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Original Research Articles

Cercariae from snails in rice fields and canal supplying water directly to *Trichopodus microlepis* fish cultured in earthen ponds in Binh Chanh district, Ho Chi Minh City, Vietnam.

Pham C. Thien[1a], Ho T. Manh[2], Duong T. Quyen[3], Nguyen T.L. Xuan[4], Nguyen T. Lan[5], Nguyen M. Hung[6]

[1] Department of Biology, Ho Chi Minh City University of Education, [2] Nguyen Thi Minh Khai High School, Tan Dong Hiep Ward, Di An City, Binh Duong Province, Vietnam, [3] Ho Chi Minh City University of Education, [4] Tan Thong Hoi High School, Cu Chi District, Ho Chi Minh City, Vietnam, [5] Nguyen Huu Canh High School, Binh Tan District, Ho Chi Minh City, Vietnam, [6] Institute of Ecology and Biological Resources, Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Cau Giay, Hanoi 10072, Vietnam

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Snails are the first intermediate host in the life cycle of trematodes. Previous research found that *Trichopodus microlepis* fish, cultured in earthen ponds in Tan Nhut Commune, Binh Chanh District, Ho Chi Minh City, Vietnam had a high infection rate with metacercariae of *Centrocestus formosanus* and *Procevorum* sp. It is necessary to investigate whether habitats supplying water to the ponds may contribute to the transmission by carrying intermediate hosts and/or cercariae into these ponds or not. A study of cercariae and snail composition in rice fields and canal supplying water directly to these *Trichopodus microlepis* ponds was carried out with six samplings in the years 2022 and 2023. A total of 1,151 snails were collected, and nine snail species belonging to 8 genera and 6 families were identified. Three snail species in both rice fields and canal were infected with trematode (cercariae stage) including *Bithynia siamensis, Lymnaea viridis* and *Indoplanorbis exustus*. The overall prevalence in the dry season (25.9%) was significantly higher than in the wet season (10.6%) ($P<0.05$). Three cercariae morphotypes were recovered from snails: xiphidio (in all three snails), echinostome (in *Lymnaea viridis* and *Indoplanorbis exustus*), and pleurolophocercous in *Bithynia siamensis*. This finding shows that *Bithynia siamensis* is one of the infected sources for *Trichopodus microlepis* fish cultured in ponds, as pleurolophocercous is also the cercariae in the family of Heterophyidae (including *Centrocestus formosanus* and *Procevorum* sp.). Further research should be done to clarify the transmission of fish-borne zoonotic diseases to cultured *Trichopodus microlepis* in other habitats including the actual fishponds, and sources of trematodes in order to produce *Trichopodus microlepis* free of metacercariae, contributing to clean aquaculture development and safe food for local people in the area.

INTRODUCTION

Binh Chanh is a suburb district where freshwater aquaculture developed most in Ho Chi Minh City, Vietnam, with a total area of 1,056.18 hectares.[1] In 2019, Pham et al. found that *Trichopodus microlepis* fish cultured in ponds in Tan Nhut Commune, Binh Chanh District, were infected with metacercariae of *Centrocestus formosanus* and *Procevorum* sp., with a prevalence of 65.5%. Snails from these ponds were collected, but no cercariae were found. Pham and Nguyen[2] carried out research in two sixth-level canals in Binh Chanh District; these canals connect the river and canal supplying water directly to fishponds. The result showed that 486 samples of snails, including 10 species, 10 genera, and 5 families, were collected and identified using the morphological method; however, all sampled snails were free of cercariae. The question still remained regarding the source of cercariae in *Trichopodus microlepis*; therefore, a study on cercariae from snails in rice fields surrounding fishponds and canal supplying water directly to fishponds of *Trichopodus microlepis* fish in Tan Nhut Commune, Binh Chanh District, was carried out to determine whether cercariae existed in snails here or not.

