Parasitic Infection in Common Lowland Frog (*Hoplobatrachus rugulosus* Wiegmann) and Disease Treatment

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Abstract:

Parasitic infection in common lowland frog (*Hoplobatrachus rugulosus* Wiegmann) in Maha Sarakham and Roi-et Provinces was studied. The samples of the present study were collected from 3 stages of frog (Tadpole, young and adult frog), totally 833 frogs. The results revealed that all stages were affected by parasites throughout the year, in which 91.72% evidently. Both ectoparasite and endoparasite were found to affect the frogs at 9.89 and 90.11%, respectively. The 2 genera of ectoparasite, belonging to ciliate protozoa including *Epistylis* and *Acineta*, while endoparasite especially intestinal parasite found 2 genera of ciliate protozoa including *Opalina* and *Balantidium* and 4 genera of parasitic rotifer including *Monostyla*, *Lecane*, *Philodina* and *Bracheonus*. The finding shows that *Opalina* and *Balantidium* were the highest prevalence and abundance in each stage. Sodium chloride, potassium permanganate and formalin were used as chemicals to treat ectoparasite and applied to control the parasites in tadpole and young frog. The results showed that 0.5-1% of sodium chloride could reduce number of parasites, showing 3-4% of tadpole and young frog died within 1 hour. Potassium permanganate and formalin could be effective to control the parasite at 10 ppm and 50-60 ppm, while the concentration at 30-40 ppm showed high survival rate after treament. For endoparasite treatment, 3 internal anti-parasite drugs including Albendazole, Metronidazole and Piperazine were used to treat *Opalina* sp. and *Balantidium* in tadpole and young frog via oral administration. This study found that Metronidazole showed high effectiveness to treat the parasites whereas another drugs were not effective to treat parasite.

Keyword: Common Lowland Frog, Ectoparasite, Endoparasite and Parasitic prevention

Introduction

Common lowland frog (*Hoplobatrachus rugulosus* Wiegmann), is an eatable frog and is one of an economically amphibians in Thailand. The frog production has widely supplied from both natural water resources and cultured frog farms especially in the northeastern and northern part. People can eat all stages (tadpole, young frog and adult frog) of this animal. Recently, frog culture has been popular in order to low cost production, short time raring and high price per unit [1]. However, an intensive farming, as high density commonly causes poor environment, animal stress and finally disease induction that can affected by bacteria, parasite, fungi, viruses and worms [2], [3].

Parasitic diseases of frog have a superior position and have received a significant attention in Thailand, one of tropical country. Away from their direct damage effect on frog tissues, parasitic agents may act as stress factors rendering the frog more susceptible to other diseases [4]. Also the drastic indirect effect played by frog parasites; their retardation of frog growth with combination of frog mortality constitute the most economical impact concerning frog production. Parasitic infection in frog could normally found both external and internal infection at all stages, the common and serious parasitic diseases in aquaculture. Ectoparasites of frog could be considered as one of the most prevalent causes of diseases affecting skin and gills cause of gill inflammation and distortion of normal anatomy which impairing their respiratory foundation. It is the primary site of nitrogenous waste excretion and plays an important roles in ionic balance [5], [2] in skin causing irritation, inflammation and loss of the surface epithelium which this in turn open the way for secondary invaders [6]. In addition, endoparasites had been some reports of helminthic parasites recovered from frogs. Some nematodes [7], [8], [9], trematodes and cestode [10] had been described from the intestine of several species of frogs.

The treatment of parasitic infection in frog is usually applied chemicals and drugs that may toxic to frogs. Fortunately, it usually takes higher concentration of the chemicals and drug to harm the frogs than it does to harm the pathogen. Chemical treatment may be linked to side effects such as toxic stress using high concentration [11] However, the chemo therapeutants are still necessary to test in controlled laboratory studies. Therefore, in the present study aimed to investigate the prevalence of parasitic diseases of cultured frog and determined the effects of some chemicals and drugs for treatment both ectoparasites and ecdoparasites on infecting frogs as eradicate of parasitic infection.

