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Application of Agar Gel Diffusion Test to the Diagnosis of Fasciolosis in Cattle and Buffaloes in the Red River Delta of Vietnam

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Abstract

The agar gel diffusion test (AGDT) was applied to detect *Fasciola* infection in several regions of Vietnam where equipment for fecal examination such as a microscope is not readily available. A 1% solution of agar dissolved in 10% saline, and the antigen prepared from adult individuals of *Fasciola* sp. were used for the test. The test was specific for *Fasciola* infection, which was detected in the sera from about 50% of the cattle and water buffaloes examined. The gel and antigen can be stored in a refrigerator for more than 3 months and used for the test. The results of this study revealed that the agar gel diffusion test could become a useful tool to diagnose fasciolosis in cattle and buffaloes at the Regional Animal Health Centers in Vietnam.

Discipline: Animal health
Additional key words: liver fluke, rumen fluke, serological test, zoonosis, herd diagnosis

Introduction

Fasciolosis of cattle and water buffalo is a very serious disease in the Red River Delta of Vietnam[4] and the neighboring countries[6] due to the economic losses associated with retardation of growth and sometimes death. Since fasciolosis is a zoonosis, humans are also infected (human fasciolosis). Tran et al.[10] have confirmed 500 cases of human fasciolosis in the central provinces of Vietnam, and reported that *Fasciola* infection is increasing in frequency and easily contaminates food due to the changes in the environmental conditions and in the number and breeds of herbivorous domestic animals. To control zoonotic fasciolosis, it is also necessary to demonstrate the presence of infection with fasciolosis in cattle in the concerned areas. Diagnosis of the infection in ruminants has been commonly performed by fecal examination, which involves the detection of fluke eggs in feces after sedimentation. In Vietnam, however, it is not easy to diagnose the disease accurately, because most of the Regional Animal Health Centers (RAHCs) in Vietnam lack facilities such as a microscope to perform fecal examination. Therefore, a simple test, as an alternative to fecal examination is required, in order to conduct epidemiological surveys over wide areas. The agar gel diffusion test (AGDT) is considered to be one of the diagnostic methods for fasciolosis. In the present study, we applied the AGDT to detect fasciolosis in several regions of Vietnam.

Materials and methods

1. Antigen

As shown in Fig. 1, adult individuals of *Fasciola* sp. ranging from 15 to 40 mm in length were collected from the bile ducts of infected cattle using a previously described technique[1]. Livers from these animals were

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sliced into strips about 1 to 2 cm in thickness and immersed in normal saline to obtain immature worms ranging from 5 to 10 mm in length. Adult individuals of *Pharamphistomum* spp. were also collected from the rumen of naturally infected cattle at the abattoir. The worms were washed with physiological saline and stored at –20°C until examination. The antigen used was extracted from adult individuals of each kind of fluke. The extract was prepared in the following manner[12]: In the first step, 0.1 g of each fluke was immersed in 5 mL of physiological saline and homogenized for 30 min. The emulsion was then frozen and thawed twice and centrifuged at 5,000 rpm for 30 min at 4°C. The supernatant of each emulsion was used as the antigen of the respective fluke. Finally, 0.01% of thimerosal was added to each antigen and stored in a refrigerator.

2. Sera and feces

Serum and fecal samples were collected from cattle (n = 30) and water buffaloes (n = 2) raised in and around Hanoi, Vietnam (Fig. 2). These samples were obtained from each animal at an abattoir in the city. Fecal samples were stored at 5°C and the sera were stored at –20°C until use. Before use, several samples of the sera to be tested were inactivated at 56°C for 30 min and diluted.

3. Number of eggs in feces

Counts of *Fasciola* eggs per gram (EPG) in feces were carried out according to the method reported by Anderson et al.[1].

4. Agar Gel Diffusion Test

Optimization of AGDT was performed in the following manner. Five mL of 1.0% agar solution (Seikagakukougyou Co., Ltd. Tokyo, Japan) in 10% saline was poured into a Petri dish 6 cm in diameter (Figs. 3 & 4). Several wells were prepared in the agar gel at a distance of 3 mm between the wells. These Petri dishes were stored in a refrigerator until use. When the effect of serum inactivation on the formation of precipitin lines in the gel was examined by using inactivated (56°C, 30 min) and untreated serum samples, precipitin lines were clearly observed in both samples. The precipitin line was formed more distinctly with undiluted serum. Therefore, undiluted and untreated serum was used in this study. Each well in the gel was filled with the antigen or serum to be tested (Fig. 5). The dish was kept in a moist chamber at room temperature for 4 days and the reaction was observed daily. When a distinct precipitin line was found in the gel, the serum was considered to have the antibody against fasciolosis. To rule out the possibility of cross-reaction with other flukes, the AGDT was also performed with the *Pharamphistomum* antigen and the positive reference sera against the *Fasciola* antigen.

