

Patological Changes in Liver and Gall Bladder Of Bali Cattle Infected by Fasciolosis

I Made Kardena^{1*}, Ida Bagus Oka Winaya¹, Anak Agung Ayu Mirah Adi¹, I Ketut Berata¹, Ida Bagus Windia Adnyana¹, I Made Sukada², Kadek Karang Agustina², Putu Agus Trisna Kusuma Antara³

¹Laboratory of Veterinary Pathology, ²Laboratory of Epidemiology Veterinary, ³Students of the Faculty of Veterinary Medicine Udayana University. Jl. PB Sudirman Denpasar-Bali

*Corresponding author: imadekardena@unud.ac.id

Abstract. Fasciolosis is a parasitic disease that infects ruminants and the disease is widely spread in the world. Fasciolosis caused by *Fasciola hepatica* and *Fasciola gigantica* that can cause macroscopic and microscopic lesions in the liver and gall bladder of Bali cattle. Samples of Bali cattle in Pesanggaran slaughter house that infected with fasciolosis were used in this study. The pathological macroscopic and microscopic changes of the liver and gall bladder were observed. The parasite found in the liver and gall bladder, thickening of bile duct mucous were observed on macroscopic examination. However, in microscopic observation found infiltration of inflammatory cells, fibrosis, necrosis, and degeneration of hepatocytes. In the gall bladder, necrosis was found in epithelial mucosal bile duct, infiltration of collagen fibers, inflammatory cells, hypertrophy and hyperplasia of the bladder epithelium were occurred.

Keywords: Fasciolosis, Liver, Gall Bladder, Bali Cattle

I. INTRODUCTION

Fasciolosis caused by *Fasciola hepatica* and *Fasciola gigantica* regarded as one of the most important parasitic diseases in the world. Fasciolosis distributed worldwide and the prevalence in ruminants is estimated ranging up to 90% in some countries, like Cambodia reached 85.2%, Wales 86%, Indonesia 80-90%, Tunisia 68.4%, and Vietnam 30-90% [1]. *Fasciola gigantica* in average has a measurement of 25-27 x 3-12 mm, a narrow shoulder, blunt posterior end, ovarian longer with many branches, while *Fasciola hepatica* 35 x 10 mm, has broad shoulders and a pointed posterior end. The Eggs *Fasciola gigantica* have operculum, with a measurement of 190 x 100 μ , while *Fasciola hepatica* eggs also have an operculum with a measurement of 150 x 90 μ [2]. Cattle that infected by *Fasciola* sp. would appear pale, listless, swollen eyes, skinny body and feather rough and dull or standing rate of growth and weight gain of livestock, decreased feed efficiency, mortality in the degree of infection is high, especially in calves and cows of reproductive age, the immune system due to anemia posed, as well as tissue damage, especially liver and gall tract

[3][4]. *Fasciola* worm infection is needed to be aware because it can cause fasciolosis in humans [5]. The life cycle of worm *Fasciola*, there are two types of snails of the family Planorbidae, namely *Segmentia trochoideus* and *Hippeutis umbilical* are contributed to it [6].

Results of research on *Fasciola* sp. have been reported in some areas of Indonesia, such as: in the district of Sukoharjo, District of Pringsewu, Province of Lampung. Aryandrie et al. (2015) reported that the prevalence of *Fasciola* sp. was 26.72%, i.e.: there were 35 positive samples infested with *Fasciola* sp. of the 131 samples tested [7]. Research conducted by Adriyati (2015) found variations necrotic lesion cells making gall duct bleeding in the lamina propria, inflammation, fibrosis, mucus gland hyperplasia, fibroblast proliferation and dystrophic calcification in infected Bali cattle [8]. Tissue changes in Bali cattle's bile duct due to varied infection of *F. gigantica* acquired by microscopic observation. Lesions in the form of necrosis was found in 35 samples (100%), hemorrhagic was found in 18 samples (51.4%), inflammatory lesions found in 35 samples (100%), and fibrosis was found in 35 samples (100%).