Materials and Methods

Sampling sites and frog collection

The 3 stages of diseased frog (tadpole, young frog; 25-45 days old and adult frog; market size) were collected from 3 farms in Nadoon District (15º40´18´´N, 103º12´41´´E; elevation of 174 m) and 1 farm in Muang district (16º11´48´´N, 103º16´08´´E; elevation of 146 m), Maha Sarakham province and 1 farm from Tawatburi district (16º02´47´´N, 103º43´33´´E; elevation of 145 m), Roi-Et province. Affected frogs, tadpole, young frog and adult frog were sampled monthly for 1 year (12 times), totally 320 tadpoles, 330 young frogs and 183 adult frogs were sampled for parasitic examination.

Parasitic examination

Diseased frogs were collected and kept in plastic containers, then transported back to the laboratory. The frogs in the laboratory were placed in the glass tanks with tap water and holding substrates for young and adult frogs. Clinical signs of skin, appendage and external organs of each specimen were firstly examined by the naked eyes for detection of any macroscopically visible lesions. Samples of mucus were scraped gently from the external organs and wound, then spread on a clean slide and freshly examined under phase contrast microscopic examination for ectoparasite. Affected frogs were euthanized with sodium pentobarbital and dissected to open body cavity, the intestine liver and stomach were removed and placed in slide glass containing a physiological solution and examined endoparasites using a compound microscope at 40, 100 and 400x magnifications. Parasitic genera were identified on the basis of their morphological characteristics according to Sirikanjana [12] Chatmongkolkul *et al*. [13] Purivirojkul [14] May [15] Keppeler *et al*. [16] Hossack *et al*. [17] and Comas *et al*. [18]. Prevalence and mean abundance were calculated according to Bush *et al*. [19].

Parasitic treatments

Experimental design

There was 2 experiments including bath challenge of 3 chemicals (sodium chloride, formalin and potassium permanganate) to control ectoparasite and oral administration of 3 anti-parasitic drugs (albendazole, piperazine and metronidazole) to control endoparasite. The experimental design was a completely randomized block with 6, 9 and 9 treatments for sodium chloride, formalin and potassium permanganate, and three replicates with 10 frogs each of tadpole and young frog. In vivo tests consisted of therapeutic baths of 8 days with various sodium chloride, formalin and potassium permanganate concentrations (Table 1). Therapeutic baths were performed in 10 L glass aquaria, with a static water system, at 25ºC and without aeration. Therapeutic oral administration using 3 anti-parasitic drugs with 7 treatments of albendazole, piperazine and metronidazole, and three replicates with 10 frog each of tadpole and young frog (Table 1) were carried out in 10 L glass aquaria, with a static water system and without aeration.

Diseased frogs: maintenance and monitoring

The 2 stages of frog (tadpole and young frog with average weight of 1.12±3.55 and 5.38±4.25 g, respectively), infested naturally with parasites or showing parasitic infection signs in plastic (PE) earthen pond from commercial frog farm in Nadoon District, Maha Sarakham Province, were used to infest experimental frog. The stock of infested frogs was held in two 250 L tanks for use in trial treatment. In addition, the tank of un-infested frog was held in 250 L tanks to provide frog for un-infested control treatments and for periodic addition to the infested stock.

Parasites were monitored by microscopic examination of both external and internal organs. Frogs were euthanized with sodium pentobarbital. In tadpole, gill arch was removed from each frog and mucus was scraped from the total left-hand side and caudal fin, and in young frog the scraping was along the skin and appendages. The tissue samples were placed on microscope slides, covered with a coverslip and examined at 40-400 magnification to search for ectoparasites. Endoparasites were examined from intestine, liver, stomach and body cavity. A total of parasites were found in the external and internal organs of frogs, with a prevalence of 100%, and mean abundance of 137.5 ± 17.2. The remaining frogs were subjected to food deprivation for 24 h prior to the start of therapeutic baths and oral administration for gastrointestinal emptying.

