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Drugs and chemicals use in frog farming in Dong Thap province

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ABSTRACT

The study was conducted to investigate the use of chemicals, drugs, and disease occurrence from frog farming in Dong Thap province. Data were collected through a questionnaire from a random sample of 30 frog farmers from January to April 2020. The results showed that the common diseases were pus in liver (66.7%), hemorrhage (66.7%), and digestive tract problems (flatulence, abdominal intestine, 40%). Farmers used antibiotics to treat diseases and chemicals to treat parasitic infection without knowledge on pathogens. Commonly used antibiotics were oxytetracycline (53.3%), doxycycline (63.3%), amoxicillin (53.3%), florfenicol (36.7%), and sulfamethoxazole/trimethoprim (46.7%). In addition, other antibiotics such as tetracycline, enrofloxacin, ciprofloxacin, rifampicin were used in some households. Farmers used iodine (26.7%), copper sulfate (20%), and BKC (16.7%) as disinfectants. There was no antibiotic residues test prior to sell to traders. It is necessary to support farmers in knowledge on diseases, drugs/chemicals use, and safety in the use of drugs and chemicals.

1. INTRODUCTION

The frog *Rana rugulosa*, also called wrinkled frog or Thai native frog, distributed in East Asia from Japan through Korea and northeastern China to southernmost of Russia (Maeda & Matsui, 1999 cited by Khonsue et al., 2001). In Thailand, frog farming has been attempted for many years with varying degrees of success (Culley, 1984) and the number of frog culture farms expanded rapidly in 1992 (Supranee & Temdoung, 1995). After that, frog culture has been practiced widely throughout South-east Asia (Somsiri, 1994). In 2001, Thai Frog (*Rana tigerina*) was experimentally cultured in Nong Lam University and spread over the Mekong Delta in 2005 (Tran Hong Thuy, 2013). This frog may be a native species in Vietnam because a study on *Rana rugulosa* was found in 1985 (Moravec, 1985). Prior to popular of Thai frog culture, the Vietnamese frog (*Rana rugulosa*) had been cultured but profit was much less than Thai frog which quickly expanded in the Mekong Delta (Le Tran Tri Thuc et al., 2013). Frog meat is a rich protein meat and be able to replace other animal original protein source (Omoniyi et al., 2012). Frog meat is also a special food with high domestic consumption and having export potential to USA, EU, Taiwan.

Thai frog has been widely cultured in many provinces in South of Vietnam, such as Tay Ninh, Binh Phuong, Binh Duong, Dong Nai and more common in the Mekong Delta, especially in Dong Thap and Tien Giang provinces (Le Minh Quoc, 2012). This species can be reared with commercial pellet feed in many different models for example farming in cement tanks, earthen ponds, or hapa (Nguyen Chung, 2007). This led to the rapid development of frog culture. Besides the development of farming, the disease situation is of concern to farmers. The diseases occurred in this species including red body, pus in liver, swollen body cavity, eye edema, deformed neck, skin ulcers (Nguyen Van Kiem & Bui Minh Tam, 2004). The causes of the frog disease may include changing weather, high stocking density, poor

preparation of the culture systems, inappropriate culture location, inappropriate feed, and feeding method (Nguyen Chung, 2007).

In aquaculture, the increase of culture density results in increasing diseases outbreak. It led to more applied drugs and chemicals. Besides, the missed use of chemicals and antibiotics could promote the development of resistant bacteria strains and especially the residue of antibiotics in the animal's body and affect the health of consumers. Currently, information on frog farming techniques, common diseases, and use of chemicals and antibiotics in frog farming is limited and should be explored.

2. MATERIALS AND METHODS

The survey was conducted in Cao Lanh (19 farms) and Thap Muoi districts (11 farms), Dong Thap Province from January to April 2020 (Figure 1). The farms were randomly selected for interviews using prepared questionnaires. Data were collected on the use of chemicals, active ingredients of drugs, purpose of use, method of application, effect on environment, and impact on health. Preliminary questionnaires were prepared and tested with three target farmers, attention was given to any new information, which was not designed to ask, but it was important and informative towards the objectives. After that, necessary modifications were made based on the feedback and the questionnaire was finalized. Collected data were analyzed by descriptive statistics including average, min, max, percentage using the SPSS software, version 18.0.

