Results

The complexity of vascular and biliary system examination has led to the use of a number of different laboratory tests based on biochemical analysis of serum parameters. These include the measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) [7, 9, 29]. Two aminotransferase enzymes (ALT and AST) are localized mainly in the cytoplasm of hepatocytes and recognized as a marker of hepatocellular injury [8]. The highest ALP activity is found on the border membranes of the bile duct whereas GGT is found in the hepatocytes and biliary epithelial cell [8, 9, 29]. Two aminotransferase enzymes (ALT and AST) are localized mainly in the cytoplasm of hepatocytes and recognized as a marker of hepatocellular injury in rats, dogs and non-human primates [3, 8].

Pathological changes mainly caused by migration of the juvenile flukes affect the complex vascular and biliary system in the liver. The juvenile flukes then migrate through the intestinal wall into the peritoneal cavity and penetrate into the liver through the liver capsule. They then migrate through the liver parenchyma to find their way to the bile ducts and develop into adult flukes [11, 25]. No significant increase in serum gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) were observed. This could reflect reduced or minimal injury of bile ducts and biliary epithelia as the flukes had reached the adult stage. Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) were not detected in the infected rabbit during the course of the experiment. Serum liver enzymes monitoring might be useful for understanding the host-parasite relationship in fascioliasis.

Gradual increase of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were observed from 3 weeks post-inoculation (WPI) and peaked at 6 WPI, which corresponded well to the period of migration and development of juvenile fluke in the liver parenchyma and the time when the young adult flukes migrated to the bile duct. However, no significant increase in serum gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) were observed. This could reflect reduced or minimal injury of bile ducts and biliary epithelia as the flukes had reached the adult stage. Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) were not detected in the infected rabbit during the course of the experiment. Serum liver enzymes monitoring might be useful for understanding the host-parasite relationship in fascioliasis.

Dynamics of serum liver enzymes in rabbits experimentally infected with *Fasciola* sp. (Intermediate form from Japan)

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**ABSTRACT.** Dynamics of serum liver enzymes in rabbits experimentally infected with metacercariae of *Fasciola* sp. (intermediate form between *Fasciola hepatica* and *F. gigantica*) were monitored. Gradual increase of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were observed from 3 weeks post-inoculation (WPI) and peaked at 6 WPI, which corresponded well to the period of migration and development of juvenile fluke in the liver parenchyma and the time when the young adult flukes migrated to the bile duct. However, no significant increase in serum gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) were observed. This could reflect reduced or minimal injury of bile ducts and biliary epithelia as the flukes had reached the adult stage. Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) were not detected in the infected rabbit during the course of the experiment. Serum liver enzymes monitoring might be useful for understanding the host-parasite relationship in fascioliasis.

**KEYWORDS:** *Fasciola* sp. intermediate form, fascioliasis, liver enzymes, rabbit

Fascioliasis is a hepatic parasitic infection in many mammalian species. The pathogenic effect on the definitive host begins with the ingestion of metacercariae, which become excysted and released the juvenile fluke in the intestinal lumen. The juvenile flukes then migrate through the intestinal wall into the peritoneal cavity and penetrate into the liver through the liver capsule. They then migrate through the liver parenchyma to find their way to the bile ducts and develop into adult flukes [11, 25]. Pathological changes mainly caused by migration of the juvenile flukes affect the complex vascular and biliary system in the liver. The complexity of vascular and biliary system examination has led to the use of a number of different laboratory tests based on biochemical analysis of serum parameters. These include the measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) [7, 9, 29]. Two aminotransferase enzymes (ALT and AST) are localized mainly in the cytoplasm of hepatocytes and recognized as a marker of hepatocellular injury in rats, dogs and non-human primates [3, 8]. ALP and GGT are the two most commonly used enzymatic marker of cholestasis in the clinical practice of small animal [8]. The highest ALP activity is found on the border membranes of the bile duct whereas GGT is found in the hepatocytes and biliary epithelial cell [8, 12]. Increased level of these enzymes is an indicator of hepatobiliary injury, epithelial damage and stasis of the bile duct [8, 17, 27]. There are many previous studies on serum liver enzymes of sheep, cattle and monkey infected with *F. hepatica* and *F. gigantica*, but few on dynamics of serum liver enzymes in rabbit fascioliasis [5–7, 9, 17, 24, 25, 29]. Shoriki et al. [18] had reported the existence of an intermediate form of *Fasciola* in Japan, which is characterized as aspermic, and having genotypic characters of both *F. hepatica* and *F. gigantica*. The clinical symptoms and enzyme profiles of the rabbit host infected by this *Fasciola* type has not yet been reported. The objectives of this study are to monitor the change of serum liver enzymes that characterize liver damage caused by *Fasciola* sp. (intermediate form) in rabbits during the invasive acute phase and to correlate the kinetics of these enzymes as a reference for interpretation of liver biochemical profiles in animal fascioliasis, especially during the migration of the larva to the bile duct.

