Prevalence of *Fasciola gigantica* Infestation in Beef Cattle at Nongduang Slaughterhouse in Vientiane Capital of Lao People’s Democratic Republic Using an iELISA Approach

Souk Phomhaksa¹, Chaiyapas Thamrongyoswittayakul¹*, Aran Chanlun¹, Suthida Chanlun², Noppadon Somphol¹, Chittraporn Yeanpet¹

Abstract

**Objective**—To determine the prevalence of *F. gigantica* in beef cattle at Nongduang slaughterhouse, Vientiane capital in Lao PDR by using iELISA approach as compared to quantitative fecal examination.

**Materials and Methods**—One hundred seventy two each of fecal and blood samples were randomly collected from slaughtered native beef cattle to determine fecal egg counts (sedimentation and modified formalin-ether concentration method) and serum IgG antibodies against *F. gigantica*-excretory-secretory antigens (Indirect enzyme-linked immunosorbent assay and immunoblot). Information regarding age, sex, body condition score, and fecal score of each cattle was recorded. All related data were normalized and analyzed by Chi-square tests, Independent t-test and Kappa statistic using SPSS version 17.

**Results**—Prevalences of *F. gigantica* infestation in beef cattle were 11.6% (20/172) and 94.7% in relation to the FEC and effective iELISA, respectively, which was insignificantly associated with sex, age, hair and skin score, BCS and fecal score. Most cattle were harbored by single and multi-parasitic infestation by *F. gigantica*, rumen fluke and nematodes (77.9%, 134/172), whereas negative cattle was 22.1% (38/172). Four main *Fg*-ES antigens (14, 22, 25, and 34 kDa molecular weight) were recognized by pooled serum IgG antibodies according to the immunoblotting approach.

**Conclusion**—The abattoir survey demonstrated a high iELISA-based prevalence of *F. gigantica* infestation in the beef cattle at Nongduang slaughterhouse, Lao PDR. The iELISA, an alternatively serological test could overcome the false negative results according to a traditional fecal examination and investigate *F. gigantica*-infected beef cattle during prepatent period. Moreover, 14- and 22-kDa excretory-secretory proteins could be alternative protein markers for detection of *F. gigantica* infestation in beef cattle.


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**Keywords:** Prevalence; *Fasciola gigantica*; Beef cattle; Slaughterhouse; Lao PDR

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ความชุกของการติดพยาธิ Fasciola gigantica ในโคเนื้อที่โรงฆ่าสัตว์หนองด้วง นครหลวงเวียงจันทน์ ประเทศสาธารณรัฐประชาชนประชาชนลาวโดยวิธี iELISA

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บทความย่อ

วัตถุประสงค์ เพื่อสํารวจความชุกของ F. gigantica ในโคพันธุ์พื้นเมือง ที่โรงฆ่าสัตว์หนองด้วง นครหลวงเวียงจันทน์ ประเทศสาธารณรัฐประชาชนประชาชนลาวโดยวิธีการ iELISA เปรียบเทียบกับการตรวจนับไข่พยาธิเชิงปริมาณ

วัสดุ อุปกรณ์ และวิธีการ การเก็บตัวอย่างเลือดและมูลโคแบบสุ่มจากโคตOTA 172 ตัวอย่างจากโรงฆ่าสัตว์เพื่อประเมินความชุกด้วยวิธีการตรวจนับไข่พยาธิจากตัวอย่างมูลโคด้วยวิธีทำให้ตกตะกอนและวิธีเข็มฉีดฟอร์มอล-อิธีร์ และวิธี Indirect enzyme-linked immunosorbent assay (iELISA) และการตรวจวัดระดับอิมมูโนในโคลิลินีจีในตัวอย่างซีรัมที่ตอบสนองต่อสารคัดหลั่งจาก F. gigantica และ Western blot พร้อมมันที่เข็มฉีดต่ำสุดที่สําคัญอุปราคาคะแนนความสมบูรณ์ของร่างกายและคะแนนมูลโค วิเคราะห์ข้อมูลด้วย Chi-square, Independent t-test และ Kappa โดยใช้ SPSS รุ่น 17

