Analysis of *Fasciola* sp. Antigen by Enzyme-Linked Immunotransfer Blot Using Sera from Experimentally and Naturally Infected Cattle

Tadashi ITAGAKI, Tsukasa SAKAMOTO, and Hiroshi ITAGAKI¹⁾

Department of Parasitology, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka 020 and ¹⁾Department of Parasitology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagamihara 229, Japan

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ABSTRACT. Soluble polypeptides extracted from adult *Fasciola* sp. were analysed by SDS-PAGE, and their antigenicity was characterized by enzyme-linked immunotransfer blot (EITB) using sera from experimentally and naturally infected cattle. Polypeptides of adult *Fasciola* sp. with estimated molecular mass of 64–52 kDa, 38–28 kDa, 17 kDa, 15 kDa, 13 kDa and 12 kDa, were recognized by sera obtained in both early and late stages of infection. Furthermore, two polypeptides of more than 160 kDa were detected by sera only in the early stage of infection. The results of EITB using sera of dairy cows naturally infected with *Fasciola* sp. suggest that polypeptides of 64–52 kDa may be candidates as serodiagnostic antigen of bovine fascioliasis.—**KEY WORDS:** bovine fascioliasis, enzyme-linked immunotransfer blot, *Fasciola* antigen.

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Fasciola species releases different antigenic components at different stages of development in mammalian hosts [1, 2, 6, 9]. Identification and characterization of the antigenic components are of fundamental importance to prepare antigen adequate for serodiagnosis and vaccine preparation against fascioliasis. The present study was designed to provide information on antigenic components of adult *Fasciola* sp. by enzyme-linked immunotransfer blot (EITB) using sera of cattle with experimental and spontaneous fascioliasis.

Adult *Fasciola* sp. obtained from slaughtered cattle were washed several times with PBS (pH 7.2) and stored at –80°C until use. The frozen flukes were homogenized in PBS (pH 7.2). The homogenate was stirred at 4°C overnight to extract soluble proteins, and then centrifuged at 12,000 g. for 30 min. The supernatant was collected and used as the extract of adult *Fasciola* sp.

The sera used in the present study were as follows: sera obtained at 2 to 4 week intervals for 23 weeks from 2 male Holstein calves about 6 months of age (Nos. 612 and 613) experimentally infected with 200 metacercariae of *Fasciola* sp., and sera from 27 dairy cows reared in Tochigi Prefecture, Japan. 17 out of these 27 cows showed positive ELISA value ranging from 0.339 to 1.008 by ELISA test described previously [4] and furthermore most of the 17 cows excreted *Fasciola* eggs in the feces. So they were considered to be spontaneously infected with the fluke. The other 10 cows, however, showed negative ELISA value ranging from 0.079 to 0.256 and negative results by fecal egg examination, so the sera from them were used as uninfected control.

The extract of adult *Fasciola* sp. was mixed with an equal volume of sample buffer (0.5 M Tris-HCl pH 6.8, 3% SDS, 10% glycerol, 5% 2-mercaptoethanol). The mixture was boiled for 3 min and applied to single wells of 12.5% polyacrylamide slab gels (7.5 cm wide, 6 cm long, 2 mm thick) in a volume of 100 µl (150 µg of protein) each. After electrophoresis at a constant current of 25 mA for about 1 hr, one of gels was stained with coomassie brilliant blue for protein analysis by SDS-PAGE and the others were used for analysis of antigenic proteins by EITB. The details were as follows. Proteins were transferred from gel

to nitrocellulose membranes at a constant voltage of 7.5 V overnight. The nitrocellulose membrane was cut into 20 strips of 4 mm wide. One of the strips was stained with India ink for total proteins, and the others were used for reaction with serum samples. The strips were then immersed for 1 hr in PBS-Tween (PBS pH 7.4, 0.05% Tween-20) containing 2% gelatin, and then incubated for 1 hr with serum samples diluted 1:400 in PBS-Tween containing 0.5% gelatin. After washed three times with PBS-Tween, the strips were made to react for 1 hr with horseradish peroxidase-conjugated rabbit anti-bovine IgG diluted 1:3,000 in PBS-Tween containing 0.5% gelatin. After rinsed with PBS-Tween, the strips were soaked in the substrate solution consisting of 10 mg 3–3' diaminobenzidine in 20 ml of PBS (pH 7.2) and 12 µl of 5% H₂O₂.

