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Hematological and Biochemical Analysis of Asymptomatic Fascioliasis of Bali Cattle (*Bos Javanicus*)

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ABSTRACT

The research was to examine the characteristic of fasciolosis (*Fasciola sp.*) in year-round irrigated rice fields in tropical areas. Fifty cattle raised in rice field areas were examined by physiologically, coproscopy, hematology and liver function tests. The results showed that 50 cattle had heart beat, respiratory and temperature rectal in normal standard, however, 41 head (82%) out of 50 cattle were found positive fasciolosis with egg per gram (epg) feces varied from 1 to 30. Indikator of the liver function such as bilirubin total, alanine amino transferase (ALT) and alkaline phosphatase (AP) were under normal condition, except γ-glutamyl transferase (GGT) higher than normal standard that indicated long term liver damage and aspartate amino transferase (AST) less than normal on two cattle. Examination of red blood cells (RBC) showed that 3 out of 20 cattle had erythrocyte of 4,30 ($10^6/\mu l$) and can be categorized as anemia, 3 out of 20 cattle had higher mean corpuscular volume (MCV) than normal, indicated as macrocytic condition. Mean corpuscular hemoglobin (MCH) in all cattle had higher than 17 (pg) that indicated hyperchromic erythrocyte, while MCHC was still at normal levels. In evaluating WBC showed that 8 out of 20 cattle showed leukocytes concentration higher than 12 ($10^3/\mu l$), 6 cattle had eosinophils number, band neutrophils, segmented neutrophils and monocyte higher than normal level and 6 cattle showed platelet concentration less than 100 ($10^3/\mu l$).

Keywords: Fasciolosis, coproscopy, hematology, liver function, cattle.

INTRODUCTION

Most of cattle raised in Lombok Island are Bali cattle (Bos javanicus). These cattle are indigenous of Indonesia, originally from banteng of Java island. These cattle have been raised as part of agriculture activities. As complementary to rice producers, cattle are raised around rice fields, around wet areas or wet agriculture areas which are endemic to fasciolosis. The liver flukes *Fasciola sp.* are parasitic trematodes that affect cattle, sheep and goats, worldwide causing significant economic losses to agriculture (Beesley et al., 2016). Fasciolosis is a liver flukes disease, spreads worldwide, can be infected not only to livestock but also in humans, with most human fasciolosis cases concentrated in South America, Africa and Asia (Hotez et al., 2008), although human fasciolosis has also been reported in other areas such as Turkey (Bosnak et al., 2016), Serbia (Pavlovic et al., 2014), Denmark (Stensvold et al., 2018), and Germany (Salzer et al., 2015).

The spread and transmission of *fasciola sp*. can occur in several ways, including 1) feces containing worm eggs, where under tropical conditions the eggs can hatch to produce larvae called miracidia. Miracidium can swim if there were water around the barn, 2) the snail would be an intermediate host for liver fluke larvae called miracidia to become redias and become several sporocysts (Wymann 2005), then finally become cercarias which left the snails, swim and stick to the grass and vegetations on the cyst form namely metacercaria. Irrigation can facilitate miracidium larvae as a medium for swimming to look for Lymnaea sp. (Admassu et al, 2015; Malone et al 1998; Ardo et al 2013).

The previous study shows that more than 76.9% of cattle suffered from fasciolosis in Central Lombok, though most of them do not show any visible symptoms, some cattle do suffer a considerable amount of weight loss (Astiti et al 2015). Fascioliasis reduces animal productivity, weight gain, and the production of meat and milk. In addition, it causes moderate icterus, metabolic disorders, and secondary infections due to decrease immunity by chronic fascioliasis and liver condemnation during postmortem inspection in slaughterhouses while the acute fascioliasis may lead to mortalities (Eman et al., 2016) Consequently, cattle suffer from fasciolosis may reduce the farmer's income by decreasing their body weight from time to time, as an infestation of *Fasciola sp*. can continuously for a cattle lifetime. It was reported that most cattle suffer fasciolosis without showing any signs of disease (Adrien et al, 2013).

Economic losses and impact of fasciolosis (Adrien et al 2013) were reported firstly, by reducing body weight (Ardo et al 2013) may reach 157gr/ fluke/years (Wamae 1996) or daily body weight gain may decrease up to 1.96± 0.8 kg (Genicot et al 1991). Secondly, milk production may reduce up 8% to 17% from their total production (Chakraborty and Podhan 2015). Thirdly, the flukes may destroy the liver, it was reported that 25% of fasciolosis was condemned totally, while 75% was condemned partially (Ssimbwa et al 2014) and resulted in the loss of 4000 USD per year (Abuna et al 2010). These indicated that fasciolosis obviously decrease farmers' income significantly, especially traditional farmers in Lombok Island which raise cattle in endemic areas of fasciolosis and feed cattle by cut and carry grasses. As this disease was without any symptoms, consequently farmers neglected the economic losses.