ผลการศึกษา จากการตรวจด้วยวิธี iELISA พบว่ามีความชุกของการติดพยาธิ F. gigantica สูงติดเป็นร้อยละ 94.7 (163/172) และจากการตรวจนับไข่พยาธิเชิงปริมาณสูงในระดับน้ําหนัก คิดเป็นร้อยละ 11.6 (20/172) โดยไม่มีความแตกต่างกันระหว่างสภาพอุปราคาคะแนนความสมบูรณ์ของร่างกายและลักษณะมูลโค นอกจากนี้ยังพบว่าโคตOAจาก 77.9 (134/172) มีการติดพยาธิไปไม่ติดและหรือพยาธิในกระเพาะหมักและพยาธิตัวกลม และมีโคที่ไม่พบไข่พยาธิแต่ติดเป็นร้อยละ 22.1 (38/172) นอกจากนี้ยังพบ Fg-ES แอนติเจนน้ำหนักโมเลกุล 14, 22, 25, และ 34 kDa ที่ทำปฏิกิริยาต่อแอนติบอดี IgG จากซีรัมโดยที่ติดพยาธิไปไม่ติดจากการตรวจโดยวิธี Western blot

ข้อสรุป ผลการสํารวจโรงฆ่าสัตว์หนองด้วง นครหลวงเวียงจันทน์ ประเทศสาธารณรัฐประชาชนประชาชนลาวสามารถประเมินความชุกของการติดพยาธิ F. gigantica ได้ดีมากโดยวิธี iELISA ซึ่งเป็นวิธีทางชีวเคมีที่สามารถใช้เป็นทางเลือกในการตรวจวินิจฉัยการติดพยาธิได้โดยที่สามารถลดปัญหาผลเฉลี่ยจากการตรวจนับไข่พยาธิในมูลโคเนื้อแบบเดิมและช่วยคงหาโคเนื้อที่ติดพยาธิได้ไม่ล่าช้าผลเลือกในการตรวจหากการติดพยาธิไปไม่ติดที่เกิดจาก F. gigantica ในโคเนื้อ
Introduction

Fasciolosis is now considered as an important parasitic disease that causes economic losses in the beef production worldwide. Normally, this health problem is caused by two liver fluke species, i.e. Fasciola hepatica and F. gigantica (trematodes). In particular, F. gigantica is known as an extremely important cause of fasciolosis in livestock in a tropical region of Africa and Asia [1]. It has involved a considerable agricultural and economic losses resulting from infection estimated at US$ 2,000–3,000 million annually [2]. The disease has undergone a sharp rise and has emerged as a major zoonotic disease in many countries [3], with an estimation of 17 million infected people and up to 180 million at risk [4]. The human form of the disease has also become a major public health problem in several parts of the world [5]. In cattle, sheep and goat, the disease also causes high economic losses from anemia, reduced production, poor performance, condemnation of liver and an increased mortality [6-8].

The typical climate of Lao PDR is a monsoon type with hot, wet summer and warm dry winter [9]; therefore, the cattle are at risk of F. gigantica infestation as in other tropical countries [1,10]. Abattoir survey has been widely used to recruit many epidemiological information regarding animal health and public health concern [11-14] especially F. gigantica-causing fasciolosis in slaughtered beef cattle. However, fasciolosis-related information in beef cattle in Lao PDR has been extremely limited. Previous reports are mainly from other international sources and unpublished information, which do not reflect the current status of the fasciolosis in beef cattle in this country. Moreover, the understanding about this liver fluke in each area is of great importance for management and control. Therefore, this study was conducted to determine the prevalence of F. gigantica infestation in beef cattle in Nongduang slaughterhouse, Lao PDR using serological approaches (iELISA and immunoblot) and quantitative fecal examination in the slaughterhouse.

Materials and Methods

Animal and study design

This cross-sectional study was implemented from September 2010 to February 2011 at Nongduang slaughterhouse, Vientiane capital of Lao PDR, in which 20-50 native beef cattle from Vientiane capital and vicinity were daily slaughtered. One hundred and seventy two beef cattle total were randomly selected during 20 visits to collect fecal, blood samples and adult F. gigantica from liver.
Information of each cattle was recorded including age, sex, body condition score (BCS) [15] and fecal scores [16].

