

ความชุกของการติดพยาธิใบไม้ตับชนิด *Fasciola* ในโคและกระบือรอบทะเลสาบสงขลา

Prevalence of a liver fluke infection (*Fasciola*) in Cows and Buffaloes nearby Songkhla Lake

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บทคัดย่อ

โรคพยาธิใบไม้ตับชนิด fasciolosis เป็นโรคที่มีความสำคัญโรคหนึ่งในปศุสัตว์และมีผลกระทบต่อสุขภาพในคน ทำให้เกิดการสูญเสียทางด้านเศรษฐกิจและสาธารณสุข การศึกษาครั้งนี้เพื่อหาความชุกของการติดพยาธิใบไม้ตับชนิด *Fasciola* ในโคและกระบือรอบทะเลสาบสงขลา โดยทำการเก็บตัวอย่างเลือดและอุจจาระจากโคและกระบือจำนวน 277 และ 95 ตัว ตามลำดับในพื้นที่รอบทะเลสาบสงขลา ผลการตรวจอุจจาระด้วยวิธี Formalin-ethyl acetate sedimentation พบโคและกระบือมีการติดพยาธิใบไม้ตับคิดเป็นร้อยละ 10.3 (28/272) และ 30.1 (25/83) ส่วนการตรวจซีรัมด้วยวิธี ELISA พบการติดพยาธิใบไม้ตับคิดเป็นร้อยละ 34.3 (95/277) และ 78.9 (75/95) โคที่มีอายุมากกว่า 7 ปี พบการติดพยาธิมากกว่าโคอายุน้อยกว่า 7 ปี แต่ในกระบือพบว่ากระบืออายุมากกว่า 7 ปีมีการติดพยาธิน้อยกว่ากระบืออายุน้อยกว่า 7 ปี ทั้งในโคและกระบือให้ผลที่สอดคล้องกันในเรื่องเพศคือตัวเมียตรวจพบการติดพยาธิใบไม้ตับสูงกว่าในตัวผู้

ABSTRACT

Bovine fasciolosis is one of the most important parasitic diseases in domestic animals and human health. The great economic losses and public health concern are caused by fasciolosis. The objective of this study is to determine the prevalence of *Fasciola* infection in cows and buffaloes nearby Songkhla Lake areas. Fecal samples and blood of 277 cows and 95 buffaloes were collected. The results of fecal examination by formalin-ethyl acetate sedimentation method shown 10.3% (28/272) of cows and 30.1% (25/83) in buffaloes were positives. However, the serological test by ELISA showed 34.3% (95/277) in cows and 78.9% (75/95) in buffaloes were positive for *Fasciola* infection. The older cows had the higher infection rate than young cows. However, the older buffaloes had the lower positive rate than young buffaloes. Both female cows and buffaloes had the highest *Fasciola* infection.

Keyword: *Fasciola*, cow, buffalo, Songkhla Lake

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INTRODUCTION

A liver fluke infection is an important health problem for animals and humans. *Fasciola* is one of the liver flukes causing fasciolosis in ruminant and many mammals including humans. The *Fasciola* infections are transmitted by ingesting water plants or water contaminated with metacercaria. In Thailand, there were reports of *F. gigantica* infections in cattle and humans (Intapan *et al.*, 2005; Kanoksil *et al.*, 2006). The prevalence of fasciolosis in Thailand was 12% (Gray *et al.*, 2008) and varied between provinces ranging from 0–85%. The northern part had the highest infection (23.4%), while the south had the lowest (4%) (Tuntasuvan and Kitikoon, 1996). In Pakpanang river basin, Nakhon si thammarat province had 8.2% of *Fasciola gigantica* infection (Worasing, 2007). Twenty-five cases of human fasciolosis were occurred in Thailand from 1967-1990. Since then, at least 10-20 new human fasciolosis cases have been confirmed in Khon Kaen University Hospital each year (Gray *et al.*, 2008). The infection rate of *Fasciola* was found 0.36% in in-patient at Siriraj hospital from 1991-1995 (Tiewchaloren and Junnu, 1996).

