

Title : A Clinical Study of Fasciolopsis buski in Thailand

Principal Investigators : Captain Andrew G. Plaut, MC  
Chalyan Kampanart Sanyakarn, M.D.

Associate Investigator : Captain George S. Manning, MSC

Advisor : Robert Desowitz, Ph.D., D.SC.

Field Support : Supit Snitwong, M.D.  
Prachern Klaisubhan, M.D.

Period of Report : 1 April 1967—31 March 1968

**Background and Objectives** Fasciolopsis buski is a large trematode which inhabits the small intestine of man and domestic animals in several Asian countries including China, Vietnam, Thailand, India and Malaysia. Man and animals are the definitive hosts, and are infected following the consumption of raw fresh water plants of many species which bear the encysted metacercariae of the fluke. Certain fresh water snails are required as intermediate hosts.

In man, the fluke attaches itself by a ventral sucker to the intestinal mucosa; its eggs may be identified in the feces, although they are nearly indistinguishable from those of Fasciola hepatica and Fasciola gigantica. Worm loads in man range from a single worm to many hundreds, rarely thousands. Definitive diagnosis requires that the adult worm be recovered following treatment.

Although numerous textbooks of parasitology declare that F. buski causes clinical illness in man, this point has not been well established in recent times, especially in those individuals with less than overwhelming worm loads. The objective of the study reported here was to determine if F. buski infection is associated with clinical symptoms, faulty growth and development, or intestinal absorption disturbances as determined by a D-xylose absorption screening test.

**Description** The study was performed in the village of Ko Ko Tau, Supanburi Province, Thailand. This province lies in the central plain, the predominate rice-growing area of Thailand, and is therefore flooded most of the year. The existence of F. buski in this province has been well established for years through the efforts of the School of Tropical Medicine in Bangkok.

**Patients** A preliminary survey of the village was undertaken to select patients for study. Stool specimens were examined for ova at the study site, or alternatively fixed in formalin and examined several days later in Bangkok. The result of this survey is shown in Table. 1.

Table 1. Age distribution and results of examination for F. buski in 568 residents of Suphanburi.

Age Group (years)	Number of Patients Examined	Number Positive for <u>F. buski</u>	Percent Positive for <u>F. buski</u>
1-4	56	6	11
5-14	312	69	22
15-24	38	7	18
25-44	87	11	13
45-60	55	3	5
61 and over	20	1	5
Totals	<u>568</u>	<u>97</u>	<u>17</u>

On the basis of this survey, 28 individuals having ova of F. buski in the stool were randomly selected. Each was paired with a control patient, (having no F. buski ova in the stool) of similar age, and in all but 4 cases the same sex. Twenty one pairs were between the ages of 4 and 13. The other 7 pairs were older. Controls and positive patients were studied in detail in the same way, and this investigation was carried out without knowledge on the part of the physicians as to who was positive.

Material and Methods The following examinations were performed on each patient:

a. History and physical examination, with the parents in attendance. The history was graded on a scale of 0 (no symptoms) to 4+ (most severe symptoms).

b. Hematocrit, hemoglobin, white blood cell count, differential count, and calculation of red cell indices.

c. Urinalysis on early morning specimen.

d. Determination, in the fasting state, of serum carotene, cholesterol, Vitamin A, calcium, phosphorus, bilirubin, glutamic pyruvic and oxalic transaminases, lactic dehydrogenase, alkaline phosphatase, albumin and globulin; serum protein electrophoresis, and blood urea nitrogen.

e. D-xylose tolerance test. The fasting subject emptied the bladder and drank 250 ml of water containing 5 grams D-xylose in solution. All urine was collected for 5 hours thereafter. An additional 250 ml of water was given after two hours to insure adequate urine output. A blood serum was obtained two hours after xylose ingestion, and together with the five hour urine collection was assayed for pentose by the method of Roe & Rice. The serum xylose examination was obtained to confirm urine excretion results.

f. Chest X-ray.

g. X-rays of bones for bone age determination. Interpretation was based on American standards since Thai standards are not yet available.

h. Repeat stool examination for ova and parasites.

i. Treatment study. Eleven subjects positive for F. buski were hospitalized at the Supanburi Provincial Hospital. A quantitative egg count was performed on a 24 hour stool collection from each patient. Thereafter, the fasting subjects were given 0.2 ml tetrachlorethylene per year of age (maximum 3 cc) by mouth. This was followed two hours later by a magnesium sulfate purge. Adult worms were recovered from the stool over the next two days, and their numbers compared to the quantitative egg count.