**Fecal examination**

The formalin-preserved samples were proceeded in order to detect helminthes eggs in feces from beef cattle according to a modified quantitative formalin/ethyl acetate sedimentation [17]. Briefly, samples were sieved by a mesh, transferred into 15-ml conical tubes, and centrifuged at 4,000 rpm for 5 min. After discarding the supernatant, the pellets were re-constituted in 7 ml of 5% formalin plus 3 ml of ethyl acetate, shaken vigorously for 1 min, and re-centrifuged at 4,000 rpm for 5 minutes. After centrifugation, the detritus rings and the supernatants were discarded, whereas the *Fasciola* egg-bearing pellets were re-suspended in 5% formalin to yield a 2.5-ml mixed volume and transferred into screw top tubes. To determine egg counts, 300 μl of the resuspended sediments were placed on microslides for egg counting. The total number of eggs per gram (EPG) was calculated according to the method modified by Elkins et al. [17].

**Indirect enzyme-linked immunosorbent assay (iELISA)**

1. **Preparation of *F. gigantica* excretory/secretory antigen (Fg-ES Ag)**

   The *Fg*-ES Ag, a metabolic antigen, was prepared according to a previous method [18]. Briefly, *F. gigantica* worms were collected from the liver of cattle, washed several times by 0.01 M PBS, pH 7.4 in order to remove all traces of blood and bile. The worms were incubated in PBS (5 worms/10 ml) at 37 °C for 6 hours and removed by sieving. The sieved E/S products-containing PBS were subsequently centrifuged at 9,660 ×g (Dynamica, Velocity 18R Versatile Centrifuge, Switzerland) for 30 minutes at 4 °C. The supernatant was filtered through a 0.2-μ Millipore filter (Sartorius Stedim, Germany), followed by a dialysis against sterile distilled water for 24 hours using 10,000 molecular weight cut off (MWCO) SnakeSkin™, Pleated Dialysis Tubing (Thermo Scientific, USA), aliquoted and stored at -20 °C until analysis. The dialyzed proteins were then determined by spectrophotometry at the 260- and 280-nm wavelengths (Life Science uv/vis spectrophotometer, Beckman Coulter™, DU™530 model) and expressed as μg/μl.

2. **Indirect Enzyme-linked Immunosorbent Assay (iELISA)**

   The iELISA was carried out according to the method modified from Chaiyotwittayakun *et al.* [19] in 96-well microtiter plates (Nunc-Immuno Plate MaxiSorp, Thermo Fischer Scientific, Denmark). Briefly, the wells were coated by 100 μl of Fg-ES Ag in 0.1 M carbonate buffer, pH 9.6 (10 μg/ml) and incubated overnight at 4 °C. The plates were washed twice with PBS/Tween 20 (0.01%, PBST) and then blocked with 100 μl/well of 0.01% bovine serum albumin (BSA) in 0.01 M PBS (pH 7.4) for 1½ hours at 37 °C. After blocking, the plates were washed three times with PBST, added 100 μl of each tested serum diluted 1:100 ratios in PBS, pH 7.4 to the wells in duplicate, and incubated for 1½ hours at 37 °C. The plates were then washed five times, followed by adding 100μl/well of anti-bovine alkaline phosphatase-conjugated rabbit IgG (Bethyl Laboratories, USA) diluted in PBS, pH 7.4 at 1:6,000 ratios, and incubated at 37 °C for 2 hours. After other five washes, as a substrate, 50 μl of p-nitrophenyl phosphate disodium
salt (1-Step™ PNPP, Thermo Scientific, USA) was added to each well and incubated in the dark for 15 minutes at 37 °C. The enzymatic reaction was subsequently stopped by 50 μl/well of 2 N NaOH. The absorbance of each well was read at a 405-nm wavelength using a microplate reader (BioRad, Model 3550, USA) and expressed as optical density (OD).

In each iELISA plate, negative and positive control diluted 1:100 in PBS, pH 7.4 were included in duplicate. Commercially available fetal bovine serum (FBS, Biochrom AG, Germany) was used as a negative control. A positive control was assigned from a tested serum with a positive gross plus fecal examination, relatively compared to the OD greater than the cut off value, which was obtained from the average OD from negative control plus three standard deviations as described in the methods [14, 19] with some modifications. The intra-assay and inter-assay coefficients of variability (CV) as a percentage of the assay were determined for precision of both within and between assays, respectively.