Research on trematode is needed because foodborne trematodiasis is an important public health problem infecting humans and mammals worldwide[3] particularly in Southeast Asia.[4] Little is known about the clinical importance of infections with minute intestinal flukes, but heavy

a Corresponding author. E-mail: thienpc@hcmue.edu.vn

infections can cause serious gastrointestinal symptoms.[5] For liver flukes, infections have significant human-health impact and cause substantial clinical or subclinical disease.[6] The life cycles of intestinal flukes and liver flukes are similar.[7] The heterophyid digeneans have a three-host life cycle involving snails as the first intermediate host, fish as the second intermediate host, and fish-eating animals and humans as the definitive host.[8]

Adult worms begin releasing eggs about one month after ingestion of infective metacercariae. The eggs are passed down the bile duct and excreted in the feces.[9] When eggs are passed from the intestinal tract in feces and into a body of water (pond, rice field, stream, canal, river, reservoir), they are ingested by a suitable snail host. Many cercariae are then produced and are released from the snail and swim into the water.[10,11] Cercariae remain active for 12-24 hours and most die after 72 hours. While they are free in the water, they are attracted to close movements of fish, attach to the fish and penetrate into the fish's tissue within 5-10 minutes.[11] It is obvious that the sources of trematodes infected fish culture in ponds can be from cercariae released from snails in ponds, snails in water supply from canals and snails in the rice fields or other natural resources around the ponds and invade fishponds through canals and run-off.

The snail intermediate hosts for the heterophyid trematode species are found variously in the previous research. *Melanoides tuberculata*, *Thiara scabra*, and *Terabia granifera* are the first intermediate hosts of heterophyids.[12] *Melanoides tuberculata* is the host of *Centrocestus formosanus*.[13] *Tarebia granifera* was found commonly infected with *Centrocestus formosanus* in Thailand.[14] *Sermyla riquetti* has been found to be the host of *Procerovum calderoni* in the Philippines.[15] *Bithynia fuchsiana*, *Parafossarulus striatulus* (Bithyniidae) and *Melanoides tuberculata* (Thiaridae) in the North of Vietnam were the hosts of 12 species of trematoda.[16]

Bui et al.[17] stated that there were 15 snail species from the research, with 12 of them collected in rice fields and small canals in Nam Dinh province, Vietnam, with most belonging to the families Bithyniidae, Stenothyridae and Planorbidae. Nguyen et al.[18] conducted research in Tuy An District, Phu Yen Province, Vietnam and found 11 snail species (canals had 7 snail species), among which *Melanoides tuberculata* and *Bithynia* sp. were infected with more cercariae of trematodes than the others. Nguyen et al.[19] surveyed the lowland areas of Binh Dinh and Phu Yen provinces in south central Vietnam and collected 13 snail species, in which cercariae were found in 5 snail species, and among these, *Indoplanorbis exustus* had the highest overall prevalence of infection (38.5%). Nguyen and Pham[20] collected 8 snail species in rice fields of Binh Khanh and Ly Nhon communes of Can Gio District, Ho Chi Minh City, Vietnam, and recovered cercariae from *Bithinya* sp. and *Melanoides tuberculata* including xiphidio cercariae, furcocercous cercariae and pleurolophocercous cercariae. Pham and Nguyen[21] stated that pleurolophocercous cercariae were the cercariae of Heterophyidae and Opisthorchidae. Therefore, Binh Chanh District, adjacent to Can Gio District, is also a district of Ho Chi Minh City, and if snails have pleurolophocercous cercariae, one of the questions for metacercariae in *Trichopodus microlepis* in this district can be answered. Consequently, the study on cercariae from snails in rice fields and canal supplying water directly to *Trichopodus microlepis* fishponds in Tan Nhut Commune of Binh Chanh District was implemented.

MATERIALS AND METHODS

STUDY AREAS

Fish was cultured in earthen ponds which got water supplied directly from canals in Binh Chanh District of Ho Chi Minh City. The infected *Trichopodus microlepis* fish in earthen ponds with fishborne zoonotic trematodes were found in Tan Nhut Commune, Binh Chanh District; therefore, Tan Nhut rice fields surrounding these ponds and Tan Nhut canal supplying water directly to these earthen fishponds were chosen for research.

