

## PREVALENCE AND SPECIES OF *Fasciolopsis buski* IN VIETNAM

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### Abstract

#### Keywords:

fasciolopsiasis,  
Fasciolopsis buski,  
Vietnam.

**PERPOSE:** To report prevalence and species of *Fasciolopsis buski* in Vietnam.

**METHOD:** Stool examination by Kato-Katz technique for detection prevalence of fasciolopsiasis, and identification species by morphology and molecular method.

**RESULT:** On 10,540 stool samples were examined in 14 provinces and adult worms of *Fasciolopsis* collected from patients in other 3 provinces. The result showed that 13/14 provinces have been patients infected *Fasciolopsis*. However, fasciolopsiasis was determined distribute in 16 provinces with prevalence of 1.23% in average (from 0.16% to 3.82%). Adult worms were identified as *Fasciolopsis buski* by morphology and confirmed by molecular analysis using of 1950 bp-18S ribosomal RNA(18S rRNA) as genetic marker.

### Introduction

Plant-borne Trematode has been found six species to affect humans: *Fasciola hepatica*, *Fasciola gigantica*, *Fasciolopsis buski* (in Fasciolidae family), *Gastrodiscoides hominis* (in Gastrodiscidae family) and *Watsonius watsoni*, *Fischoederius elayatus* (in in Paramphistomidae).

Fasciolopsiasis is common fluke causing by *Fasciolopsis buski*. This disease is found in Central and Southern China, Taiwan, Banladesh, India, Vietnam, Thailand, Laos and some other foci in South East Asia and affect some 10 million people.

Foodborne trematode is common in Vietnam as *Clonorchis/Opisthorchis* in more than 32 province (prevalence is highest of 37%), Hetrophyidae/Echinostomatidae in more than 18 provinces (prevalence is highest of 53.4%), *Paragonimus* in more than 10 provinces (prevalence is highest of 15%) (Nguyen Van De et al, 1996, 2003, 2006). *Fasciola gigantica* was the first report in 1928 (Codvelle et al, 1928) and during 1997 to 2012, *Fasciola gigantica* distributed in more than 52 provinces. *Fasciolopsis buski* was the first report in 1908 (Barios and Noc, 1908) and during 2000 to 2006, *Fasciolopsis buski* was determined distribution in more than 16 provinces (in this report).

### Methodology

Human prevalence surveys: Fasciolopsiasis survey in community is used methods stool examination by Kato-Katz technique.

Identification of the species: Adult worms collected from patients for identification of species by morphology and molecular methods.

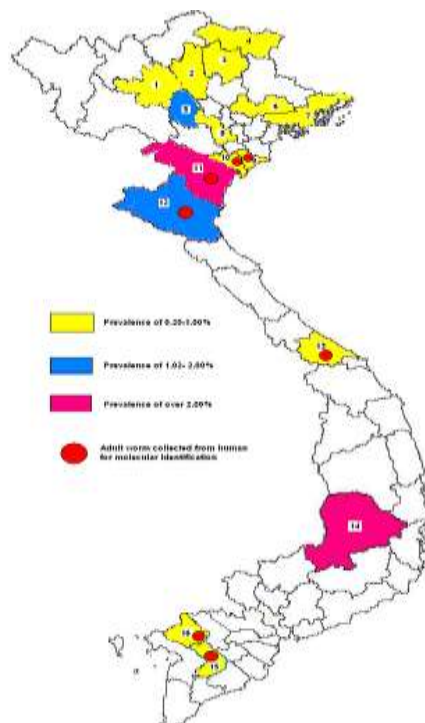
Identification of *Fasciolopsis spp* used 18S Ribosome genom. Total genomic DNA was extracted from the adult worms using a DNAaesey Tissue Kit (Quagen). A 1,950 bp fragment of 18S rDNA was amplified by polymerase chain reaction (PCR) using conserved oligonucleotide primer. After purification, PCR products were cloned using a TA Cloning Kit (Introgen) and plasmids was extracted using QIAprep Spin Miniprep Kit (Qiagen). DNA inserts was subsequently sequenced using T7 and M-13 reverse primers and an ABI 377 automated DNA sequencer and BigDye sequencing chemistry (ABI, Foster City, CA, USA). The sequences was aligned with a sequence for *Fasciolopsis buski* 18S rDNA deposited in the GenBank database (Accession No.L06668).

**RESULTS***Prevalence of Fasciolopsis by province***Table 1. The result of stool examination of Fasciolopsis buski egg by Kato-Katz technique**

No	Study site (province)	No examine	Result of stool examination	
			Infected people	% infection
1	Yen Bai	499	2	0.40
2	Tuyen Quang	704	4	0.57
3	Cao Bang	492	1	0.20
4	Bac Kan	627	2	0.32
5	Phu Tho	604	7	1.16
6	Quang Ninh	545	3	0.55
7	Bac Giang	686	5	0.73
8	Ha Tay	603	3	0.50
9	Nam Dinh <sup>a</sup>	615	5	0.81
10	Thanh Hoa <sup>a</sup>	558	17	3.05
11	Nghe An <sup>a</sup>	1,376	14	1.02
12	Ha Tinh	724	0	0
13	Dak Lak	1,494	57	3.82
14	An Giang <sup>a</sup>	1,013	10	0.97
	<b>Total</b>	<b>10,540</b>	<b>130</b>	<b>1.23</b>

Remark: <sup>a</sup> adult worms collected from this province for identification species.