Treatment of frog parasites

To test the efficiency of 3 chemicals on natural infested frogs, with the treatment concentrations present in Table 1. The experimental frogs were test in 3 chemicals for 8 days baths and feed daily, in order to collect the gill, appendage, fin, mucus and skin, one frog of each replicate was daily used to evaluate the efficacy of the parasitic treatments at day 0, 2, 4, 6 and 8. Samples were scraped gently, then spread on a clean slide and freshly examined under phase contrast microscopic examination for ectoparasite. After the therapeutic baths, the remaining frogs were kept for a recovery period of 8 days, with clean water. At the end of this period, the parasitic load of the frog, the occurrence of mortality were also evaluated.

To test the efficiency of 3 anti-parasitic drugs on natural infested frogs by fed with floating commercial feed mixed with the drugs using the concentrations present in Table 1. The experimental frogs were fed 2 times a day (at 8.00 and 16.00) for 7 days, in order to collect the internal organs, one frog of each replicate was daily used to evaluate the efficacy of the parasitic treatments at day 0, 2, 4, 6 and 8. Samples were euthanized with sodium pentobarbital and dissected to open body cavity, the intestine liver and stomach were removed and placed in slide glass containing a physiological solution and examined endoparasites using a compound microscope. After the therapeutic feeds, the remaining frogs were kept for a recovery period of 7 days, with clean water. At the end of this period, the parasitic load of the frog, the occurrence of mortality were also evaluated.

Statistical analysis

Data were expressed as mean of 3 replications ± s.d. (standard deviation). All statistical analyses were conducted using one-way ANOVA. Differences among groups were compared using Duncan's Multiple Range test. A p-value of less than 0.05 was taken to indicate statistical significance.

Table 1 Treatment concentrations of anti-parasitic chemicals and drugs for bath and oral administration Challenges

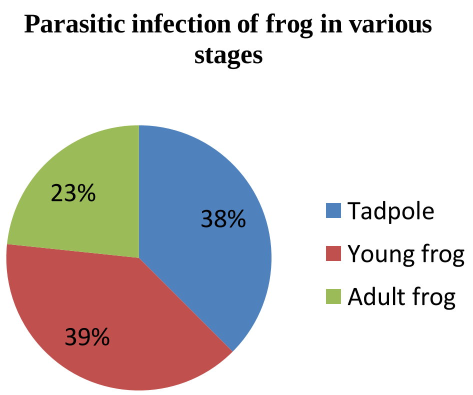
Anti-parasitic chemicals and drugs	Concentrations (% and ppm)								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Bath challenge									
Sodium chloride: NaCl (%)	0	0.5	1	1.5	2	3	4	-	-
Formalin (ppm)	0	10	20	30	40	50	60	70	80
Potassium permanganate: $KMnO_4$ (ppm)	0	0.5	1	2	3	4	6	8	10
Oral administration									
Albendazole (ppm)	0	12.5	25	50	100	200	400	-	-
Piperazine (ppm)	0	12.5	25	50	100	200	400	-	-
Metronidazole (ppm)	0	12.5	25	50	100	200	400	-	-

Results and Discussions

The distribution and prevalence of parasitic infection of frog

Inspection a total of 833 frogs, including tadpole, young frog and adult frog 320, 330 and 183 frogs, respectively, were collected from Maha Sarakham and Roi-Et Provinces throughout the year. The results revealed that all stages were affected by parasites throughout the year, in which 91.72% evidently. Both ectoparasite and endoparasite were found to affect the frogs at 9.89 and 90.11%, respectively (Figure 1). Disease frogs from Nadoon 1, Nadoon 2, Nadoon 3, Muang 1 and Tawatburi 1 were collected and found in 200, 202, 191, 65 and 175 infested frogs with infection rates 24.68, 24.4, 22.58, 8.13 and 20.21%, respectively. The distribution and prevalence of the parasites revealed infection with 2 genera of ectoparasite, belonging to ciliate protozoa including *Epistylis* and *Acineta*, while endoparasite especially intestinal parasite found 2 genera of ciliate protozoa including *Opalina* and *Balantidium* and 4 genera of parasitic rotifer including *Lecane*, *Monostyla*, *Bracheonus* and *Philodina* with prevalence 8.88, 8.4, 86.55, 59.06, 5.16, 4.32, 3.72 and 1.92%, respectively (Table 2). Mixed infection with more than 2 parasitic genera among ectoparasies, endoparasites or both were commonly occurred. The rotifer is zooplankton that can found in natural water resources but some of rotifer could become pathogenic parasites to caused damage of various kind of host such as the genera *Brachionus*, *Volvox*, *Lecane*, *Monostyla* and *Philodina* [15], [16], [20].