SAMPLING OF SNAILS IN RICE FIELDS SURROUNDING TRICHOPODUS MICROLEPIS FISHPONDS

Two cross-sectional studies on snails were carried out in October 2022 (the wet season) and in April 2023 (the dry season) in Tan Nhut rice fields. Snail sampling was done by using hand net and hands with gloves to collect snails in the standard cell of 0.4m wide x 10.0 m long x 0.1 m deep along the bank of rice fields. Fifteen points for sampling were done in each rice field with a distance of 500m between the two sampling sites. Each sample was washed, and collected snails were transferred to cloth bags and transported to the laboratory for analysis. Snails were identified to species following the keys of Dang et al.[22]

SAMPLING OF SNAILS IN CANAL SUPPLYING WATER TO TRICHOPODUS MICROLEPIS FISHPONDS

Snail sampling was conducted using a 25-cm wide dredge to scrape the canal bottom from 1.0 m out from the canal bank. Two cross-sectional studies were carried out in the dry season (January and March 2023) and two in the wet season (June and August 2023) in Tan Nhut canal. A total of three such samples were taken in this canal with a distance of 100 m between sampling points. The first sampling point was at the supplying water monk to the *Trichopodus microlepis* fishponds, while the two subsequent sampling points were on the right and on the left of the monk, each with a distance of 100 m. Each sample was washed, and collected snails were kept in cloth bags and transported to the laboratory for analysis. Snails were identified to species using keys provided by Dang et al.[22]

EXAMINATION OF SNAILS FOR CERCARIAE

Snails were examined for trematode infection (cercariae stage) using shedding method[17,23] in 100 mL small plastic beakers and left for 12 hours for shedding. Cercariae were checked twice per day at 8:00 AM and 12:00 AM for three

Table 1. Snail composition in Tan Nhut rice fields and small canal supplying water to *Trichopodus microlepis* fish cultured in ponds in Binh Chanh district, Ho Chi Minh City, Vietnam in 2022-2023.

Family	Genus	Species	In rice field		In canal (2023)			
			Oct 2022	Apr 2023	Jan	Mar	Jun	Aug
Thiaridae	*Melanoides*	*Melanoides tuberculata*	0	26	4	10	5	0
	Thiara	*Thiara scabra*	0	1	9	6	4	2
Viviparidae	*Filopaludina*	*Filopaludina sumatrensis*	11	25	10	6	7	8
		Filopaludina martensi	30	41	5	8	8	12
Ampulariidae	*Pomacea*	*Pomacea canaliculata*	124	181	22	15	15	15
	Pila	*Pila ampullacea*	0	0	5	3	9	16
Bithyniidae	*Bithynia*	*Bithynia siamensis*	370	47	3	5	15	14
Lymnaeidae	*Lymnaea*	*Lymnaea viridis*	0	16	5	1	0	0
Bulinidae	*Indoplanorbis*	*Indoplanorbis exustus*	1	3	6	4	8	10
		Grand total number of snails	536	340	69	58	71	77

days. Cercariae were recognized by using systematic key references.[23,24]

DATA ANALYSIS

Data entry was performed using Microsoft Excel 2010, and data analysis was conducted using SPSS (Statistical Package for Social Sciences version 20; SPSS Inc., Chicago, Illinois). The Chi-squared test was used to compare the difference in prevalence between seasons, with a value of $P<0.05$ considered significant.

RESULTS

SNAIL COMPOSITION IN RICE FIELDS AND CANAL

Nine snail species belonging to 8 genera and 6 families were collected from Tan Nhut rice fields and canal. Eight snail species were found in both research rice fields and canal except for *Pila ampullacea* which was not found in Tan Nhut rice fields (**Table 1**).

A total of 1,151 samples of snails were collected in Tan Nhut rice fields (876 snails) and canals (275 snails). In the rice fields, *Bithynia siamensis* had the highest percentage, accounting for 69.0% in the wet season, while *Pomacea canaliculata* occupied 53.2% in the dry season. In the canals, *Pomacea canaliculata* and *Bithynia siamensis* were the most prevalent in the wet season, whereas *Pomacea canaliculata* dominated in the dry season (**Table 2**).

CERCARIAE MORPHOTYPES INFECTED IN SNAILS

Three snail species in both rice fields and canals were infected with trematode (cercariae stage), including *Bithynia siamensis, Lymnaea viridis*, and *Indoplanorbis exustus*. However, each snail species infected in one season and free cercariae in the other season, and vice versa, in both rice fields and canals. For the overall calculation, the prevalence in the dry season (25.9%) was significantly higher than in the wet season (10.6%) ($P<0.05$) (**Table 3**).

The result showed that three cercariae morphotypes were released from snails in the research. Xiphidio cercariae were recovered from *Bithynia siamensis*, *Indoplanorbis exustus*, and *Lymnaea viridis*. Echinostome cercariae were found in *Lymnaea viridis* and *Indoplanorbis exustus*. Pleurolophocercous cercariae were found in *Bithynia siamensis* in both rice fields and canal (**Table 4**).