Infection of *F. gigantica* can result in changes on gross pathology and histopathology of the gall bladder and liver

of ruminants. Gross pathology changes of hepatomegaly and liver damage can be observed. Gall duct obstruction and hardening of the gall can also be observed in the infection of fasciolosis [9][10]. Histopathological examination revealed track-like lesions varying from acute hemorrhagic necrosis to active granulomas with organized fibrotic areas generated by eggs and worms the parasite in the parenchyma of the liver. Most of the chronic lesions showed the presence of lymphocytes and macrophages [11]. The cause of the epithelial hyperplasia is a chronic irritation induced by existence worms *Faciola* sp. in the gall duct [12]. It is an indication of a chronic trauma as a result of persistent worms suck the blood at the same place. Dystrophic calcification occurs because the calcium deposits are found in areas that have previously been degeneration or necrosis [13][14][15]. This study aim was to determine the variation of lesions in the liver and gall bladder bali cattle infected by the worm *Fasciola gigantica*.

II. RESEARCH METHOD

The samples used in this study were the gall and liver of bali cattle infected with *F. gigantica* obtained from Slaughterhouse Pesanggaran Denpasar. Determination of *F. gigantica* infection was done by observation of the liver and gall bladder that showed the presence of the parasite, *F. gigantica* in the organs. At the same time, liver and gall bladder in bali cattle that did not reveal any infestation of the parasite were also taken for comparison (liver and gall bladder relatively normal). The organ samples were then fixed into containers contained with 10% neutral buffered formalin, before it then further process for making preparations for microscopis observation using hematoxylin and eosin staining (HE). Histopathologic examination was performed with a dissecting microscope magnification of 100x and 400x with three different visual field tissue. Variations lesions that found were documented and analyzed descriptively.

III. RESULTS AND ANALYSIS

The parasitic agents, *F. gigantica*, were found in the livers and gall ducts of the infected bali cattle. Examination of the infected gall bladder bali cattle showed different conditions compared to the non-infected gall tissue. The bile ducts of the *F.gigantica* infection were observed thicker and in elastic compared to the non-infected bile ducts. The worms, *F.gigantica*, were found attached to the wall of the gall bladder gall and caused bleeding on the wall of gall.



Fig.1. Description: (a). There *F. gigantica* worms found in the bali cattle liver. (b). The worm *Fasciola gigantica* (c). mucosal thickening of the gall bladder's wall (d). bleeding in the mucosa of the gall bladder.

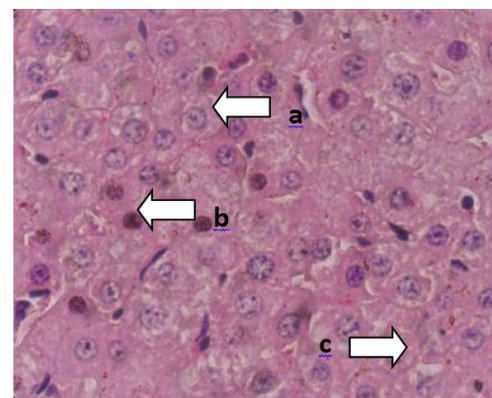


Fig. 2. Microscopic bali cattle's liver tissue observed with 400x magnification (a) normal hepatocyte cells, (b) piknotic hepatocyte cells, (c) cell necrosis of hepatocytes [15].