This finding shows that *Opalina* was the highest prevalence and abundance in each stage, followed by *Balantidium*, *Epistylis*, *Acineta*, *Lecane*, *Monostyla*, *Bracheonus* and *Philodina*, respectively. Except the genus *Acineta* could found only in tadpole. Frog infected with ciliate protozoa *Epistylis* and *Acineta* showed slimy pale skin with sever blood spots scattered on the body especially at the base of appendage and skin. While the endoparasites especially *Opalina* and *Balantidium*, the frogs became skinny, lean, loss of appetite or anorexia and apathy. In severe case there was no food in the intestine and large amount of parasites were found, however, these was not caused to be death. Concerning seasonal prevalence in investigated that highest infection rate with *Opalina* and *Balantidium* was recovered in October and September followed by April, May, March and June. The lowest infection was November, December, January and February, respectively. The mixed infection of the parasites was recorded in rainy season prevalence of 53.7%. There was found that farm management system affected to parasitic infection, especially water quality management. Cysts of these ciliates protozoa are passed in faecal material but they are difficult to detect and identify. In contrast, trophozoites present in colonic content can be differentiated on the basis of their morphological characteristics. It would therefore be better to collect samples of at postmortem rather than faecal samples to screen for enteric ciliates in frogs. The examination of postmortem samples would also facilitate the detection of endocommensal opalinids present within the colon and rectum of amphibian hosts [21]. Similar to Siririkanont [22] reported that parasitic infection of frog in the southern part of Thailand found 3 genera of *Opalina* sp., *Protoopalina* sp. and *Balantidium* sp. On the other hand, stocking density and water quality management was influence to parasitic infection. However, Goldberg *et al*. [23] report that the endoparasites in ranid frogs from Papua New Guinea were found 1 species of Cestoda, 3 species of Digenea, 18 species of Nematoda, 2 species of Acanthocephala and 1 species of Pentastomida. Other hosts are listed in Bursey *et al*. [24] and include frogs, lizards, and a mammal from Australia. Rhabdias australiensis was described by Moravec and Sey [25] from Rana daemeli collected in Queensland, Australia. Papua New Guinea is a new locality record, and New Britain is a new island record. Seuratascaris numidica is known from a variety of anurans from Europe, the Orient, and Australia [26].



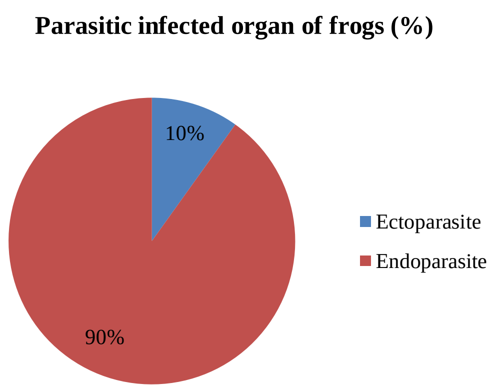


Figure 1 Parasitic infection and distribution of frog in Maha Sarakham and Roi-et province

Table 2 Parasitic infection, distribution, prevalence and mean abundance of various stages of frog

Frog	Isolated frogs/ Infected habitats	Infected frogs	Total parasites	Parasitic genera	Prevalence (%)	Mean abundance ±SD
All stages	833 / skin	74	1464	*Epistylis*	8.88	19.78 ± 2.85
		55	1045	*Acineta*	8.4	19.00 ± 3.35
	intestine	721	36458	*Opalina*	86.55	50.01 ± 2.74
		492	14465	*Balantidium*	59.06	29.40 ± 3.18
		43	*625*	*Lecane*	5.16	14.53 ± 4.18
		36	358	*Monostyla*	4.32	9.94 ± 2.81
		31	298	*Bracheonus*	3.72	9.32 ± 3.36
		16	143	*Philodina*	1.92	8.94 ± 2.45

Effect of 3 chemicals on ectoparasitic treatment in frog.