The *Fasciola* fluke used in our study has been identified as *Fasciola* sp. (intermediate form between *F. hepatica* and *F. gigantica*) following the PCR-RFLP and multiplex PCR method designated intermediate form described by Shoriki et al. [18]. Metacercariae of *Fasciola* sp. were collected from laboratory-bred and artificially infected snail by encystation on the wall of the polyethylene bag of emerged cercariae following the protocol described by Taira et al. [23]. Briefly, infected lymnaeid snails
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Fasciola infection has two distinct phases in which the signs and symptoms are different. The initial or parenchymal phase occurs when the parasite perforates the liver capsule and begins to migrate through the liver parenchyma towards the large biliary duct. After the ductal phase begins, parenchyma lesions resolve and eggs may be found in stools. Discrimination of the phases has been aided by measuring the activity of enzymes released by damaged hepatic cells in the serum. These may be used as markers of the different stages of Fasciola infection [7, 11]. Increases of ALT and AST have been previously reported to appear by 4–6 weeks post infection in sheep, buffalo and monkey [5–7, 24, 29]. In our study, significant increases in serum ALT and AST appeared at 3 WPI and reached a peak value at 6 WPI (Fig. 1). The elevation of these enzymes relates to the liver inflammatory state and to

| WPI a) | EPG |
|--------|-----|
| Rabbit No.1 | Rabbit No.2 | Rabbit No.3 |
| Inoculated 20 MC b) | Inoculated 20 MC | Inoculated 12 MC |
| 6 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 |
| 8 | 22 | 8 | 0 |
| 9 | 108 | 103 | 0 |
| 10 | 138 | 222 | 0 c) |
| 11 | 219 | 171 | - |
| 12 | 190 | 214 | - |
| 13 | 139 | 290 | - |
| 14 | 284 | 317 | - |
| 15 | 199 | 322 | - |
| 16 | 136 | 161 | - |
| 17 | 98 | 121 | - |

a) Weeks post-inoculation, b) Metacercariae, c) Rabbit No.3 died at 10 WPI, 3 flukes from liver parenchyma & 2 from bile ducts recovered respectively.
tissue destruction provoked by parenchymal migration of juvenile flukes during the first stages of fascioliasis [6, 24]. At 7 WPI, ALT and AST progressively returned to normal values probably due to the migration of juvenile flukes to the bile ducts and since the liver is an organ that regenerates itself comparatively quickly ALT and AST returning to the normal level might reflect this observation [6, 7].

Yang et al. [29] reported an increase of GGT in buffalo at 8–26 WPI after daily infection with 60 metacercariae for over 20 days. Takemoto et al. [24] reported that serum ALP increased at 6–10 WPI and remained high until 17 WPI in monkeys infected with 20–100 metacercariae. On the contrary, we observed no significant increase in serum GGT nor ALP in our study with rabbits infected only once with 12–20 metacercariae (Fig. 2). This may imply that no injury of the bile ducts had occurred or that the adult flukes did not continue to destroy the biliary epithelial cell due to the hardening of the biliary duct wall. Moreover, the adult flukes might not be very motile, and thus might not be harmful to the biliary epithelium as compare with juvenile flukes [5].

Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) are used as the tumor markers for cholangiocarcinoma in human
There are reports, which suggested that the parasites that localizes in the bile duct such as, *Clonorchis sinensis*, and *Opisthorchis viverrini* can induce cholangiocarcinoma [19, 20, 26, 30]. Chen *et al.* [4] proved that rabbits can also produce AFP, albeit in viral infection. In this study, no change in the value of AFP and CEA was observed for all the rabbit sera. Our data are in agreement with previous case reports indicating that tumor markers including AFP and CEA were at normal value in human fascioliasis [1, 14, 15, 28]. This suggested that there might be no relationship between fascioliasis and cholangiocarcinoma, at least at the acute phase.

The *Fasciola* intermediate form may evolve its a new valid species with a given names but at the present moment, since the characterization is still being investigated we will leave the speciation subject for future discussion. Our data indicated that the serum levels of liver enzymes might be a useful parameter for understanding the host-parasite relationship in the final host. Although the number of rabbits seemed to be insufficient for statistical analysis after 11 WPI, we could obtain the trend of the serum enzymes in infected rabbit in this study. This trend could be used as indicators for the interpretation of the liver biochemical profiles of final host at the different stages of *Fasciola* sp. infection.

Fig. 2. Serum enzymes activities of GGT and ALP of rabbits during the course of infection with *Fasciola* sp. (intermediate form). Data represent mean ± SD (n=3 at 0–10 WPI, n=2 at 11–17 WPI).
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