**SDS-PAGE and Immunoblotting**

The dialyzed proteins were then characterized and analyzed by SDS-PAGE according to the manufacturing instruction and modified from a protocol [19]. Briefly, Fg-ES Ag was prepared using a 1:1 ratio in Laemmli sample buffer (BioRad, USA) plus 5% (v/v) of β-mercaptoethanol (AppliChem, Germany). The mixtures were heat-denatured at 95°C for 5 min prior to loading (23 μg of total protein in 25 μl/well) on pre-casted Tris-HCl, acrylamide-bis mini gels (12%, Amersham™ ELC™, GE Healthcare, Israel). The electrophoresis was carried out in the Amersham™ ELC™ Gel Box (GE Healthcare, Israel) using electrophoresis buffer (0.025 M Tris, 0.192 M Glycine and 0.1% SDS, Vivantis, Malaysia) under 160 volts, 45 mA, for 1 hour (Power supply, Model: PowerPac 3000, Bio-Rad, USA). The protein bands were then visualized by silver staining (Silver Stain Plus, Bio-Rad, USA) according to a manufacturing instruction and compared with the molecular weight markers (Precision Plus Protein™ Standards 10-250 kDa; Bio-Rad, USA).

Western blotting was performed to characterize interaction between bovine IgG antibody and Fg-ES antigens by following the manufacturing instruction and a modified protocol [19]. The electrophoresed proteins were transferred from gel to polyvinylidene difluoride (PVDF) mini membranes (0.2μ, invitrogen™, USA) in dry blotting system under 20 volts for 7 minutes using the iBlot™ transfer device (invitrogen™, USA). The transferred PVDF membranes were rinsed by Tris buffered saline (TBS, 50 mM Tris, 150 NaCl, pH 7.4) and submerged in a blocking buffer containing 3% BSA in TBS/Tween 20 (0.1%, TBST) at room temperature for 1 hour to ensure the specific binding. One membrane was probed by primary antibody prepared from 5 pooled Fg-positive sera, the other was probed by FBS at a 1:1,000 optimized dilution in TBST. Secondary antibody (anti-bovine alkaline phosphatase-conjugated rabbit IgG, Bethyl Laboratories, USA) was subsequently used at a 1:6,000 dilution in TBST. Each probing step was carried out for 1 hour at a room temperature. Moreover, between each individual step, the PVDF membranes were immersed twice in TBS/Tween 20 (0.05%) for 10 minutes to get rid of excess BSA and antibodies. The enzyme substrate reaction was then visualized by reacting the BCIP/NBT phosphate substrate (0.21 mg/ml of 5-bromo-4-chloro-3-indoxyl-phosphate (BCIP) and 0.42 mg/ml
of nitroblue tetrazolium (NBT) in an organic base/Tris buffer, KPL, USA) and stopped by immersing in reagent quality water for 20 minutes. The results were analyzed by ChemiDoc™ XRS Plus system with Image Lab™ software (Bio-Rad Laboratories, Inc., USA).

**Data Analysis**

The descriptive statistic was used to analysis and explained a data frequency, percentile, mean, and standard deviation for data as regards sex, age, BCS, and fecal score. Pearson Chi-square test was used to individually determine the difference of sex, age, BCS, and fecal score. Difference serum IgG between cattle with shedding and non-shedding *Fasciola* eggs was tested using an independent t-test. McNemar Chi-square test was applied to analyze consistency of the results of serum IgG and FEC grouping. Kappa statistic was also used to determine the agreement of iELISA and FEC results. All statistical analyses were performed using SPSS version 17. Significant difference was indicated when $P < 0.05$.

**Results**

There were 30 male and 142 female slaughtered cattle. FEC-based results found that *F. gigantica* infestation in older cattle (>2 years old) was higher 11% (19/172) than in young cattle (<2 years old) 0.6% (1/172). Most cattle (67.4%, 116/172) were poor to fair condition (BCS ≤ 2) and 8.1% (14/172) positive. In addition, age, BCS and fecal scores were not different between the infested and non-infested cattle (Table 1).