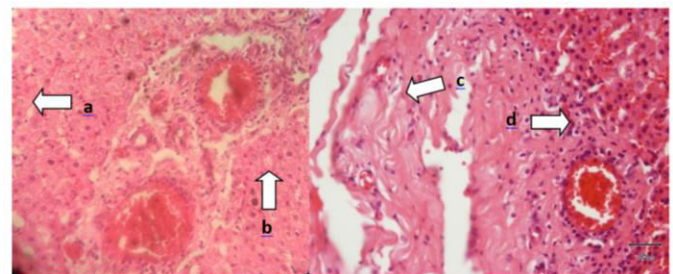


Fig. 3. Microscopic bali cattle liver tissue infected by *Fasciola gigantica* at 400x magnification: (a) fatty degeneration in the hepatocytes; (b) necrotic of hepatocytes; (c) the occurrence of fibrosis; (d) infiltration of inflammatory cells.

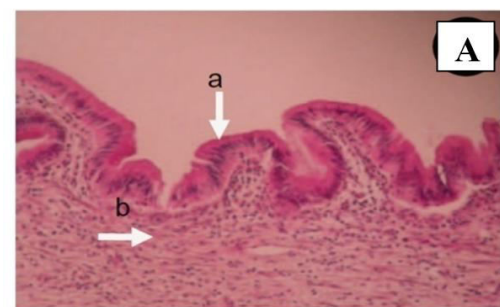


Fig. 4. A. gall bladder bali cattle 400 x (a) visible patterned villi mucous membranes of the gall bladder is short and (b) seem thin collagen fibers in the mucosa of the gall bladder.

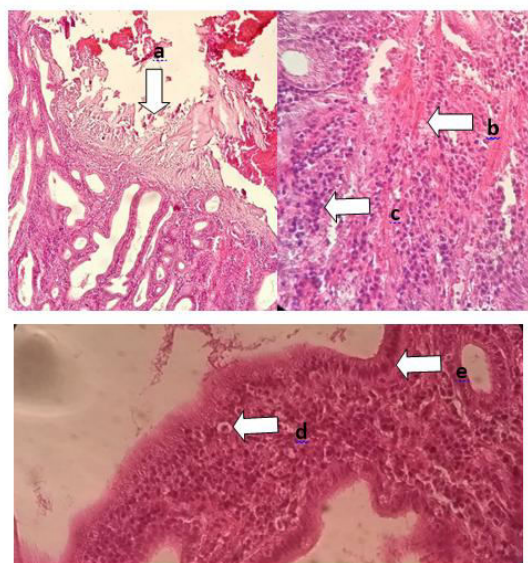


Fig.5. *Fasciola gigantica* infected gall at 100x and 400x magnifications: (a) necrotic of the gall bladder 100x (b) seemed proliferation of collagen fibers 400x (c) infiltration of inflammatory cells 400x (d) hypertrophic epithelium gall 400x (e) epithelial hyperplasia gall 400x.

Microscopic examination of the Bali cattle's liver infected by *F. gigantica* slaughtered in slaughterhouses Pesanggaran contained inflammatory cell infiltration, fibrosis, necrotic and fatty degeneration in hepatocyte cells. Histopathological structure of the gall infected by *F. gigantica* showed various lesions. Lesions observed: epithelial necrosis, proliferation of collagen fibers, inflammatory cell infiltration and hypertrophy and hyperplasia of the epithelium of the gall bladder.

Fasciola sp infection in Indonesia are generally caused by *F. gigantica*. *F. hepaticus* is not found in Indonesia, which may be due to the intermediate host, *Lymnaea truncatula*, is not found in the area. Anatomically, *Fasciola gigantica* found measuring around 25-27 x 3-12 mm, has a narrow shoulder, blunt posterior end, ovarian longer with many branches [2]. The anatomic structure of the parasite found in this study were in the range and its likely associated with the lesions in the liver and gall bladder of the infected cattle. The anatomical form and nature of the worm migration are also associated with lesions caused in the infected hosts. Mendes et al., (2012) reported that infection of *F. gigantica* can cause various lesions like: necrosis, acute hemorrhagic, and fibrosis in the liver parenchymal area which associated with the parasite.