On 10,540 human stool samples were examined by Kato-Katz technique in 14 provinces. The result showed that *Fasciolopsis* infection rate of 1.23% in average and one of 14 provinces no case (Tab 1). Beside 14 above provinces for stool examination, there are 3 provinces, which were Ninh Binh<sup>a</sup>, Thua Thien - Hue<sup>a</sup> and Can Tho<sup>a</sup>, where determined fasciolopsiasis patients, who collected adult worms for identification (from all above provinces, adult worms collected from 7 provinces).



**Figure 1:** Distribution of *Fasciolopsis buski* by province in Vietnam up to 2006

**Identification of *Fasciolopsis* adult worms from patients.**

Adult worms from patients in 7 provinces were identified by morphology and confirmed by molecular method as follow:

**Identification species by morphology**

Adult worms of *Fasciolopsis* are pink color, average body size was 12.5 mm in length and 5.5 mm in wide. Oral sucker of 0.5 mm and ventral sucker of 1.5 mm. There are 2 testes, which are highly branched, located one behind the other in the posterior region of the body, the ovary is also branched and is located in front of the testes to the right of the mid-line of the body, whilst the vitellaria are located laterally from the anterior to the posterior. The testine is turning and to the end of the body. Melite form is big and located in the central body (Fig 2).

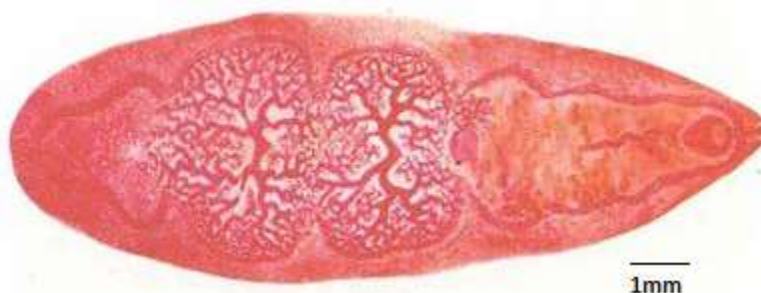


Figure 2. A adult of *Fasciolopsis buski* collected from patient

**Identification species by molecular method**

Table 2: List of adult worm samples collected from patients for molecular identification

Morphology	Code	Local origin	Form sample
<i>Fasciolopsis</i> sp	FspNDHuma	Nam Dinh	Adult worm
<i>Fasciolopsis</i> sp	FspNBHuma	Ninh Binh	Adult worm
<i>Fasciolopsis</i> sp	FspTHHuma	Thanh Hoa	Adult worm
<i>Fasciolopsis</i> sp	FspNAHuma	Nghe An	Adult worm
<i>Fasciolopsis</i> sp	FspHHuma	Thua Thien-Hue	Adult worm
<i>Fasciolopsis</i> sp	FspCTHuma	Can Tho	Adult worm
<i>Fasciolopsis</i> sp	FspAGHuma	An Giang	Adult worm

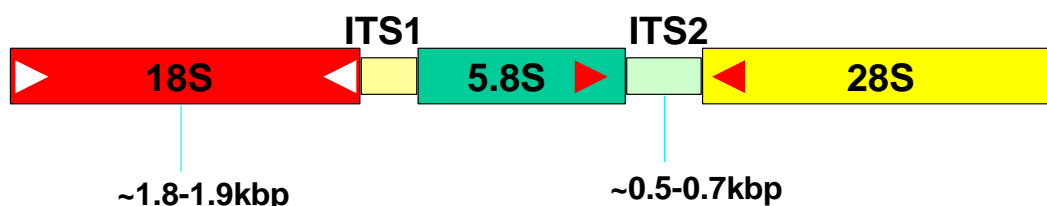


Figure 3: Ribosome genome (18S, 5.8S and 28S) and internal transcribed spacer (ITS1, ITS2). 18S (length of 1.8-1.9kbp) and ITS2 (length of 0.5-0.7kbp) are important molecular makers for study of identification of species.

Total of 18S Ribosome (18S rARN) genome (1950 nucleotide pair) of adult worms of *Fasciolopsis* from patients in Nam Dinh, Ninh Binh, Thanh Hoa, Nghe An, Thua Thien-Hue, Can Tho and An Giang provinces (Table 2) were sequenced and compared with this genome of *Fasciolopsis buski* in Gen Bank. The result showed that nucleotide genome sequences of 18S rARN from all study samples were similarly (100%) and compared with 18S rARN of *Fasciolopsis buski* in Gen Bank (code number: L06668), they difference of two nucleotide only (2/1950 nucleotide), the difference rate of 0.01%. However, Vietnamese *Fasciolopsis* from patients is *Fasciolopsis buski* and published in Gen Bank with code number AY 311386, AY618841, AY618842 and AY618843 (Fig. 3).