## Progress

Clinical History Gastrointestinal symptoms and signs were common but mild in both groups. These included nausea, vomiting, anorexia, abdominal pain, abdominal swelling, diarrhea, melena, painful defecation, constipation, and weight loss. Jaundice was reported by one patient and steatorrhea not at all. 20% of the patients reported occasional red blood in the stool, but there was no significant difference between those positive and negative for F. buski. Patients positive for F. buski had a somewhat greater severity of gastrointestinal symptoms, but the difference was quite small.

Physical Examination Overall development, as determined by general appearance height and weight, arm span, skin fold thickness, and dental age was not different in the two groups. Signs of vitamin deficiency were not observed in study subjects, although angular cheilosis, atrophic mucus membranes and overt beri-beri were seen in several adult members of the village. Signs of malnutrition were not observed in the study group. Seven subjects with F. buski and three without had a palpable liver, but none were of a consistency or size suggesting that the liver was the seat of disease. One subject without F. buski had splenomegaly plus hepatomegaly. Otherwise there were no physical findings which could be attributed to intestinal parasitism or other gastrointestinal illnesses in the study group.

Urinalysis The only abnormalities, infrequently noted, were trace proteinuria and scattered leukocytes in the sediment. The two groups did not differ with respect to these findings.

Hematology Data are summarized in Table 2. Anemia (hematocrit below 36 in prepubertal children) was noted in four children without and 2 children with F. buski. Four of these subjects (two in each group) had a hypochromic microcytic anemia, and the remainder were normochromic and normocytic. One adult female with F. buski also had a normochromic normocytic anemia. There was a slightly higher mean peripheral eosinophile count in the F. buski group.

Serum biochemistry Data are summarized in Table 2. There were no significant differences in the two groups. In particular, mean total serum protein, albumin and globulin were identical in the two groups. Serum protein paper electrophoresis showed no abnormalities in any subject.

Table 2. Clinical values in 28 F. buski patients and 28 controls.

Determination	<u>F. buski</u> positive	<u>F. buski</u> negative
	Mean value and standard deviation	
Blood urea nitrogen (mg per 100 ml)	9.3±2.8	9.0±2.2
Serum Calcium (mg per 100 ml)	9.8±.53	9.6±.29
Serum Phosphorus (mg per 100 ml)	4.6±.7	4.6±.6
Total serum bilirubin (mg per 100 ml)	0.3	0.3
Serum glutamic oxalic transaminase (Sigma-Frankel Units)	22.1±4.8	22.7±4.5
Serum glutamic pyruvic transaminase (Sigma-Frankel Units)	16.8±2.8	23.1±7.7
Serum lactic dehydrogenase (Sigma Units)	291±83	308±67
Serum Alkaline Phosphatase (Sigma Units)	3.9	4.7

(Table 2. Continued)