DISCUSSION

It is interesting that total snail species from rice fields (N=8) in this study was the same as what Nguyen and Pham[20] found in two rice fields in Can Gio District, also belonging to Ho Chi Minh City. However, it was lower than the research result by Nguyen et al.,[18] who found 9 snail species in An Hoa rice field of Tuy An District, Phu Yen Province, or the findings on rice fields in Nam Dinh Province by Bui et al.[17] with 10 snail species. It was much lower than what Nguyen et al.[19] done in the lowland areas of Binh Dinh and Phu Yen provinces in south central Vietnam with 13 snail species. It can be commented that the total snail species in rice fields might be similar in the same area, but they were different from the different areas. Nevertheless, more research on snails in rice fields needs to be done to confirm the similarity of number of snail species in rice fields in Vietnam.

As for the snails in canals, the total snail species in the research canals (N=9) was much lower than in the small canals in Nam Dinh province with 12 snail species.[17] However, it was slightly higher than the study by Pham et al.[25] from small canals in Cu Chi District of Ho Chi Minh city with 8 snail species. Comparing it to the other canals in the same research district, it was a slightly lower than the finding from Pham and Nguyen[2] in Ba Lao and Ba Ty canals, which link the river and the research canal with 10 snail species. It can be said that total snail species in different geographical areas varies.

Although the total species of snails was similar between rice fields in Can Gio and Binh Chanh districts of Ho Chi

Table 2. Percentage contribution of each snail species in Tan Nhut rice fields and small canal supplying water to *Trichopodus microlepis* fish cultured in ponds in Binh Chanh district, Ho Chi Minh City, Vietnam in 2022-2023.

Snail species	*In rice field*						*In canal (2023)*					
	Oct 2022 (N)	*(%)*	*Apr 2023 (N)*	*(%)*	*Jan 2023 (N)*	*(%)*	*Mar 2023 (N)*	*(%)*	*Jun 2023 (N)*	*(%)*	*Aug 2023 (N)*	*(%)*
Melanoides tuberculata	0	0	26	7.6	4	5.8	10	17.2	5	7.0	0	0.0
Thiara scabra	0	0	1	0.3	9	13.0	6	10.3	4	5.6	2	2.6
Filopaludina sumatrensis	11	2.1	25	7.4	10	14.5	6	10.3	7	9.9	8	10.4
Filopaludina martensi	30	5.6	41	12.1	5	7.2	8	13.8	8	11.3	12	15.6
Pomacea canaliculata	124	23.1	181	53.2	22	31.9	15	25.9	15	21.1	15	19.5
Pila ampullacea	0	0	0	0	5	7.2	3	5.1	9	12.7	16	20.8
Bithynia siamensis	370	69.0	47	13.8	3	4.3	5	8.6	15	21.1	14	18.2
Lymnaea viridis	0	0	16	4.7	5	7.2	1	1.7	0	0.0	0	0.0
Indoplanorbis exustus	1	0.2	3	0.9	6	8.7	4	6.9	8	11.3	10	13.0
Grand total	536	100	340	100	69	100	58	100	71	100	77	100

Table 3. Trematode prevalence in snails in Tan Nhut rice fields and small canal supplying water to *Trichopodus microlepis* fish cultured in ponds in Binh Chanh district, Ho Chi Minh City, Vietnam in 2022-2023.

Snail species	*In rice field (wet season) Oct 2022*		*In rice field (dry season) Apr 2023*		*In canal (wet season) June and Aug 2023*		*In canal (dry season) Jan and Mar 2003*	
	Infected snails/ Collected snails	*(%)*	*Infected snails/ Collected snails*	*(%)*	*Infected snails/ Collected snails*	*(%)*	*Infected snails/ Collected snails*	*(%)*
Bithynia siamensis	38/370	10.3	0	0	0	0	1/3 (Jan)	33.3
Lymnaea viridis	0	0	4/16	25.0	0	0	1/5 (Jan)	20.0
Indoplanorbis exustus	0	0	1/3	33.3	2/8 (Jun) 1/10 (Aug)	25.0 10.0	0 0	0 0
Grand total	38/370	10.3	5/19	26.3	3/18	16.7	2/8	25.0

Table 4. Number of snails infected with different cercariae morphotypes in Tan Nhut rice fields and small canal supplying water to *Trichopodus microlepis* fish cultured in ponds in Binh Chanh district, Ho Chi Minh City, Vietnam in 2022-2023.