The damage of microscopic liver tissue was mainly occurred in the parenchyma of the liver. The hepatocytes found hemorrhage, degeneration and necrotic; and infiltrated by inflammatory cells. The changes are similar with the *Fasciola* sp infection in liver tissue of the female cattle FH which showed damage in the liver cells surrounded by connective tissues. Necrosis and fatty degeneration in the liver were the result of chronic infection of fasciolosis [17][18]. Bleeding can also be experienced at the liver that infested by *Fasciola* sp. besides infiltration of

inflammatory cells. Meeusen et al., (1995) reported that the infection of fasciolosis affected the increase of the inflammation cells, in which the number of eosinophil increased, and infiltrate mainly in the area of the parasite migration [19].

The gall bladder was the organ where the *Fasciola gigantica* found more compare to the liver. Almost in every observation of the infected Bali cattle, the fasciola were found in the gall bladder. The gall bladder and its ducts are the main places of the *Fasciola* worm in the infected host. Therefore, the organ or tissue damages in liver and gall bladder can be determined pathological changes for the pathological tissue damage as indication of the *Fasciola* worm infection [20].

Histopathological changes in the case of the cattle infected by *F. gigantica* mainly caused by the migration activity and spiny tegument of the *F. gigantica* which may irritation of the gall bladder mucous membranes of the cattle. The migration and the spiny can lead to changes of the tissue: necrotic, hemorrhage, inflammation, hyperplasia, and cellular proliferation [14]. The infestation and the existence of *F. gigantica* commonly can be chronic and its associated with lesions, such as necrotic of the mucous membranes of the gall bladder. Additionally, the distribution of necrotic in the tissue organs is largely determined by the number of worms that infect the liver and the secreted toxic substance. Irritation of the mucous membranes in the lamina propria of the gall bladder may lead to rupture of blood vessels and ended up with bleeding. Bleeding can also occur due to of the activation of blood sucking worms *F. gigantica*. Severe bleeding can happen because of destruction of most blood vessels in the gall bladder. Clinically, this usually results in hypochromic normocytic anemia [21].

Proliferation of collagen found spread in the lamina propria, which may be associated with the stimulation of proline secreted by worms *F. gigantica*. Modavi and Isseroff (1994) argued that the proliferation of collagen microscopically characterized by the thickening of the mucous membranes in the gallbladder [22]. In chronic cases, a change of heart can also be observed. The changes may resemble the cases of liver cirrhosis due to massive collagen proliferation as a response of the migration of *Fasciola* worms. There appears to be an association between infection by *Fasciola* worms with the change of heart, including the incidence of cirrhosis in infected cattle [23].

Infiltration of inflammatory cells in the liver and gall bladder was also observed in microscopic observation. Macrophages and eosinophils play an important role in the inactivation of the parasite by freeing cytotoxic molecules to the surface of the worm body [24]. Lymphocyte infiltration was also found in lamina propria and generally associated with the chronic response to the presence of adult worms of *F. gigantica* over time in the mucous membranes of the gall bladder [25].

Hypertrophy and hyperplasia lesions on the epithelium were also found in this study. The hypertrophy and hyperplasia can be induced by the presence of chronic irritation of the worm *Fasciola* sp. on the gall [14]. This is an indication of a chronic trauma as a result of persistent worms suck the blood at the same place [13]. In epithelial of the mucous, hyperplasia occurred which characterized by a structure that resembled to many glands [26]. Proliferation mio-fibroblast can also be observed in the fasciolosis infection. this proliferation is also associated with the chronic phase of the infection, which may result in liver fibrosis [27].

There are various factors that can affect the liver and gall bladder lesions of infected cattle fasciolosis. Lesions caused *Fasciola* worms of the liver or gall bladder is not only influenced by the number of worms that infect, but also the infection period, geographical conditions where cattle raising and the weather conditions of a region [28].