Determination	<u>F. buski positive</u>	<u>F. buski negative</u>
	Mean value and standard deviation	
Serum Total Protein (Grams per 100 ml)	7.3±.4	7.3±.3
Serum Albumin (Grams per 100 ml)	3.9±.3	3.9±.4
Serum Globulin	3.4±.4	3.4±.4
Hemoglobin (grams per 100 ml of blood)	12.4±1.1	12.3±1.1
Hematocrit (% volume packed red cells in whole blood)	39.0±3.1	39.0±2.8
White blood cells (number per mm <sup>3</sup> of blood)	10,000±3,000	9,400±2,700
% Polymorphonuclear leukocytes	47	48.5
% Lymphocytes	38.2	38.5
% Monocytes	4.4	4.8
% Eosinophiles	10.2	7.6
Red Blood Cells (number per mm <sup>3</sup> whole blood)	4.6±0.7×10 <sup>6</sup>	4.7±0.7×10 <sup>6</sup>
Mean Corpuscular Hemoglobin Concentration (percent)	31.8±1.3	31.5±1.2
Mean Corpuscular Volume (cubic microns)	86.3±10.9	84.8±14.0
Mean Corpuscular Hemoglobin (micro micrograms)	27.4±3.8	27.0±4.5
Urine D—Xylose (Grams excreted in five hours after 5 gram oral dose)	1.87±.37	1.80±.44
Serum D—Xylose (mg per 100 ml 2 hours after 5 gram oral dose)	15.8±5.8	15.9±4.8
Serum Carotene (micrograms per 100 ml)	118.3±47.3	111.6±45.4
Serum Cholesterol (mg per 100 ml)	171±34.6	167±27.1

Xylose tolerance test Data are summarized in Table 2. The two groups did not differ in their capacity to absorb D-xylose. Mean excretion corresponds to that observed in normal Thai subjects in Bangkok, hence also to that in normal subjects in the United States.

Bone age and Chest X-ray Evaluation of bone age\* showed that many subjects positive and negative for F. buski had retarded bone development. Bone age evaluation did not distinguish between the two groups. It should be emphasized that American standards were used. Data are shown in Table 3. The actual numerical data is given in detail to allow for future comparisons when normal values become available for Thai subjects.

Chest X-rays were normal in all subjects with one exception. This patient, negative for F. buski (subject number 42), had enlarged hilar nodes of unknown etiology.

\* Dr. Chitti Palawatana, Royal Thai Army Hospital; Bangkok, Thailand

Table 3. Bone age determinations in 23 patients with F. buski and 23 controls.

Patient number	Chronological age of subject (month)**	Calculated bone age of subject (month)	Minimal normal bone age* for this group (month)	Interpretation		
2	66	40.6	48.2	retarded		
1	72.3	48.4	54.5		retarded	
4	68.3	58.1	50.5	normal		
3	66	58.1	48.2		normal	
10	92.0	67.2	74.6	retarded		
11	89.0	75.0	71.8		normal	
14	101.7	80.6	79.1	normal		
13	101.7	68.4	79.1		retarded	
16	103.8	74.0	81.8	retarded		
15	110	92.5	87.8		normal	
20	141.4	80.6	120.6	retarded		
22	147.5	132.1	126.3		normal	
28	180.7	162.0	152.3	normal		
27	172.5	168.5	144.9		normal	
6	101	74.5	82.8	retarded		
37	102.0	67.0	83.8		retarded	
36	144.5	138.4	123.7	normal		
45	116.0	80.6	93.2		retarded	
35	118.0	104.9	95.2	normal		
47	166.5	150.5	146.5		normal	
48	22 years			normal		
30	24 years				normal	
50	111.0	80.6	88.6	retarded		
43	92.5	80.6	70.9		normal	
49	82.0	58.1	75.4	retarded		
54	70.4	58.1	52.8		normal	
40		adequate X-ray not obtained				
55	68.3	40.6	50.3		retarded	
26	132.1	118.0	111.1	normal		
62	132.1	118.0	111.1		normal	
25	154.5	150.5	132.5	normal		
64	125.7	75.46	106.1		retarded	
52	108.0	104.9	85.8	normal		
59	113.0	104.9	90.4		normal	
24	103.8	92.5	81.8	normal		
5	102.8	86.6	80.8		normal	
51	101.8	56.2	79.8	retarded		
7	85.6	50.3	64.8		retarded	
12	87.0	65	70.0	retarded		
38	119.0	104.0	97.4		normal	
23	52.3	39.5	36.3	normal		
60	46.4	34.5	32.0		normal	
42	66.4	56.2	48.4	normal		
21	92.5	56.2	70.9		retarded	
41	58.3	44.8	41.1	normal		
46	123.0	104.9	100.8		normal	
				Total retarded	9	8
				% retarded	41	35

\* The figure represents the mean for this age minus two standard deviations according to Grenlich, M.W. and Pyle, S.I. Radiographic Atlas of The Skeleton Development of the Hand and Wrist, 2nd Edition. Stanford University Press, California and Oxford University Press, London.