Snail species	*Morphotypes of cercariae*					
	In rice field			*In canal*		
	Oct 2022	*Apr 2023*	*Jan 2023*	*Mar 2023*	*Jun 2023*	*Aug 2023*
I. exustus		xiphidio			echinostome	echinostome
L. viridis		echinostome	xiphidio			
B. siamensis	xiphidio pleurolophocercous		pleurolophocercous			
Infected snails (N)	38	5	2	0	2	1

Minh City, the species composition was very different. In rice fields of Can Gio, the dominant snail species were *Sermyla tornatella* (47.6%), *Pomacea canaliculata* (27.2%), and *Melanoides tuberculata* (14.0%).[20] Whereas in the study rice fields, *Bithynia siamensis* had the highest percentage of 69.0% in the wet season, while *Pomacea canaliculata* occupied with 53.2% in the dry season. Similarly, a study by Nguyen et al.[18] found the three most abundant snail species in rice fields of Phu Yen Province were *Bithynia* sp., *Pomacea* sp., and *Tarebia grannifera*. This indicates that snail species and composition vary across different geographic areas. In the research canal, *Pomacea canaliculata* and *Bithynia siamensis* were also dominated, which contrasts with the sampling result from Ba Lao and Ba Ty canals in the same district. In those canals, the three most abundant snail species were *Tarebia granifera* (25.9%), *Melanoides tuberculata* (25.5%) and *Sermyla tornatella* (18.5%). The differences in natural conditions between the canals, such as depth, water-flow velocity, habitats, and other water parameters, might influence the snail species composition.

The golden apple snail (*Pomacea canaliculata*) was abundant both in rice fields and canals in this research. While this snail species poses a threat to rice crops,[26] all *Pomacea canaliculata* and the other five snail species examined were free from cercariae. However, three snail species, including *Bithynia siamensis, Indoplanorbis exustus* and *Lymnaea viridis*, were found to be infected with trematode (cercariae stage) in both rice fields and canal.

Bithynia siamensis showed trematode infection in the research rice field during the wet season and in the small canal during the dry season. This finding is consistent with Nguyen and Pham,[20] who reported that *Bithynia* in rice fields had the highest prevalence. Similarly, Bui et al.[17] and Nguyen et al.[18] observed high prevalence of *Bithynia* in canals and ponds. *Indoplanorbis exustus* was also found to be infected (11.1%) in Phu Yen Province in the central Vietnam[18] and had the highest overall prevalence of infection (38.5%) in the lowland areas of Binh Dinh and Phu Yen provinces in south central Vietnam,[19] marking the first time it has been observed in the Ho Chi Minh City area. *Lymnaea viridis*, although not infected with trematodes in Cu Chi District of Ho Chi Minh City,[27] or in Nha Be Districts of Ho Chi Minh City,[28] exhibited cercariae infection in the research rice fields and canal in Binh Chanh District may be more susceptible to trematode infection compared to other areas of Ho Chi Minh city.

Regarding cercariae infection, the overall prevalence in the dry season was significantly higher than in the wet season ($P<0.05$). This finding aligns with Nguyen et al.,[18] who noted a high infection rate of trematode larvae in snails during the dry season and a lower rate during the wet season in Tuy An district, Phu Yen province. The research result was also similar to what Nkwengulila and Kigadye[29] in Ruvu basin of Tanzania, and Pham[28] also found that the trematode prevalence in snails in Nha Be district was higher in the dry season and lower in the wet season ($P>0.05$). However, this contrasts with the findings of Nguyen and Pham,[20] who reported a higher prevalence in the wet season than in the dry season in Can Gio district. Despite Binh Chanh, Nha Be and Can Gio districts belonging to Ho Chi Minh City, the effects of seasonality on trematode were not consistent. Further studies are needed to better understand how seasonality influences trematode prevalence in snails.