Although the impacts of fasciolosis have been recognized by researchers and investigators, this disease may still being neglected (Mas-Coma et al 2009) by farmers as they do not know that their cattle suffer from fasciolosis. It was indicated that fasciolosis in this study without showing the physiological effect on the cattle. It was reported previously

that chronic form is more common (Admasu et al 2015) and without any clinical signs (Adrien et al 2013). The effect to the liver was when at least 52 flukes (Alemu and Abebe 2015) or 56± 28 flukes (Marcos et al 2007) and cirrhosis liver when infested at least by 116± 30 flukes.

From that descriptions, studies have been performed to evaluate Fasciolosis of Bali cattle raised under rice field conditions in Central Lombok of Indonesia. This study aimed to identify fasciolosis by fecal coproscopy, examination of hematology and liver function test to evaluate the characteristic of Fasciolosis of Bali cattle under rice field conditions.

MATERIALS AND METHODS

This research was performed in a farmers' communal barn which surrounded by rice field. Farmers fed cattle by cutting grass around rice fields, where there are snails Lymnaea sp. around and the fields were irrigated year-round. The areas have been reported as endemic to Fasciolosis (Astiti et al 2015)

Fifty mature cattle mixed male and female were used in the study, by examination of fluke eggs by a sedimentation technique (Avcioglu et al, 2014). Fecal sample was collected by insertion of finger covered with plastic glove into rectum, approximately of 10 gr feces was preserved by 3 drops of formaldehyde 1%. The worm eggs identification was performed by using 6 gr of feces suspended and mixed with 250 ml water, then filtered 3 times through a tea sieve. The filtrate was allowed to stand for 3 minutes then decanted the solution, the sediment was collected and stained with two drops of methylene blue. The stained filtrate was then mixed using a pipette and dropped into object glass and examined under binocular with 100x magnification and *Fasciola sp*. eggs would show yellowish color.

Physiological examination of 50 cattle including rectal temperature (°C), heart rates and respiratory rates. Examination of rectal temperature was performed by thermometer when it showed 35°C then inserted into rectum and ready to read when it was in the rectum for 5 minutes. Examination of heart rate was performed by listening using a stethoscope at the left front breast of cattle and counting systolic sound for one minute. The respiratory examination was performed by putting the right hand in front of calf nose, as it can be felt by hand and expiration was counted for one minute. Rectal temperature was considered under the normal condition when it was between 38°C and 39,5°C, while heart rates and respiratory rates were between 100 and 140 beats, 24 and 26 expirations per minute.

Liver function test was also evaluated in 7 cattle randomly, using an auto-analyzer (ILab 300 Plus Chemistry system) for Bilirubin T (Total), aspartate amino transferase (AST),

alanine amino transferase (ALT), alkaline phosphatase (AP), serum γ-glutamyl transpeptidase or transferase (GGT).

Hematology examination was performed of 20 cattle randomly by automatic hematology machine (SYSMEX KX-21) from blood collected from jugular vein. The blood sample was dispensed into a 10 ml vacuum tube containing *ethylene diamine tetra acetic acid* (EDTA). Hematology examination was red blood cells count (RBC), the concentration of hemoglobin (HGB) and corpuscular parts or hematocrit (HCT), white blood cells (WBC), basophils, eosinophils, neutrophils, lymphocyte and monocyte.

RESULTS AND DISCUSSION

Prevalence of Liver Flukes

The results of coproscopy (Figure 1) showed that 41 (82%) of 50 cattle were found fasciolosis positive, with epg varied from 1 to 30 eggs per gram feces (epg). This prevalence of liver flukes is higher than that of previous study in Lombok Island. It was reported up to 67.5% (Astiti et al 2015). The prevalence rate of liver flukes is influenced by seasons and livestock grazing conditions. Adedokun et al. 2008 reported that the prevalence of fascioliasis in winter (52.3%) was higher than in dry season (21%) in Nigerian cattle. Elshraway and Mahmoud, 2017 also reported that the prevalence rate of seasonal fasciolosis was highest in winter, autumn, spring and summer, respectively. This finding may be associated with the rainy season where fasciolosis is highest in winter (around the rainy period) and/or humid areas due to the dispersal of snail hosts (Oryan et al., 2011; Mochankana and Robertson, 2016).

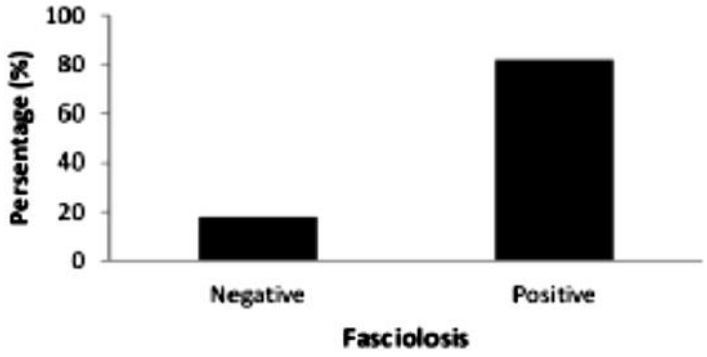

Figure 1. Result of fecal examination eggs of *Fasciola sp.* of Bali cattle.