\*\* Paired subjects as described in text. In this table, the subject negative for F. buski is on the top of each pair shown, the positive subject is below.

Treatment Study Quantitative egg count data and total worms passed by patients are summarized in Table 4. 3 patients having no eggs seen on quantitative count nevertheless passed adult worms following treatment. Adult worms were not recovered from an additional 3 subjects. Table 5 also shows that certain clinical and biochemical observations did not vary with regard to number of worms recovered.

A single course of tetrachlorethylene was effective in eradicating the parasite from 9 of 11 subjects based on re-examination of stools for ova at 2 week and 6 week intervals following treatment. Two stools were examined from each subject at the 6 week period. Dizziness was the predominate side effect noted with tetrachlorethylene administration.

Table 4. Pre-treatment egg counts and results of Tetrachlorethylene treatment in 11 patients with *F. buski* infection.

Patient number	Age (years)	Eggs/gram of stool	Adult worms recovered after treatment	Stool exam for <i>F. buski</i> six weeks post treatment
43	5	11,200	84	-
38	10	6,000	7	+
44	11	3,000	10	-
22	12	800	35	-
5	8	300	6	-
39	58	<300	1	-
18	11	<300	1	-
27	14	<200	1	+
11	7	<200	none	-
19	8	<200	none	-
7	7	<200	none	-

Table 5. Comparison of clinical parameters and worm burden in 11 patients with *F. buski* infection.

Patient number	Adult worms Recovered	Xylose Absorption (urinary excretion in 5 hours in grams)	Total serum protein (Gms/100 ml)	Blood Hemoglobin (Gms/100 ml)	Clinical symptoms	Eosinophile (% of total white blood cells)
43	84	1.88	7.9	11.5	4+	8
22	35	2.18	7.3	10.9	3+	22
44	10	1.61	7.4	12.7	1+	2
38	7	1.20	7.0	12.1	2+	12
5	6	1.74	7.2	11.5	4+	15
39	1	1.49	7.9	12.5	1+	4
18	1	1.94	7.2	11.5	1+	20
27	1	2.35	6.7	13.5	4+	10
11	0	1.42	6.9	11.9	3+	22
19	0	1.87	7.3	12.6	2+	17
7	0	2.04	7.0	12.6	3+	8

Comment In this investigation which has dealt with fasciolopiasis in randomly selected individuals in an endemic area, little if any evidence has been found that this parasite is harmful to man if present in less than massive numbers. To be sure, health problems were noted in the study group, whether positive or negative for *F. buski*, but the worm did not appear to be responsible for these problems. The only suggestion that a difference exists between subjects bearing or not bearing the worm is that the former

reported more abdominal pain and diarrhea; the difference however was small. Otherwise these two groups demonstrated similar and normal xylose absorption and serum biochemical examinations. Hematologic abnormalities were similar in the two groups, therefore not readily attributable to the presence of F. buski. The same is true for the frequently observed retardation of bone development. In this regard, it is well to note that American standards were applied. To categorically state that so many children were retarded in their bone development awaits the establishment of normal Thai standards. In fact, the study subjects did not show gross bone age abnormalities as a group when compared to normal Bangkok children currently under study.

Summary Twenty-eight individuals with F. buski infection and 28 matched controls were studied in a search for clinical and biochemical abnormalities attributable to this parasite. Clinical examination, evaluation of growth and development, hematologic studies and screening tests for malabsorption syndrome did not reveal significant differences between affected and control subjects. It is concluded that F. buski (in the age group studied) in less than massive numbers probably is not directly responsible for overt clinical disease in the area studied.