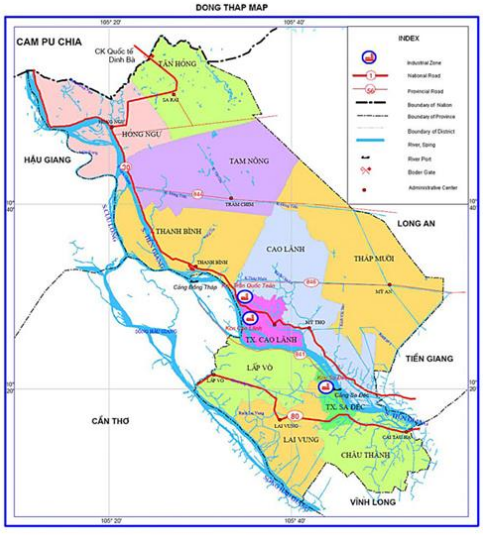

Figure 1. Survey location in Dong Thap province (Cao Lanh and Thap Muoi districts)

3. RESULTS AND DISCUSSIONS

3.1. General information of frog farming in Dong Thap province

According to farmers, the froglet is an important factor in culture, the weak froglet may lead to a low survival rate in grow out stage. The results showed that 100% of farmers used artificial froglet. The advantage is that the amount of froglet can satisfy the demand of household and can be purchased locally with the size of 5-6 g/individual. However, the farmers stated that the quality of froglet is not quarantined due to lacking assessment regulation. Besides the quality of froglet, the stocking density is also important, the stocking density was 198±23 frogs/m^2. To culture this frog, the farmer placed hapas (32 – 48 m^2) in an earth pond (500- 2,100 m^2) (Table 1).

Table 1. General information of frog farming in Dong Thap province

Items	Values (n=30)
Farming experience (year)	4.9±4.2 (1 - 20)
Hapa size (m^2)	42.9±6.1 (32 - 48)
Pond area (m^2)	665±574 (500-2100)
Number of hapas (hapa/farm)	21.1±9.4 (6 - 40)
Density (frog/m^2)	198±23 (150 - 250)
Size of froglet (g/froglet)	5.4±0.5 (5 - 6)
Productivity (kg/m^2)	18.1±2.23 (14.6-21.9)
Survival rate (%)	70.9±6.0 (60-85)
FCR	1.29±0.08 (1.2-1.4)
Harvest size (g)	167-333

Data expressed as mean ± standard deviation (min-max)

According to farmers, the culture duration ranged from 50 to 75 days. Frogs were fed 30% crude protein commercial pellet feed, purchased from popular companies such as Tongwei (40%), CP (65.7%), Cargill (23.3%), Ewos (33.3%), and Afiex (26.7%). The survival rate of the frogs was 70.9 ± 6.0% (60-85%). The low survival rate reported by some households could be by prolonged high temperature in the Mekong Delta, leading to frequent disease occurrence. The feed conversion ratio (FCR) was 1.29 ± 0.08 (1.2–1.4). The average yield was 18.1 ± 2.23 kg/m^2 (14.6-21.9 kg/m^2). Frogs were stocked and cultured year-round.

The market size of frogs depended on the consumer and price, ranging from 167 to 333 g, corresponding to 3-6 ind./kg. According to farmers, harvested frogs were not checked the residues of antibiotics, and the retailers also did not consider that before trading. This posed an issue on food hygiene and safety

management in frog products for consumers. In case of using antibiotics before harvest, it may lead to the possibility of antibiotic residues in frog products.

Farming experience varied widely from 1-20 years. Nearly 50% of farmers have attended trainings and seminars organized by different organizations. The training courses are mainly organized by fisheries administrators in the province, drug/chemical supply agents, and feed companies. The training contents were mainly advertised for drugs/chemical and farming techniques, but there was little guidance on the safe use of drugs for the farmers and consumers.

3.2. Frog disease occurrence

The common diseases in frog culture included pus in liver (66.7%), septicemia disease, which named hemorrhage by the farmer, (66.7%), digestive tract problems (flatulence, abdominal intestine) (40%) (Table 2). The results indicated that the cultured frog may suffer in the poor culture environment, e.g. high culture density, low quality of feed. According to Varga et al. (2019), the amphibian skin is a mucosal surface in direct and continuous contact with a microbially abundant aquatic and terrestrial environment. Therefore, frog skin is an important innate immune organ and first line of defense against pathogens in the environment. The skin disease may result from over load in the immune system capacity of this organ.