## 18SrARN (*Fasciolopsis buski*)

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                *      20      *      40      *      60      *      80      *
Fb (GB) : GTAGTCATATGCTTGTCTCAGAGATTAAGCCATGCATGCTCTAAGTACAAACCTTTAAACGGTGAACCGCGAATGGCTCATTAAATCAGCTAT : 93
FbNAHuma : ..... : 93
FbHNPig : ..... : 93
FspNBHum : ..... : 93
FspTHHum : ..... : 93
FspHHum : ..... : 93
FspCTHum : ..... : 93
FspAGHum : ..... : 93
    
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-----(((abridgment)))-----

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                1400      *      1420      *      1440      *      1460      *      1480
Fb (GB) : ATTTGCTGGTTAATCCGATAACGAAATGAGACTTTGGCCTGCTAAATAGTATGCCTGTCCCTCTGTGCTCGTGCAGGTTTCGTTGTCCATTGC :1488
FbNAHuma : ..... :1488
FbHNPig : ..... :1488
FspNBHum : ..... :1488
FspTHHum : ..... :1488
FspHHum : ..... :1488
FspCTHum : ..... :1488
FspAGHum : ..... :1488
    
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                *      1500      *      1520      *      1540      *      1560      *      1580
Fb (GB) : CTCCTCGTGGGGTAGTGGTTACGTTGACCGCGCAATGCGGCGCAGGTAATTACTTCTTAGAGGGACAAGCGGCCTTCAGTCGCACGAAAATGA :1581
FbNAHuma : ..... :1581
FbHNPig : ..... :1581
FspNBHum : ..... :1581
FspTHHum : ..... :1581
FspHHum : ..... :1581
FspCTHum : ..... :1581
FspAGHum : ..... :1581
    
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                *      1600      *      1620      *      1640      *      1660      *
Fb (GB) : GCAATAACAGGTCTGTGATGCCCTTAGATGTCCGGGGCCGCACGTGCGCTACAATGACGGTTTCAGCGAGTTTGGAATCCTGGCCTGAGCAGG :1674
FbNAHuma : ..... :1674
FbHNPig : ..... :1674
FspNBHum : ..... :1674
FspTHHum : ..... :1674
FspHHum : ..... :1674
FspCTHum : ..... :1674
FspAGHum : ..... :1674
    
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                1680      *      1700      *      1720      *      1740      *      1760
Fb (GB) : TCGGGTAAACTGTATCATAACCGTCGTGACTGGGATCGGGGCTTGCAATTGTTCCCGTGAACGAGGAATTCCTGGTAAGTGCAAGTCATAAG :1767
FbNAHuma : ..... :1767
FbHNPig : ..... :1767
FspNBHum : ..... :1767
FspTHHum : ..... :1767
FspHHum : ..... :1767
FspCTHum : ..... :1767
FspAGHum : ..... :1767
    
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                *      1780      *      1800      *      1820      *      1840      *      1860
Fb (GB) : CTTGCGCTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGGTTTAGCGAGGTCCTCGGATTGGTGCCATTGT :1860
FbNAHuma : ..... :1860
FbHNPig : ..... :1860
FspNBHum : ..... :1860
FspTHHum : ..... :1860
FspHHum : ..... :1860
FspCTHum : ..... :1860
FspAGHum : ..... :1860
    
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*          1880          *          1900          *          1920          *          1940          *
Fb (GB) : AGTGGCTTCGGCCGCTCGACCGGTGCTGAGAAGACGACCAAACCTTGATCATTAGAGGAAGTAAAAGTCGTAACAAGGTTCCGTAGGTG :1950
FbNAHuma: .....:1950
FbHNPig : .....:1950
FspNBHum: .....:1950
FspTHHum: .....:1950
FspHHum : .....:1950
FspCTHum: .....:1950
FspAGHum: .....:1950

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**Figure 3. Sequencing gen 18S Ribosome of study samples and comparison with pig *Fasciolopsis buski* in Gen Bank. Note: Fb(GB): *Fasciolopsis buski* from GenBank; FbNAHuma: *Fasciolopsis buski* collected from patient in Nghe An; FbHNPig: *F. buski* collected from pig in Hanoi; FspNBHum: *Fasciolopsis spp* collected from patient in Ninh Binh; FspTHHum: *Fasciolopsis spp* collected from patient in Thanh Hoa; FspHHum: *Fasciolopsis spp* collected from patient in Thua Thien –Hue; FspCTHum: *Fasciolopsis spp* collected from patient in Can Tho and FspAGHum: *Fasciolopsis spp* collected from patient in An Giang.**

## Conclusion

Prevalence of Fasciolopsiasis in 16 provinces of the country in Vietnam with 1.23% and adult flukes collected from patients were identified as *Fasciolopsis buski* using morphology and molecular method.

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