Chemicals are routinely used in aquaculture for treating parasitic and fungal infections of aquatic animals. This study was intended to assess the effect of 3 of the commonly used chemicals to control parasitic infection in common lowland frog.

Ectoparasitic treatments with sodium chloride of frogs in this study shown at the concentration of 0.5-1% could totally eliminate number of parasites at day 8 and 4-6, respectively in both tadpole and young frog, at concentration of 1-1.5% parasites were eliminated at day 4 but at 1.5% tadpole and young frog were died at day 4 and 6 post exposure. While at concentration 3-4% tadpole was eradicated parasite at day 2 and died within 3 days, and young frog was eradicated at day 4 and died at day 5-6 (Table 3). The prevalence of replicates of control was 60 to 100%, while in treatments 2-7, the prevalence was reduced to zero on skin and gills. In the analysis performed per infected organ, the highest prevalence was found on skin 46%, appendage 24% and gills 30%. The concentrations 0.5-1% was effective for the control of parasites. Vargas *et al.* [27] used sodium chloride a dose of 3% sodium chloride for 10 min reduced the prevalence of ciliate protozoa fron 46 to 2%. The action mechanism of sodium chloride, which eliminates protozoa and bacteria [28] controlled by an osmotic pressure difference. Color changes also occurred in fish exposed to doses of sodium chloride treatments at different doses, observed as darkening of the skin in response to the treatments. Nouh and Selim [29] presented similar results in a study by exposing to *O. niloticus* (25 ppm) of formalin, with organisms presenting a darkening of the skin among other symptoms. The change in color can have various causes, including the presence of toxic substances in wastewater [30], [31], [32], such as chlorine. However, the sodium chloride application could reduce the mortality then mantain the high survival rate of the juvenile that infected by *Argulus* for early days of infection. On the other hand, highest dose of sodium chloride give more protection for fish that attacked by parasite that is showed by highest survival rate and salt could improve the osmoregulation of fish and probably inhibit the parasites [33]

Formalin is an effective chemotherapeutic agent used to treat various external parasitic infections in fish and other aquatic animals. The concentrations typically used for prolonged bath treatment are 25-500 ppm [34], [35], whereas the concentration suggested for short-term bath treatment is 250 ppm for duration of not more than 60 min [36]. However, in this study, formalin was proven effective against ectoparasites; *Epistylis* and *Acineta*, natural infested in tadpole and young frog found that at the concentration of 30-40 ppm was not effective post exposure from 1-8 days but shown high survival rate of both tadpole and young frog. While at the concentration of 50-60 ppm parasite was reduced and eradicated in young frog but tadpoles were died at day 8 and 6. At concentration of 70-80 ppm could control the parasites at day 2 but after day 3 and 4 all tadpole were died. The survival of the parasites was very high at the suggested range of concentration and duration of the treatment (Table 4). Therefore formalin is considered a suitable treatment for ectoparasites of frog at 50-60 ppm for long bath but at 70-80 ppm may use for short period or less that 36 h. In addition, Rowland *et al.* [37] suggested that the used of formalin 30 ppm could control ichthyophthiriosis, but at 20 ppm remained infested with theronts and trophonts on day 17 and survival at both concentrations was 100%.

The action mechanism of formalin is alkylation of chemical groups of proteins, and nucleic acids. Alkylating agents are generally attached to the methyl or ethyl group of proteins and DNA, translating these molecules as nonfunctional and cause the death of the microorganism [38]. As for possible damage, observations of the gills in organisms exposed to formaldehyde treatments showed inflammation in the gill lamellae. Formalin is used as chemotherapeutic agent and may cause damage to branchial tissue because these are substances that have a toxic effect on the fish or others as observed by Mert *et al.* [39].