Results

The AGDT enabled to detect *Fasciola* infection in 15 out of 32 serum samples examined (Table 1), although no significant correlation between antibody detection by AGDT and the number of worms in the liver was found. In general, two or more precipitin lines were observed between the antigen and antiserum (Fig. 6). No positive reaction was observed between the *Pharamphistomum*

Table 1. Serological examination of cattle and water buffaloes harboring *Fasciola* worms in their livers

Sample	No. of adult and immature worms	AGDT	Eggs per gram feces
1	188	+	60
2	139	–	3
3	115	–	228
4	96	+	27
5	94	–	42
6	59	+	3
7	49	–	45
8	48	–	72
9	45	–	45
10	36	+	18
11	35	–	18
12	31	–	9
13	29	+	90
14	29	–	18
15	24	+	0
16	20	+	15
17	18	+	0
18	17	+	24
19	17	–	0
20	15	–	0
21	15	+	9
22	15	+	9
23	15	–	0
24	13	–	0
25	13	+	18
26	8	+	0
27	6	–	0
28	4	–	12
29	3	+	0
30	2	–	0
31*	2	–	0
32*	2	+	0

*Water buffalo

Fig. 1. *Fasciola* worms collected from an abattoir in Hanoi

Fig. 2. An endemic area of fasciolosis in Bac Ninh Province

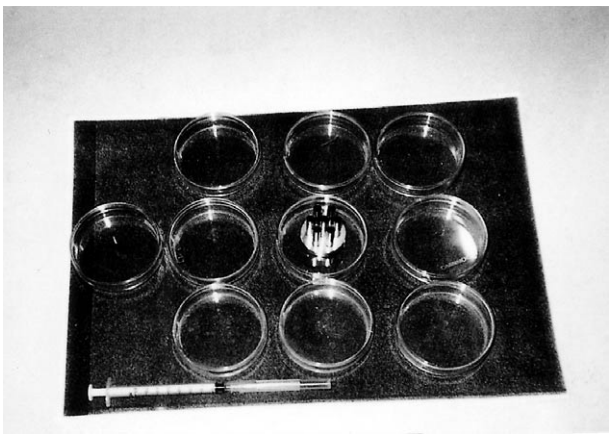

Fig. 3. Tools for AGDT

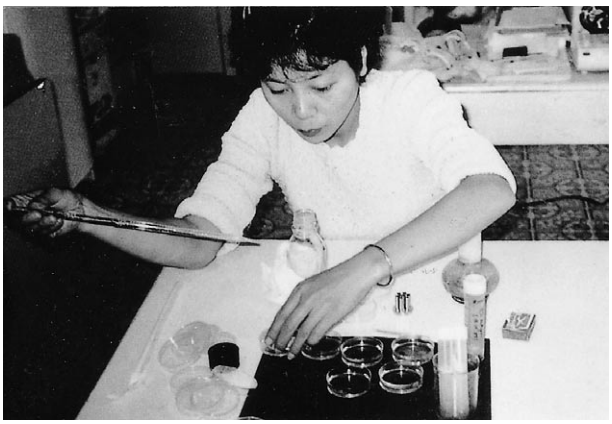

Fig. 4. Agar solution being poured into Petri dishes in the laboratory at the National Institute of Veterinary Research