Table 2. Common diseases reported by frog culture farmers

Common disease symptoms	Reported farmers (%) (n=30)
Pus in liver	66.7
Hemorrhage	66.7
Eye and neck problems	33.3
Digestive tract problems	40
Skin ulcers	23.3

The symptoms of frog hemorrhage disease are described by the farmer as follows: frog belly is enlarged with water, the kidneys are swollen, hemorrhage in the lungs, but the liver color kept unchanged. Frogs usually lost their appetite, reduced activity, and died after a few weeks. The results of this study were different from the results indicated in the study of Supranee and Temdoung (1995) which found 6 disease occurred in cultured frog in Thailand included red leg, white patch, infectious dropsy, intestinal rot, pale skin disease, and paralysis. It may be assumed that the digestive tract problems found in this study are infectious dropsy (caused by *A. hydrophila*) or intestinal rot (yet unknown causative). The name of disease described by farmers in this study was different from that of the study by Supranee and Temdoung (1995), it may due to lack of diagnosis performance and in this study area. According to Crumlish and Inglis (1999), the common disease of frogs farmed in Southeast Asia was a septicemia disease which is commonly referred to 'red leg'. The clinical signs of this disease include ascites, loss of appetite, general lethargy and, haemorrhagic lesions on the hind legs and abdomen of infected frogs (Gibbs, 1963). According to Nguyen Chung (2007), the diseases may result from changing weather, high stocking density, poor preparation of ponds, and inappropriate feeding method. In addition, the source of water was supplied directly from the river and was not applied any water treatments. This led to pathogens easily spreading from neighboring farm households, causing great damage to the farmers.

Generally, disease occurs with the frequency of 1 and 4 times in a crop, especially in high-temperature season. However, very few farmers know pathogens that cause disease; farmers normally choose antibiotics as a treatment. According to Tran Hong Thuy (2007), when stocking frogs was at high density, frogs started to appear some bacterial diseases such as frog ulcers caused by *Aeromonas hydrophila*. This pathogen makes the frog reducing appetite, slow motion, skin sores, and haemorrhage in the abdomen. According to Roberts (1993), *A. hydrophila* is an opportunistic pathogen, natural distribution in fresh water, and often associated with hemorrhagic septicemia in stressed or immunocompromised aquatic animal. This bacterium occurs as part of the normal frog flora and is recovered from the internal organs of farmed *R. rugulosa* (Crumlish and Inglis, 1999). *A. hydrophila* is isolated from infectious dropsy frog and also is pathogen of skin sores are highly sensitive to florfenicol, doxycycline and tetracycline (Luu Thi Thanh Truc, 2008; Tu Thanh Dung et al., 2014). In general, it is essential to have appropriate disease management and farmer training to limit disease outbreaks which resulted in economic loss and to reduce the over-use of chemicals and antibiotics in disease prevention and treatment.

3.3. Chemicals use in frog farming in Dong Thap province

3.3.1. Antibiotics

There were nine types of antibiotics used to treat frog diseases. The application method is to mix these antibiotics in frog feed. Commonly used antibiotics were oxytetracycline (53.3%), doxycycline (63.3%), amoxicillin (53.3%), florfenicol (36.7%),

and the mixture of sulfamethoxazole and trimethoprim (46.7%). The other antibiotics such as tetracycline, enrofloxacin, ciprofloxacin, and rifampicin were used in some farms (Table 3). The farmers stated that the dosage of the antibiotic used is in accordance with the manufacturer's and the seller's instructions. In general, all farmers use antibiotics in the prevention after stocking the froglet and treatment of diseases in frogs. Two types of antibiotics belonging to the list of banned drugs issued in 2012 by the Ministry of Agriculture and Rural Development were enrofloxacin and ciprofloxacin (Ministry of Agriculture and Rural Development, 2016). The number of used antibiotics in frog farming is much lower than the number of antibiotics used in aquaculture in Vietnam, i.e., 9 types compared with 31 types of antibiotics uses (Rico et al. 2012). Although frog culture is not as popular as snakehead fish culture, the number of antibiotic use is slightly higher. In a survey of drugs and chemical use in snakehead fish done by Nguyen Quoc Thinh at al. (2020), there was 8 antibiotics found comparing with 9 antibiotics used in frog culture in this study. It may due to the attitude of aquaculture farmers, according to them, applying drugs is the first effective method to treat aquatic animal disease. The issue should be concerned is that two banned antibiotics presented in the list of used drugs, so local administrators should check chemicals/drugs stores and train the farmer about prohibited drugs and chemicals.