There were many techniques for diagnose *Fasciola* infection including fecal, immunological and molecular techniques. The faecal examination is the referent method for detection of *Fasciola* infection. This method based on formalin-ethyl acetate sedimentation (Bonita and Taira, 1996). The test was diagnosed by finding the egg of parasites. The serological methods such as ELISA were developed to diagnose fasciolosis in early infection. The assay was more sensitive for *Fasciola* infection than fecal examination but it was less practical for the field survey (Intapan *et al.*, 2003). Several antigens were used including crude antigen, tegument, egg and excretory-secretory antigen (ES Ag). There were many ES Ag from *Fasciola spp.* The molecular weight of ES protein was 15-101 kDa (Awad *et al.*, 2009). However, the twenty-seven kDa proteins of ES protein was the specific Ag of *Fasciola spp.* The 27 kDa ELISA had high sensitivity (93-94.9%), high accuracy (96%) and no cross reaction between *Fasciola gigantica* and *Paramphistomum epiclitum*, but that assay was not practical in the field diagnosis (Estuningsih *et al.*, 1997; Cornelissen *et al.*, 1999; Dixit *et al.*, 2002).

Songkhla Lake is the largest lake in Thailand and has the environment facilitating *Fasciola* life cycle. The lake promoted increasing population and freely spreading in the pasture of water snails, and vegetating of water plants. Many cows and buffaloes were reared nearby the lake. There were cattle in nearby Songkhla Lake areas, which died from *Fasciola* infection. In the present study, we aim to detect

Fasciola spp. infection in cows and buffaloes by using fecal technique and serological method (ELISA) and determine the risk factor for the transmission of *Fasciola* infection in nearby Songkhla Lake areas.

MATERIALS AND METHODS

Blood Samples and Study areas

Five districts including Sathing Phra (ST), Ranot (SR), Singha Nakhon (SS), Krasaesin (SA) and Khuan Niang (SK) districts of Songkhla province (Figure 1) were assigned and 277 blood samples of cows (ST=54, SR=99, SS=49, SA=59 and SK=16) and 95 of buffaloes (ST=29, SR=19, SS=27 and SA=20) were collected. Blood samples were allowed to clot at room temperature for one hour, centrifuged at 1,448 G for 20 min, separated for sera, and stored at -20°C until tested.

Coprological examination

Two hundred and seventy-two fecal samples of cows and 83 fecal samples of buffaloes were collected directly from rectum of the animals in the study areas and examined for the presence of helminthic eggs by formalin-ethyl acetate sedimentation method. Fecal samples were washed in normal saline, strained into 15 mL centrifuge tubes, and centrifuged at 1,448 G for 5 min. The supernatant was decanted, and 10% formalin was added to a volume of 10 mL into the tube and mixed. The suspension was added 2 mL with ethyl acetate, shaken and centrifuged at 1,448 G for 5 min. Finally, loosening the debris plugs at the top layer and 10% formalin were decanted. The remaining pellets were mixed with 10% formalin, added a drop of suspension on a glass slide and examined under light microscope.

Collection of adult worms

Adult worms of *Fasciola* were collected from liver and bile ducts of cattle at the slaughter houses and transferred into phosphate buffer saline (PBS, pH 7.2). Alive flukes were washed with normal saline to remove host contents. Thereafter, the flukes were washed five times in PBS (pH 7.2) and processed for obtaining adult worm regurgitant for isolation and purification of *F. gigantica* ES proteins

Isolation of *Fasciola* excretory/secretory antigen

Adults of *Fasciola* were washed cultured to obtaining ES antigen which followed the protocol by Raina *et al.*, 2006. Briefly, the flukes were incubated in RPMI1640 (100 unit/mL penicillin) at 37 ° C overnight. The media containing ES Ag product was collected and centrifuged at 10,000 G for 30 min at 4 ° C. Then, the supernatant was precipitated by two steps alcoholic precipitation. Finally, this product was resuspended with PBS. The suspension was aliquotted and stored at -80 ° C until test. Protein concentration of each prepared antigens was determined by Bradford method.