Xiphidio cercariae were the most prevalent in this research. This finding was similar to the previous studies that xiphidio was dominated in the research by Nkwengulila and Kigadye,[29] and in Kenh Lang – Ben Muong canal in Cu Chi district of Ho Chi Minh city,[25] and in Can Gio district of Ho Chi Minh City.[2] Echinostome cercariae were recovered in only some snails in the research area, but they were found commonly in Phu Yen district[18] and in Nam Dinh province in the North of Vietnam.[30] One more interesting finding was that pleurolophocercous cercariae, identified as cercariae of Heterophyidae according to Pham and Nguyen,[21] were fortunately found in *Bithynia siamensis* from the rice fields and canal in the research area. Pham et al.[31] reported that *Trichopodus microlepis* fish cultured in ponds in Binh Chanh District have been found to be infected with metacercariae of *Centrocestus formosanus* and *Procevorum* sp., both of these species belong to Heterophyidae.[32] It can be inferred that pleurolophocercous cercariae in *Bithynia siamensis* in this study serve as one of the sources of trematode infection in *Trichopodus microlepis* fish cultured in ponds in Tan Nhut Commune of Binh Chanh District. These findings provided the information that cercariae from water sources outside the ponds might be the risk factors which could follow water current to cause fish infected with trematodes, so it should be taken into account of fish culture practices. However, further studies are necessary to determine whether *Trichopodus microlepis* juveniles are infected before being released into the ponds or if the infection occurs afterward. Additionally, conducting more research on cercariae in snails in other water bodies in Binh Chanh District during different months is essential to gain better understanding of why *Trichopodus microlepis* is susceptible to trematode infection.

The study has already classified snail species composition and their trematode prevalence in Tan Nhut canal and rice fields in Binh Chanh district in the dry and the wet season. However, this research still has some limitations. Two cross-sectional studies in rice fields and four sampling times in canal could provide a snapshot, but it could not fully capture the annual variation in the trematode prevalence and snail distribution. Furthermore, sampling points might not capture the full populations of snail and trematode infections in the entire Tan Nhut canal and rice fields. Moreover, some factors like water quality, predators' presence, or human activities that could affect snail diversity and infection rates were not mentioned in the study. In addition, no applications of molecular techniques were used, but morphological methods were applied for the identification of snail and cercariae, which could be sometimes less precise. Further research should consider minimizing the above limitations and implement deeper study.

CONCLUSIONS

A total of nine snail species belonging to 8 genera and 6 families were collected and identified. Among them, three snail species were found to be infected with cercariae in both rice fields and canal: *Bithynia siamensis, Lymnaea viridis* and *Indoplanorbis exustus*. The overall prevalence of infection during the dry season (25.9%) was significantly higher than during the wet season (10.6%) ($P<0.05$). Three cercariae morphotypes recovered from infected snails: xiphidio (present in all three snail species), echinostome (found in *Lymnaea viridis* and *Indoplanorbis exustus*), and pleurolophocercous (detected in *Bithynia siamensis*). The identification of pleurolophocercous cercariae from *Bithynia siamensis*, belonging to Heterophyidae, suggests one of the sources of infection for *Trichopodus microlepis* fish cultured in ponds. However, further research is needed to investigate other habitats, including the fishponds, concurrently to identify all sources of trematodes infection in *Trichopodus microlepis* fish in earthen ponds. This research can contribute to the development of fish culture practices to reduce the trematode prevalence in *Trichopodus microlepis* fish and other fish species in the research area.

AUTHORS' CONTRIBUTION

Conceptualization: Pham C. Thien (Equal), Ho T. Manh (Equal), Duong T. Quyen (Equal), Nguyen T.L. Xuan (Equal). Methodology: Pham C. Thien (Equal), Ho T. Manh (Equal), Duong T. Quyen (Equal), Nguyen T.L. Xuan (Equal), Nguyen M. Hung (Equal). Formal Analysis: Pham C. Thien (Equal), Ho T. Manh (Equal), Duong T. Quyen (Equal), Nguyen T.L. Xuan (Equal). Investigation: Pham C. Thien (Equal), Ho T. Manh (Equal), Duong T. Quyen (Equal), Nguyen T.L. Xuan (Equal). Writing – original draft: Pham C. Thien (Equal), Ho T. Manh (Equal), Duong T. Quyen (Equal), Nguyen T.L. Xuan (Equal), Nguyen T. Lan (Equal). Writing – review & editing: Pham C. Thien (Equal), Nguyen M. Hung (Equal). Funding acquisition: Pham C. Thien (Lead). Resources: Pham C. Thien (Lead). Supervision: Pham C. Thien (Lead).

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No competing interests were disclosed.

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Research had no vertebrates.

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All authors and institutions have confirmed this manuscript for publication.

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All are available upon reasonable request.

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