health measures, especially when trade contributes to the spread of ranaviruses.

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