Potassium permanganate (KMnO4) has been tested as a anti-parasite and fungicide on several species of aquatic animals. Effective dosages have been found to vary with the fish species tested. In this study the effect of KMnO4 to the ectoparasites of frogs found that at concentration of 10 ppm could control the parasite at day 1-5 after that the parasites could be increased, the other concentration were reduced number of parasite but increased later days (Table 5). KMnO4 is an oxidizing agent that has been used for many years in aquaculture. As an oxidizer, it is able to chemically "burn up" organic material. This includes undesirable organic matter such as bacteria, parasites, and fungus, as well as desirable material such as gill tissue and mucus [40]. Because the chemical cannot distinguish between desirable and undesirable organic matter, it is up to the individual to use the chemical in a manner that results in maximum benefit and minimum harm to treated aquatic animals. KMnO4 can be administered at a concentration of 2 ppm as a long-term bath (four-hour minimum) in fresh water or salt water systems. KMnO4 is also reasonably safe to use in recirculating systems and has minimal impact on biofilters when used at 2 ppm. Treated water should retain the purple coloration for at least four hours. If a total application of 6 ppm KMnO4 does not result in maintenance of the purple color for at least four hours, the system should be cleaned [34]. Most of the organisms that are treated with KMnO4 thrive in an organically rich environment; therefore, improved sanitation can have a tremendous impact on treatment efficacy. Potassium permanganate can also be used as a short-term bath at concentrations of 10 mg/L for 30 minutes. At this concentration, careful observation of fish is mandatory to avoid mortality [41].

Table 3 Number of ectoparasite survived in the Sodium chloride (NaCl) treatment of tadpole and young frog

NaCl (%)	**Days post exposure of tadpole**					**NaCl** (%)	**Days post exposure of young frog**				
	0	**2**	**4**	**6**	**8**		**0**	**2**	**4**	**6**	**8**
0	++++	++++	++++	+++	++++	0	++	++	++	+	++
0.5	+++	+++	++	+	-	0.5	++	++	+	+	-
1	+++	++	-	D	D	1	++	++	+	-	-
1.5	+++	++	D	D	D	1.5	++	++	+	-	D
2	+++	+	D	D	D	2	++	+	-	D	D
3	+++	-	D	D	D	3	+	+	-	D	D
0	+++	-	D	D	D	0	++	+	-	D	D

Number of parasite: ++++ = >50 (Most); +++ = 31-50 (More); ++ = 11-30 (Moderate); + = 1-10 (low); - = 0 (Non) and D= Death

Table 4 Number of ectoparasite survived in the formalin treatment of tadpole and young frog

Formalin (ppm)	**Days post exposure of tadpole**					Formalin (ppm)	**Days post exposure of young frog**				
	0	**2**	**4**	**6**	**8**		**0**	**2**	**4**	**6**	**8**
0	++++	+++	++++	++++	++++	0	++	++	+	++	++
10	++++	+++	+++	++++	+++	10	++	++	+	++	+
20	+++	+++	++	+++	++	20	+	+	+	+	+
30	++++	+++	+++	-	-	30	+	++	+	+	+
40	++++	+++	+	-	-	40	++	+	+	-	-
50	+++	++	++	-	D	50	++	+	+	-	-

60	+++	+	-	D	D	60	++	+	-	-	D
70	+++	-	D	D	D	70	+	-	-	D	D
80	++++	D	D	D	D	80	++	-	-	D	D

Number of parasite: ++++ = >50 (Most); +++ = 31-50 (More); ++ = 11-30 (Moderate); + = 1-10 (low); - = 0 (Non) and D= Death

Table 5 Number of ectoparasite survived in the potassium permanganate ($KMnO_4$) treatment of tadpole and young frog