IV. CONCLUSION

Fasciolosis infection in bali cattle affects its liver, gall bladder and the gall ducts, which in macroscopic observed the parasitic agents in the organs, gall duct walls less elastic, thickened and bleeding. However, microscopic observation on the parenchyma liver is infiltrated by inflammatory cells, fibrosis, necrosis and fatty degeneration on hepatocytes. In addition, on gall bladder found proliferation of collagen fibers, infiltration inflammation cells, hypertrophy, hyperplasia and necrotic on the epithelium of the gall bladder.

REFERENCES

- [1] Nguyen, T.G.T. (2012). Zoonotic fasciolosis in Vietnam: Molecular Identification and Geographical Distribution. [Disertasi]. Faculty of Veterinary Medicine. Universitas Gent.
- [2] Baker, D.G. (2007). Flynn's Parasites of Laboratory Animals. Second edition. American College of Laboratory Animal Medicine. USA: Blackwell Publishing.
- [3] Guntoro, S. (2002). Maintenance of Bali Cattle. Yogyakarta: Kanisius.
- [4] Hambal, M., Arman, S., Agus, D. (2013). Susceptibility of Bovine and Bubalis spp on *Fasciola gigantica* in Lhoong Sub-District Aceh Besar. *Jurnal Medika Veterinaria* 7(1):49-53
- [5] Schmidt, G.D., Roberts, L.S. (1989). Foundations of Parasitology. 4th ed. times mirror/mosby college publishing. St . Louis, Toronto, Boston, Los Altos. pp: 283-287.
- [6] Gilman, R.H., Mondal, G., Maksud, M., Alam, K., Rutherford, E., Gilman, J.B., Khan, M.U. (1982). Endemic focus of *Fasciolopsis buski* infection in Indonesia. *Am . J.Trop. Med. Hyg.* 31(4): 796-802.
- [7] Aryandrie, D.F., Santosa, P.E., Suharyati, S. (2015). Liver Fluke Infestation Level of Bali Cattle in Sukoharjo Sub-District, Pringsewu Regency, Lampung Province. *Jurnal Ilmiah Peternakan Terpadu.* 3(3): 134-139.
- [8] Adriyati, G.A.A.P., Winaya, I.O., Berata, I.K., (2015). Histopatological Studies of Bile Duct Mucosa Membrane in Bali Cattle That Infected With *Fasciola Gigantica*. *Indonesia Medicus Veterinus.* 4(1): 54-65.
- [9] Balqis, Ummu, Darmawi, Sitti, A., Muhammad, H. (2013). Pathology anatomy changes in liver and bile duct of Aceh cattle infected with *Fasciola gigantica*. *Agripet:* 13(1): 53-58.
- [10] Martindah, E., Widjajanti, S., Estuningsih, S.E., Suhardono. (2005). Meningkatkan Kesadaran dan Kepedulian Masyarakat Terhadap Fasciolosis Sebagai Penyakit Infeksius. *Wartazoa.* 15.
- [11] Mendes, E.A., Vasconcelos, A.C., Lima, W.D.S. (2012). Histopathology of *Fasciola hepatica* Infection in *Meriones Unguiculatus*. *Revista de patologia tropical.* 41(1): 55-62.
- [12] Kardena, I.M., Winaya, I.B.O., Elyda., Adhiwitama, I.D.M., Mirah Adi, A.A.A., Berata, I.K. (2016). Histopatological Illustration of Gall Bladder Mucous Membrane In *Fasciola Gigantica* Infected Bali Cattle. *J. Vet.* 17 (1): 16-21.
- [13] Sukhdeo, M.V.K., Sangster, N.C., Mettrick, D.F. (1988). Permanent Feeding Sites of Adult *F. Hepatica* in Rabbits. *Int. J Parasitol* 18: 509-512.
- [14] Shaikh, A.A., Bilqees, F.F., Khan, M.M. (2004). Bile Duct Hyperplasia an Assosiated Abnormalities in Buffaloes Infected with *Fasciola gigantica*. *Pakistan J. Zool* 36(3): 231-237.
- [15] Marcos, L.A., Terashima, A., Lequia, G., Ganales, M., Espinoza, J.R., Gotozzo, E. (2007). Hepaticfibrosis and *Fasciola hepatica* Infection in Cattle. *J. Helminth.* 81: 381-386.
- [16] Putri, K.A., Berata, I.K., Kardena, I.M. (2015). The Comparative Degree of Autolysis between Musculus and Hepar in Bali Cattle Observed on Some Period of Time. *Indonesia Medicus Veterinus.* 4(5): 393-400.
- [17] Dow, C., Ross, J.G., Todd, Jr. (1967). The pathology of experimental fascioliasis in calves. *J. Comparative Pathology.* 77(4): 377-386
- [18] Tarmudji, Ginting, N. (1983). Liver Damage Level Due to Fasciolosis in Holstein Friesian cows Females in Malang, East Java. *Research Institute of Animal Diseases. Bogor. Penyakit Hewan.* 15(25): 83-85.
- [19] Meeusen, E., Lee, C.S., Rickard, M.D., Brandon, M.R. (1995). Cellular Responses During Liver Fluke Infection in Sheep and Its Evasion by The Parasite. *Parasite Immunol.* 17: 37-45
- [20] Dinaol, B., Tilaye, D., Hagos, A., Ababayehu, T. (2015). Comparative Pathological Study of Liver Fluke