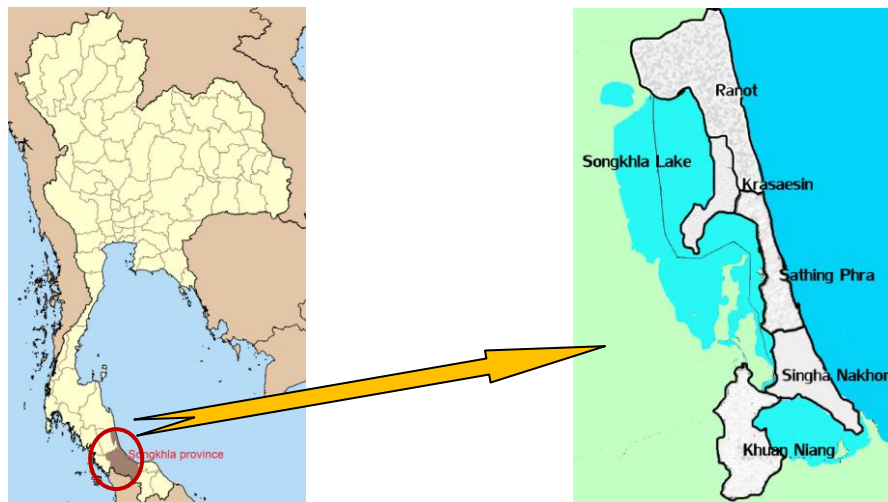


Figure 1 Map of Thailand and Songkhla Lake in Songkhla province

Enzyme linked immunosorbent assay (ELISA)

The sera from cows and buffaloes were evaluated by ELISA. The ELISA was performed as per the method described by Raina *et al.* (2006) with some modifications. Briefly, ELISA plates were coated with 100 μ L of 0.25 μ g ES Ag in 0.1M carbonate buffer (pH 9.5) per well, incubated at 4 $^{\circ}$ C overnight. The plates were washed five times with 200 μ L/well washing buffer (0.05% Tween-20 in PBS) and blocked with 100 μ L/well blocking buffer (3% skimmed milk powder in PBS) for 1 h at 37 $^{\circ}$ C. The plates were washed five times with washing buffer. One hundred μ L of serum dilution (1:200 dilutions in blocking solution) was added into the wells and incubated at 37 $^{\circ}$ C for 1 h. The plates were washed five times. The anti-bovine IgG peroxidase conjugate was added into the wells and the plates were incubated at 37 $^{\circ}$ C. Finally for 1 h, after five washes, 100 μ L of substrate (3,3',5,5'-tetramethyl benzidine, TMB) and plates were incubated at room temperature for 30 min in the dark. The reaction was stopped by adding 100 μ L of 1N HCl into each well. The absorbance was read at 450 nm by ELISA plate reader. The positive control was chosen from the positive sample tested by fecal examination which the cattle showed single helminthic infection. The negative control was chosen from the sample which was not found any parasite in the stool.

Statistical Analysis

The prevalence of fasciolosis was determined and analyzed by from the ratio of positive results and total number of animals, and data were analyzed using the chi-square test, according to age and sex by Number Cruncher Statistical System programs (NCSS) version 2000 (Kaysville, UT).

RESULTS

The prevalence of *Fasciola* infections in cows and buffaloes was 10.3% (28/272) and 30.1% (25/83), respectively by the fecal exam (Table 1). Compared to the fecal examination, the

seroprevalence was increasing to 34.3% (95/277) and 78.9% (75/95) in cows and buffaloes by ELISA (Table 1). The *Fasciola* infection occurred in all districts. The percentage of infected cows were 17.5% (11/63) in male cows and 39.25% (84/214) in female cows (Table 1). The male cows were lower positive numbers than the female cows but in buffaloes, the male (81.8%) was higher than the female (78.6%). The old cows (46.9%) showed more infected numbers than the young cows (31.4%). In buffaloes, the young buffaloes showed the highest positive percentage of *Fasciola* infection. The ages and sex group differences were significant ($p=0.0013$ and $p=0.0032$, respectively) in cows. However, in buffaloes, the ages and sex group differences were not significant ($p=0.8$ and $p=0.76$, respectively).