$KMnO_4$ (ppm)	**Days post exposure of tadpole**					$KMnO_4$ (ppm)	**Days post exposure of young frog**				
	0	**2**	**4**	**6**	**8**		**0**	**2**	**4**	**6**	**8**
0	++++	+++	++++	++++	+++	0	++	++	++	++	+++
0.5	+++	+++	+++	+++	++	0.5	++	++	++	++	++
1	+++	+++	+++	+++	++	1	++	++	++	++	+
2	+++	+++	+++	+++	+++	2	+	++	+	+	-
3	++++	+++	+++	++	++	3	++	++	+	++	+
4	++++	+++	++	+	+	4	++	++	+	+	+
6	+++	++	++	++	++	6	++	++	+	+	-
8	+++	+++	++	++	-	8	++	++	+	+	+
10	+++	++	++	+	-	10	+	++	+	-	-

Number of parasite: ++++ = >50 (Most); +++ = 31-50 (More); ++ = 11-30 (Moderate); + = 1-10 (low) and 0 = 0 (Non)

Metronidazole, albendazole and piperazine are a commercially available antibiotic used to eliminate anaerobic bacteria and protozoans from human and animal hosts. We hypothesized that administration of metronidazole to an common lowland frog host in culture frog farm would result in the clearance of its ectoparasites population, especially *Opalina* and *balentedium*.

In present study, oral administration with albendazole and piperazine feed to control internal parasite found that these anti-parasitic drugs could not reduce *Opalina* and *balentedium* in the intestine as same as control groups in both tadpole and young frog (Table 6-7). However, Albendazole and piperazine are anti-anthelmintic or anti-worm medication. It prevents newly hatched insect larvae (worms) from growing or multiplying in your body and used to treat certain infections caused by worms such as pork tapeworm and dog tapeworm. Therefore, these could not effective to ciliate protozoa in frog,

Metronidazole could effective to *Opalina* and *balentedium* in the intestine at 100-200 ppm with feed that was reduce number of parasites and eradicated within day 6 and 4, respectively, while 400 ppm was also reduced the parasite and eradicated but frog were less uptake in both stages. The concentration of 12.5 and 25 ppm were not effective to the parasites, but at 50 ppm could reduce the number of parasites (Table 8). Metronidazole is a versatile drug that is bacteriocidal, trichomonacidal, and amoebicidal. The termicidal, when used medically, refers to something that kills rather than suppresses (static) the organisms it targets. The cidal action against susceptible bacteria appears to disrupt DNA and nucleic acid synthesis in the bacteria. Metronidazole is especially effective against anaerobes (bacteria that live in the absence of oxygen) and is considered to be one of the drugs of choice for anaerobic infections. The mode of antiprotozoan action is unknown [42]. However, Nickol and Tufts [6] reported that 10 ppm of metronidazole injected orally by micropipette into posterior oropharynx could effective to control Opalinids in juvenile Woodhouse's toads (*Bufo woodhousii*). Moreover, these suggested that the treating juvenile *B. woodhousii* with a single oral dose of metronidazole results in rapid, reliable, and well-tolerated clearance of opalinids from the gastrointestinal tract. While, Kolmstetter *et al*. [43] indicated that a metronidazole dosage of 20 mg/kg PO q 48 hr should be adequate for the treatment of most anaerobic infections in yellow rat snakes (*Elaphe obsoleta quadrivitatta*) by injection. The most recent study was performed on red rat snakes and found that an oral dose of metronidazole at 50 mg/kg every forty-eight hours was more than enough to control susceptible anaerobic bacteria and protozoa [42]. Jacobson and Kollias [44] reported deaths in indigo snakes at dosages above 100 mg/kg; however, dosages at 40 mg/kg have been given safely. However, in this study showing high dosage to treat the parasites that may cause by the given method that need to get high concentration.