- Infection in Ruminants. *Indian Journal of Veterinary Pathology*. 39(2):113-120.
- [21] Lotfollazadeh, S., Mohri, M., Bahadori, S.R., Desfouly, M.M., Tazik, P. (2008). The Relationship Between Normocytic Hypochromic Anemia and Iron Concentration Together with Enzyme Activities in Cattle Infected with *Fasciola hepatica*. *J. Helminth*. 82: 85-88.
- [22] Modavi, S., Isseroff, H. (1994). *Fasciola hepatica*: Collagen Deposition and Other Histopathology in The Rat host's and by Proline Infusion. *Exp Parasitology* 58(3): 239-244.
- [23] Raji, M.A., Salami, S.O., Ameh, J.A. (2012). Pathological Conditions and Lesions Observed in Slaughtered Cattle in Zaria Abattoir. *J. Clin. Pathol. Foren. Med.* 1(2): 9-12.
- [24] Piedrafita, D., Parson, J.C., Sandeman, M., Wood, P.S., Estuningsih, E., Partoutomo S, Spithil, T.W. (2001). Antibody Dependent Cell Mediated Cytotoxicity to Newly Excysted Juvenile *Fasciola hepatica* in Vitro Mediated by Reactive Nitrogen Intermediate. *Parasite. Immunol.* 23: 473-482.
- [25] Clery, D, Torgeasan, P., Merclahy, G. (1999). Immune response of chronically infected adult cattle to *Fasciola hepatica*. *Vet Parasitol.* 62(1): 71-82.
- [26] Mahmoud, A.Z., Uoussef, M.S., Dibrahim, M.K. (1989). Pathological Studies of Some Liver Affection in Camel Parasitic Hepatitis (Distomiasis). *Egypt J Comp Pathol and Clinic.* 2(1): 94-106.
- [27] Golbar, H.M., Izawa, T., Juniantito, V., Ichikawa, C., Tanaka, M., Kuwamura, M., Yamate, J. (2013). Immunohistochemical Characterization of Macrophages and Myofibroblasts in Fibrotic Liver Lesions Due To *Fasciola* Infection in Cattle *J.Vet Med. Sci.* 75(7): 857-865.
- [28] Affroze S, Begum, N., Islam, M.S., Rony, S.A., Islam, M.A., Mondal, M.M.H. (2013). Risk Factors and Gross Pathology of Bovine Liver Fluke Infection at Netrokona District, Bangladesh. *J Anim Sci Adv.* 3(3): 83-90.