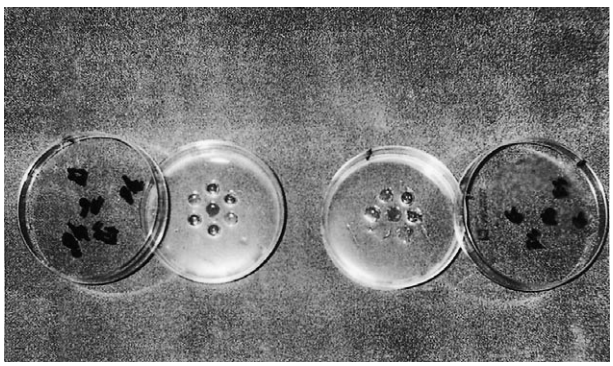

Fig. 5. Agar gels with antigen and test sera in a moist chamber

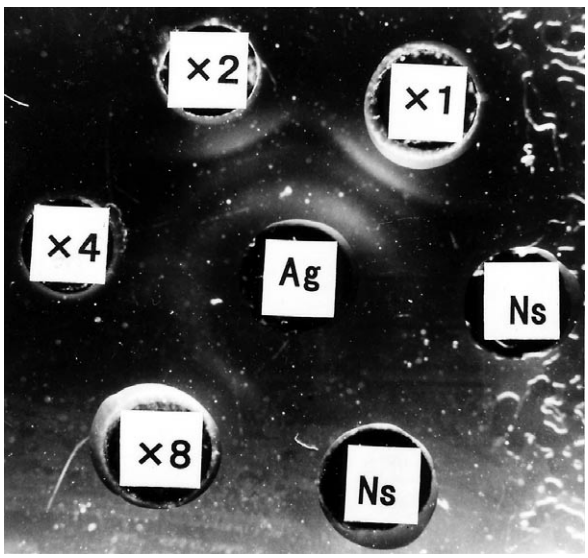

Fig. 6. Agar gel diffusion test
Ag: Antigen, ×1–×8: Diluted positive serum.
Ns: Normal serum.

antigen and the sera reacting against the *Fasciola* antigen. In some cases, antibody against the *Fasciola* sp. was detected even though eggs were not detected in the feces, and vice versa.

When the gel and antigen were stored in a refrigerator for 3 months and then used for AGDT, clear reactions could still be observed and there was no fungal development.

Fasciola eggs were detected in the feces of 20 out of 32 animals examined (62%).

Discussion

Ouchterlony precipitation in gels has been effectively employed for the serological diagnosis of certain parasitic infections. In spite of being less sensitive, the procedure in general is more specific than either the indirect hemagglutination or indirect fluorescent antibody tests and offers a considerable potential as a diagnostic technique[5]. In addition, AGDT is also a simple and low-cost technique.

In the present study, AGDT was positive for *Fasciola* antibody in about 50% of the sera from the cattle and buffaloes harboring worms in their livers. The reaction was considered to be specific for *Fasciola* infection because the *Pharamphistomum* antigen did not react with sera from the cattle infected with *Fasciola* sp. The results show that the test can be applied to the diagnosis of fasciolosis.

In our tests, the sera were used without dilution because serum dilution is time-consuming and laborious. In the present study, however, AGDT was only used as a qualitative technique for diagnosis.

AGDT has been used to diagnose *Fasciola gigantica* infection in sheep in Iraq[3] and buffaloes in India[8]. In these examinations, normal saline solution (0.85%) was used to prepare the agar gel. Clear precipitin lines were obtained by using gels containing 10% sodium chloride in the present study. According to Soulsby[7], sera from cattle and sheep gave clearer precipitin lines at high concentrations of sodium chloride (10–14%). Our results for the sera from cattle and buffaloes were in agreement with the findings of Soulsby on the sera from cattle and sheep.

In the present study, the detection rate of fasciolosis by AGDT was lower than that by fecal examination. It was reported that in general, the serological test for fasciolosis can detect the acute phase of infection earlier than fecal examination[11], even in sera from calves with very low fecal egg counts[2], while fecal examination is useful when the infection is rather heavy[9]. In the present study, serum samples were collected only from cattle and buffaloes with severe macroscopical changes on the surface of the livers, such as thickening of the bile ducts. Therefore, the persistence of antibodies in infected animals and the sensitivity of AGDT are still under investigation in the chronic phase of the infection.

The advantage of the serological techniques is that they can be easily applied to a large number of samples at once[11], and are useful especially in areas which lack facilities for fecal examination, like in the Red River Delta of Vietnam. It is estimated that several hundred serum samples can be tested within a day using AGDT at the RAHCs. In addition, antigen and gels for AGDT can be stored at least for 3 months in a refrigerator.

From the results of the present study, it is concluded that AGDT could become a useful technique for herd diagnosis of fasciolosis in cattle and buffaloes by veterinarians and animal health workers at RAHCs belonging to the Department of Animal Health in provinces that lack suitable equipment for fecal examination. It seems likely, furthermore, that this simple and low-cost technique could be effectively employed for herd diagnosis of fasciolosis in domestic animals in the neighboring countries.

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