Table 1 Seroprevalence of *Fasciola* infection using fecal examination and ELISA in cows and buffaloes

Parameter				Statistic	
Fecal examination					
cows		272	28(10.29)		
buffaloes		83	25(30.12)		
ELISA					
cows	ages	<1	9	1(11.1)	$X^2=10.26$, $df=1$, $p=0.0013$
		1-7	204	64(31.4)	
		>7	64	30(46.9)	
sex	Male	63	11(17.5)	$X^2=6.89$, $df=2$, $p=0.0032$	
	Female	214	84(39.3)		
	total	277	95(34.3)		
buffaloes	ages	<7	37	31(83.8)	$X^2=0.06$, $df=1$, $p=0.8$
		>7	58	44(75.9)	
sex	Male	11	9(81.8)	$X^2=0.09$, $df=1$, $p=0.76$	
	Female	84	66(78.6)		
	total	95	75(78.9)		

DISCUSSION

Songkhla Lake, which is the largest lake in Thailand, covered area of 1,040 km² and bordered Songkhla and Pathalung provinces. There are many wildlifes living in this area. Several domestic

animals were reared nearby and in Songkhla Lake. In the morning most domestic animals including cattle, buffaloes and goats were grazed in the field nearby the lake (fig 2), and at night, cows and buffaloes came back into the pens by themselves. The animals lived, fed, defecated and slept nearby Songkhla Lake. The feces contaminated with parasitic eggs dropped on the ground or into the water in the lake. The parasites were spread around the lake by the flowing of water (Patz *et al.*, 2000). Then, the lake was the reservoir of parasite. Humans lived nearby Songkhla Lake which ate vegetable from the lake and the domestic animals could graze water plants nearby the lake, which were a potential risk to the infection of *helminthic parasites including Fasciola gigantica*. Cattle and humans nearby Songkhla Lake areas had many risk factors for *Fasciola* infection such as fresh water snails and water plants (Claxton *et al.*, 1997). The environment of Songkhla Lake, where was large lake and had water all the year round, promoted proliferation and spreading of fresh snails and water plants. The fresh water snails and water plants had importance for the transmission of liver flukes.



Figure 2 The environment nearby Songkhla Lake.

The previous study nearby Pakpanang river, Nakhon si thammarat province in the south of Thailand had low prevalence (8.2%) of *Fasciola* infection (Worasing, 2007). The prevalence of bovine fasciolosis (34.3% and 78.9% in cows and buffaloes, respectively) in the present study was greater than the previous study in Nakhon si thammarat. The water in Pakpanang river flowed all times. The flow of running water in river might have carried some the infected snails (Rondelaud *et al*, 2005). That habitat was not appropriate for distributing of fasciolosis. However, in Songkhla Lake, the water was flowed in the lake and had water all year round. The migration of the infected snails was in the same area. The geography of Songkhla Lake supported the fasciolosis distribution but no data on human fasciolosis in Songkhla province was reported. However, the data in the present study showed high prevalence of

bovine fasciolosis. The people nearby Songkhla Lake had risked to the liver flukes infection. Therefore, they should avoid ingesting raw water plants which grew nearby the lake.

The positive result of immunological assay in our study was higher than fecal examination. The fecal examination was simple method for detecting the intestinal parasites and the formalin-ethyl acetate sedimentation was practical for field survey. The disadvantage of fecal examination was not detecting in the early stage of fasciolosis and showed high false negative in chronic infection (Awad *et al.*, 2009). ELISA could diagnose fasciolosis in the early stage, current and chronic infection (Arias *et al.*, 2007). The infected buffaloes with *Fasciola gigantica* were detected in three week post infection by using 27 kDa Ag iELISA (Kumar *et al.*, 2008). The ES-ELISA had high sensitivity (100%) and specificity (near 100%), compared to coprological and bile examinations (Ridi *et al.*, 2007). ELISA was high sensitivity, specificity and no cross reaction with others flukes (Intapan *et al.*, 2003). Then, ELISA is a recommendatory method for diagnosis bovine fasciolosis than fecal examination.

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