Table 3. Antibiotics used in frog aquaculture

Antibiotic	Ratio of household applied (%) (n=30)
Doxycycline	63.3
Oxytetracycline	53.3
Sulfamethoxazole + Trimethoprim	46.7
Amoxicillin	53.3
Florfenicol	36.7
Tetracycline	23.3
Enrofloxacin	20
Ciprofloxacin	10
Rifampicin	3.3

Results indicated that farmers used drugs and chemicals mainly based on their experiences (86.7%), the number of farms used chemical according to extension administrator was 43.3% (Table 4). Most farmers often buy drugs from stores (63.3%), but they often do not have separate drug and chemical storage cabinets. The farmers' knowledge about banned drugs and chemicals in aquaculture is still very weak, many farmers still do not know which drugs have been banned for use in aquaculture. Most households do not use labor protection in chemicals and drugs application. In spite of not using labor protection, most frog farmers stated that there were no clinical problems when using chemicals. It is recommended that farmers should store drugs and chemical in cupboards or containers with lid, and the container should be away from the living and cooking area. In addition, it is necessary to wash and clean all related equipment after any chemical touching.

Table 4. Attitude of frog farmer on chemical use (%)

	Ratio of household applied (%) (n=30)
Use chemical according to	
Extension administrator	43.3
Experience	86.7
Chemical/drug supplier	20
Chemical/drug use record	36.7
Storing chemical for use	63.3
Use protected equipment (mask, protected clothes, etc.)	0
Knowledge on banned chemicals	26.7
Experienced on abnormal clinical	0

3.3.2. Chemicals for disinfection, probiotics and others

Chemicals, disinfectants and anti-parasitic compounds used in frog culture included 8 types (Table 5).

Table 5. Chemicals for disinfection and supplementary

	Ratio of farm use (%) (n=30)
Disinfectants and water treatment agents	
$CaCO_3$	73.3
NaCl	63.3
Iodine	26.7
$CuSO_4$	20
BKC	16.7
Yuca products	13.0
$KMnO_4$	6.7
Chlorin	3.3
Supplementary and Vitamins	
Vitamin remix	90
Mineral	90
Probiotic (*Bacillus* spp.)	40

Antiseptic chemicals were widely used in frog farming, e.g., lime $CaCO_3$ (73.3% reported farmers), salt (63.3%), iodine (26.7%), copper sulfate (20%). In addition, frog farmers also use $KMnO_4$ and chlorine as disinfection agents. Many farmers used

nutritional supplements such as remix vitamins and minerals (90%), probiotic i.e., *Bacillus* spp. was also used (40%). The method of application was mixing 3-5 g of nutritional product into 1 kg of feed and feed it every 2 days to improve the growth and frog health (Table 5). Being similar to antibiotics use, the number and types of disinfection chemical used in frog culture is also similar to snakehead fish culture (Nguyen Quoc Thinh et al. 2020). That may due to farmer bough drugs and chemical in the same in drugs and chemical store in local area. However, the number of chemicals was lower than that used in aquaculture in Vietnam. According to Rico et al. (2012), there were 28 chemicals used in aquaculture in Vietnam. The reasons may due to that many managed water quality chemicals are not applied in frog culture such as alkalinity maintained chemicals, minerals, pesticides or inorganic fertilizers.

4. CONCLUSIONS AND RECOMMENDATIONS

The common frog disease symptoms included pus in liver, hemorrhage, and digestive tract problems. Nine antibiotics were used to control frog disease. The most commonly used antibiotics were amoxicillin, doxycycline, and oxytetracycline. Farmers used iodine, copper sulfate and BKC as disinfectant. Harvested frogs are not tested for residues of antibiotics may lead to a risk to customer health. Local administrator should train the frog culture farmer on knowledge of frog diseases and the use of drug and chemical in term of farmers' health and food safety.

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