Table 6 Number of endoparasite survived in the Albendazole (Alben.) treatment of tadpole and young frog

Alben. (ppm)	Days post exposure of tadpole					Alben. (ppm)	Days post exposure of young frog				
	0	**2**	**4**	**6**	**8**		**0**	**2**	**4**	**6**	**8**
0	++++	++++	++++	++++	++++	0	++++	++++	++++	++++	++++
12.5	++++	++++	++++	++++	++++	12.5	++++	++++	++++	++++	++++
25	++++	++++	++++	++++	++++	25	++++	++++	++++	++++	++++
50	++++	++++	++++	++++	++++	50	++++	++++	++++	++++	++++
100	++++	++++	++++	++++	++++	100	++++	++++	++++	++++	++++
200	++++	++++	++++	++++	++++	200	++++	++++	++++	++++	++++
400	++++	+++	++++	+++	+++	400	++++	+++	++++	+++	+++

Number of parasite: ++++ = >101 (Most); +++ = 51-100 (More); ++ = 21-50 (Moderate) and + = 1-20 (low)

Table 7 Number of endoparasite survived in the Piperazine (Piper.) treatment of tadpole and young frog

Piper. (ppm)	Days post exposure of tadpole					Piper. (ppm)	Days post exposure of young frog				
	0	**2**	**4**	**6**	**8**		**0**	**2**	**4**	**6**	**8**
0	++++	++++	++++	++++	++++	0	++++	++++	++++	++++	++++
12.5	++++	++++	++++	++++	++++	12.5	++++	++++	++++	++++	++++
25	++++	++++	++++	++++	++++	25	++++	++++	++++	+++	++++
50	++++	++++	++++	++++	++++	50	++++	++++	++++	++++	+++
100	++++	++++	++++	++++	++++	100	++++	++++	++++	+++	++++
200	++++	++++	++++	++++	++++	200	++++	++++	++++	++++	+++
400	++++	++++	++++	+++	+++	400	++++	++++	++++	+++	+++

Number of parasite: ++++ = >101 (Most); +++ = 51-100 (More); ++ = 21-50 (Moderate) and + = 1-20 (low)

Table 8 Number of endoparasite survived in the Metronidazole (Metron.) treatment of tadpole and young frog

Metron. (ppm)	Days post exposure of tadpole					Metron. (ppm)	Days post exposure of young frog				
	0	**2**	**4**	**6**	**8**		**0**	**2**	**4**	**6**	**8**
0	++++	++++	++++	++++	++++	0	++++	++++	++++	++++	+++
12.5	++++	++++	++++	++++	+++	12.5	++++	++++	++++	++++	+++
25	++++	++++	++++	+++	+++	25	++++	++++	++++	+++	+++
50	++++	+++	++	++	+	50	++++	+++	++	++	+
100	++++	++	+	-	-	100	++++	++	++	-	-
200	++++	++	-	-	-	200	++++	+	+	-	-
400	++++	++	-	-	-	400	++++	+	-	-	-

Number of parasite: ++++ = >101 (Most); +++ = 51-100 (More); ++ = 21-50 (Moderate) and + = 1-20 (low)

Conclusions

It is concluded that parasitic infection of common lowland frog from plastic-earthen ponds could occur throughout the year, in which 91.72% evidently. There was found ectoparasite and endoparasite at 9.89 and 90.11%, respectively, according to water quality and farm management. The ectoparasite, belonging to ciliate protozoa including *Epistylis* and *Acineta*, while endoparasite found 2 genera of ciliate protozoa including *Opalina* and *Balantidium* and 4 genera of parasitic rotifer including *Monostyla*, *Lecane*, *Philodina* and *Bracheonus*. Ectoparasite treatment challenge using NaCl, $KMnO_4$ and formalin to control the parasites in tadpole and young frog showed that 0.5-1%, 10 and 30-50 ppm were effective to control the disease. More higher concentrations may damaged and caused of death. Endoparasite treatment using 3 internal anti-parasite drugs: Albendazole, Piperazine and Metronidazole to tadpole and young frog with oral administration found that Albendazole, Piperazine could not effective to the parasites, only metronidazole showed effective to treat the parasites at 100 ppm and above concentration could eradicated parasite but the frog was less of apatite.

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