A sedimentation method using formalin-fixed fecal samples resulted in 11.6% of the *F. gigantica* positive cattle (20/172) including only 5% (1/20) of single *F. gigantica* infestation and 95% (19/20) of multi-parasitic infestation (mainly liver fluke, rumen fluke and round worm) as shown in Table 2. Based on the simple sedimentation technique, most cattle were positive for major parasites, while others were negative shedding for any parasitic eggs, accounted for 77.9% (134/172) and 22.1% (38/172), respectively.

iELISA yielded 94.7% of positive sera (163/172) using the OD at a cut-off point of 0.17. Both methods shared similar result of the 20 infected cattle. In addition, iELISA could detect 83.1% false negative (143/172) and 5.2% of true negative (9/172) sera from the cattle without *Fasciola* egg shedding in feces (Table 2). Average serum IgG absorbance (±SE) in cattle with FEC-based positive and negative were significantly different ($P<0.01$) accounted for 0.48±0.03 and 0.14±0.01, respectively.

Results indicated that Fasciola positive cattle using fecal examination were related with the IgG absorbance of infected cattle detected by iELISA. According to Independent t-test, serum IgG against *Fg*-ES antigens was significant different between the cattle shedding and non-shedding liver fluke eggs ($P<0.05$). However, serum IgG and log-transformed FEC was insignificantly associated as regard a Linear regression (data not shown). Results of serum IgG grouping test were inconsistent with results of the egg grouping test ($P > 0.05$), based on McNemar Chi-square test.

Six main proteins (14, 15, 22, 24, 52 and 53 kDa) were found according to SDS-PAGE followed by silver staining (Figure 1). Moreover, western blotting analysis revealed a detection of *Fg*-ES antigens with molecular weights of 14, 22, 25, and 34 kDa (Figure 2) after probing by pooled serum IgG antibodies, whereas none was found by FBS IgG (data not shown).
### Table 1. *F. gigantica* Infestation in 172 Slaughtered Beef Cattle Categorized by Sex, Age, BCS, and Fecal Scores Based on Fecal Egg Counts

<table>
<thead>
<tr>
<th>Items</th>
<th>Category</th>
<th>n</th>
<th>Infected cattle</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>30</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>142</td>
<td>18</td>
<td>12.7</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;2 years old</td>
<td>4</td>
<td>1</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>≥2 years old</td>
<td>168</td>
<td>19</td>
<td>11.3</td>
</tr>
<tr>
<td>BCS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>52</td>
<td>8</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>[16]</td>
<td>64</td>
<td>6</td>
<td>9.4</td>
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<td>[17]</td>
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<td>5.7</td>
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</tr>
<tr>
<td></td>
<td>5</td>
<td>16</td>
<td>4</td>
<td>25.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>BCS = body condition score

### Table 2. Results of Fecal Examination and iELISA (Using Sera and Fg-ES Antigens) in 172 Slaughtered Beef Cattle from Nongduang Slaughterhouse, Lao PDR

<table>
<thead>
<tr>
<th>Fecal examination</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>iELISA Negative</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Positive</td>
<td>143</td>
<td>20</td>
<td>163</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>20</td>
<td>172</td>
</tr>
</tbody>
</table>
Figure 1. SDS-PAGE & Silver Staining of Fg-ES Antigens

Using 12% Tris-Glycine mini gel, 160 volts, 45 mA, 1 hour; M = Protein marker, Lane 1-2 = Fg-ES antigens

Figure 2. Western Blot and Colorimetric Analysis Fg-ES Antigens

Probed by pooled Fg-positive sera followed by anti-bovine alkaline phosphatase-conjugated rabbit IgG
Discussion

Abattoir survey was widely accepted for determination of parasitic infection especially liver fluke in being slaughtered beef cattle [11-14]. Because it was useful for determination the prevalence, risk assessment and prevention and control strategic planning of parasitic infection [13]. Fasciolosis survey at the Nongduang slaughterhouse, Lao PDR also provided relevant information as in previous studies [13,20-22]. In the north of India, epidemiological studies on *F. gigantica* infection found 10.8% and 13.9% in cattle and buffaloes, respectively [20]. Sukhapsena et al. [23] reported 13.9% and 8.9% in cattle and buffalo, respectively. Neamjui et al. [24] also found higher prevalent in buffaloes (13.9%, 25/180). Overall while *F. gigantica*-causing fasciolosis in buffalo and cattle were 8.6% [24] and 42.1% [25]. Besides, high prevalences of *Fasciola* infestation was reported (30-90%) [14]. In contrast, a low prevalence rate was also reported as minimal as 1.5% in Nigeria [13], 2.4% in Central Iran [12], and 3.33% (6/180) in Thailand [24].