Polypeptide pattern of adult *Fasciola* sp. analysed by

Fig. 1. SDS-PAGE of soluble polypeptides extracted from adult *Fasciola* sp. Gel was stained with coomassie brilliant blue. Molecular markers are indicated in kilo dalton (kDa) on the right side.

SDS-PAGE is shown in Fig. 1. More than 30 polypeptides with estimated molecular mass from ≥ 200 kDa to <14 kDa were observed in a gel stained with coomassie brilliant blue.

EITB patterns using sera obtained from the two experimentally infected calves are shown in Fig. 2. The patterns of the two calves were almost the same each other and varied with time after infection. Two polypeptides of ≥ 160 kDa reacted with sera from 6 to 10 weeks after infection. Bands of 64–52 kDa were dominant and detected at weeks 6 to 23. Bands of 38–28 kDa appeared

at week 10 and increased in intensity after week 12. Bands of 17 kDa, 15 kDa, 13 kDa and 12 kDa were detected at weeks 10 to 23. Band of 26 kDa was observed at week 23 in one (No. 612) of the two calves.

EITB patterns using sera from the 17 dairy cows with spontaneous fascioliasis are shown in Fig. 3. All the sera recognized 64–52 kDa bands with different intensity. The bands of 38–34 kDa, 31–28 kDa, 26 kDa, 17 kDa, 15 kDa, 13 kDa and 12 kDa were detected by 13, 12, 3, 11, 5, 2 and 4 out of these 17 sera tested, respectively. Sera from 10 uninfected control cows did not recognize these bands

Fig. 2. Enzyme-linked immunotransfer blot (EITB) of adult *Fasciola* sp. antigens using sera taken from 2 experimentally infected calves (Calf 612 and 613) at 2–4 weeks intervals after infection. Strip numbers represent weeks post infection when sera were obtained. Molecular markers are indicated in kilo dalton (kDa) on the right side.

Fig. 3. EITB of adult *Fasciola* sp. antigens using sera taken from 17 dairy cows with spontaneous fascioliasis. 'N' and 'P' represent a negative and a positive control serum (a serum of 'calf 612', 21 weeks after infection), respectively. Molecular markers are indicated in kilo dalton (kDa) on the right side.

(Data not shown).

In sheep experimentally infected with *F. hepatica*, sera in both early and later stages of infection recognize the same components of adult *F. hepatica* antigen [8]. However, the present results of EITB using sera from 2 experimentally infected calves showed that sera obtained in early and late stages of infection recognized some different antigenic polypeptides. The difference between the present and the previous studies [8] may come from that in recognition between cattle and sheep, or that in released components between *Fasciola* sp. and *F. hepatica*. Moreover, it has been reported that most of the polypeptides detected in the present study are also recognized by sera of cattle [7], sheep [8], rabbit [5, 6] and mouse [5] experimentally infected with *F. hepatica*.

Hillyer and Galanes [3] recommended 17 kDa polypeptide from *F. hepatica* as a serodiagnostic antigen of acute and chronic human fascioliasis. However, the present study suggested that 17 kDa-polypeptide seemed to be inadequate for a diagnostic antigen in bovine fascioliasis, because the polypeptide reacted with only 5 out of the 17 sera with spontaneous fascioliasis. Polypeptides of 64–52 kDa were recognized by all of the

17 sera with spontaneous fascioliasis in the present study, so they may be useful antigens for serodiagnosis of fascioliasis, unless cross-reaction with other parasites will be detected in future studies.

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