However, the prevalence of liver fluke infection in beef cattle could be varied by many factors, including the animal’s age, numbers of infected cattle, stages of infection maturation status of the fluke, locations, differences in landscape (such as swampy areas, agricultural irrigation practices), season, density of the animal in particular area, management, preventive control strategies, poor meat inspection facilities, and uncooperative attitudes of butchers [13,21,22,24]. In accordance with previous studies, more prevalent fasciolosis was observed in the older cattle [14,22,24,25].

Although, fecal examination has been routinely used for diagnosis of fasciolosis for several decades, certain limitations of this method were clearly mentioned including time consuming, suitable amount of feces, its low sensitivity, and false negative [18]. Theoretically, the eggs can be found in the feces 3½ month after a primary infection [22]. For the cattle with false negative sera, it could be explained that the cattle could be in prepatent period (early infection and maturation of the fluke), low infection, chronically infected, having adult flukes laying no or less number of eggs, intermittent shedding of *Fasciola* eggs in bovine feces, or ectopic fasciolosis, as previously discussed [22]. Furthermore, in chronic fasciolosis, a pathological change of the common bile duct can inhibit the egg release into the duodenal lumen and mix with feces even in the heavily infected animal harboring by adult flukes in the liver [22].

Among those techniques, ELISA technique has become one of the most widely used tests and effective tools for diagnosis of fasciolosis in ruminants with its simplicity and reliability [14,18,26,27], including iELISA. At least, the infestation can be diagnosed 2 week as early as after infection by ELISA [28]. Demonstrated the iELISA using crude adult antigen for diagnosis of experimental or naturally occurring *F. gigantica* infestation in cattle, was superior to a fecal egg examination. Therefore, it could overcome the limitation of the conventional methods especially the fecal egg count [29]. In this study, intra-assay and inter-assay CVs of iELISA were 7.3% and 2.4%, respectively, which were within the general acceptable levels (below 10% and 15%) [30].

Generally, Kappa statistic is used to determine the agreement of results from each paired test. As
a rule of thumb values of Kappa from 0.40 to 0.59 are considered moderate, 0.60 to 0.79 substantial, and 0.80 outstanding. The Kappa of the present result was 0.014, which was lower than previous reports of k=0.74 [31] and k=0.53 [32]. However, in this study, Kappa statistic may not be precise because of some factors affecting the results of each test as regards, sensitivity (iELISA versus FEC) [14,18,26,27], stage of infection, egg shedding rate, animal’s age, and a pathological change in bile duct [22]. Descriptive analyses could be then more appropriate. As a result, the study revealed that iELISA is still effective diagnostic tool for bovine fasciolosis by using Fg-ES antigens.

SDS-PAGE followed by silver stain of Fg-ES Ag provided predominant 14, 15, 22, 24, 52 and 53 kDa protein bands (Figure 1) and yielded high quality of protein bands than Coomassie Blue staining used in previous reports [19,26]. Although studies suggested that a 28-kDa cysteine proteinase, an ES product was used to immunodiagnose for fasciola infestation in ruminant [26,28,33], such protein was not observed in this study. However, the current study demonstrated the interaction between 4 main Fg-ES antigens with molecular weight of 14, 22, 25, and 34 kDa and bovine IgG antibodies. Only 14- and 22-kDa Fg-ES antigens were detected in both silver staining and immunoblotting procedures. In particular, the detection of 14-kDa [33] and 34-kDa [34] antigens were corresponded to previous studies. Therefore, these two antigens (14 and 22 kDa) could be considered as alternative protein markers for fasciolosis diagnosis in beef cattle. Further research is warranted to understand the interaction between F. gigantica and a host response in beef cattle.

The abattoir survey demonstrated a high iELISA-based prevalence of F. gigantica infestation in the beef cattle at Nongduang slaughterhouse, Lao PDR. The iELISA, an alternatively serological test could overcome the false negative results according to a traditional fecal examination and investigate F. gigantica-infected beef cattle during prepatent period. Moreover, 14- and 22-kDa excretory-secretory proteins could be alternative markers for detection of F. gigantica infestation.

Acknowledgements

The study was financially supported by the child’s dream foundation and the thesis research fund from the Faculty of Veterinary Medicine, Khon Kaen University. The authors would like to acknowledge Asst.Prof.Dr Kwankate Kanistanon for the assistance on data analysis and Nongduang slaughterhouse, Lao PDR for the sampling permission.

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