Furthermore, Livestock grazing conditions with high water levels or frequent flooding are at high risk of becoming infected with liver fluke. The reason is that the snails acting as intermediate hosts are amphibious and need humid habitats that are periodically

submerged or flooded. Irrigation may facilitate larva miracidium as media to swim to find snails Lymnaea sp. and as media cercaria may swim to find grass or other vegetations (Admassu et al, 2015; Ardo et al 2013) so that surrounding grasslands remain infected and livestock that is permanently housed can also become infected through contaminated grass.

The reason for asymptomatic fasciolosis in the present study that the increase of rainfall was positively rises the deep of water and correlated to the increase snails (Lymnea sp.) may increase cases of fasciolosis. While the decrease of rainfall during dry season may reduce the snail and less infestation of Fasciolosis on Bali cattle (Surana et al 2019).

In addition to these factors, diagnostic techniques also greatly determine the level of accuracy of prevalence fasciolosis. There was no accurate technique to diagnose fasciolosis, the technique available was sedimentation test to evaluate epg, however this test with high specificity or positive cases being positive up to 100%, while the sensitivity or negative cases being negative was only 67,8% (Alemu and Abebe, 2015) depend on sample collection and eggs release by flukes.

Physiology and Liver Function of Fasciolosis Cattle

The results of physiological examinations such as heart rates, respiratory rates and rectal temperature, can be seen in Table 1 were still under normal conditions (Kahn et al., 2010). Taimur et al. (1993) reported that low infestation of liver flukes without showing any sign of disease, the symptoms of fasciolosis were detected when infestations were higher than 300 epg. In this present study, epg was found only between 1-30 and in the present study as the cattle without signs of diseases, it was neglected by farmers. Another study showed with prevalence of 31.14% and detected with egg of 133.03± 9.04 showed signs of disease by poor health (Affroze et al. 2013). The severity of fasciolosis is largely influenced by the infectious dose, and the age and immune status/response of the host (Behm et al., 1999).

Table 1. Heart rates, respiratory rates and rectal temperature (means ± SD) of fasciolosis cattle (n=20).

No	Examination	Means ± SD	Min-max	Kahn et al, 2010
1	Heart rates/ minute	49,00 ±7,31	42-60	48-84
2	Respiratory rates/minutes	22,00 ±4,21	21-29	26-50
3	Rectal temperature (°C)	38,19 ±0,70	36-38,2	36,7-39

Normal standard (Kahn et al, 2010).

The other reason was that liver tissues have the ability to remove death cells regenerate and produce new hepatocytes to replace the damage. The pathogenesis of fasciolosis depends on the ability of regeneration and repair of tissues damaged by juvenile

F. hepatica infection and the liver has a high capacity to regenerate damaged tissue (Michalopoulos, 2007). Consistent with that, Alvarez Rojas et al. (2015) also found a significant up-regulation in infected livers of genes associated with the cell cycle and mitosis. They also noted that tumor necrosis factor-alpha (tnfα) contributed to the restoration of functional liver mass by promoting hepatocyte proliferation and liver regeneration.

Liver function test was also evaluated using an auto-analyzer (ILab 300 Plus Chemistry system) for bilirubin T (Total), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (AP), serum γ-glutamyl transpeptidase or transferase (GGT). The results of the liver function test of cattle fasciolosis showed in table 2.

Table 2: Liver function test of cattle fasciolosis (n=7).

No	Liver function test	Means ± SD	Min- max	Kahn et al, 2010
1	Bilirubin T (mg/100 ml)	0.25 ± 0.10	0,2-0,4	0-1,6
2	AST (mg/100 ml)	75.67 ± 7.09	51-84	60-125
3	ALT (mg/100 ml)	27.00 ± 3.46	20-29	6,9-35
4	AP (mg/100 ml)	110.67 ± 33.56	72-148	18-153
5	GGT (mg/100 ml)	33.00 ± 14.11	18-46	6,0-17,4

Normal standard (Kahn et al, 2010).

Bilirubin total, alanine amino transferase (ALT) and alkaline phosphatase (AP) under normal conditions. Two cattle out of seven showed aspartate amino transferase (AST) less than normal. All cattle (7 head) showed γ-glutamyl transferase (GGT) higher than normal value, indicating long-term liver damage. Hepatic enzymes can be used to indicate the presence of diseases such as Fasciolosis. Overall, all liver function indicators showed average values under normal conditions. This condition can be explained that the liver plays a central role in host physiology, being responsible for most of the amino acids, carbohydrate and lipid metabolism, urea synthesis, detoxification, ketogenesis, albumin and glutathione synthesis, and it is also an important organ with respect to innate immunity (Arias at al., 2009). The liver contains many cell types, including sinusoidal endothelial, hepatic stellate (HSC), hepatic parenchyma (hepatocytes), Kupffer, natural killer and natural killer T cells (Crispe et al., 2009).

Hematological Indices of Cattle Fasciolosis

The results of the hematological indices determined for Fasciola-infected and non-infected cattle are presented in Table 3. The results of red blood cells evaluation that 3 out of 20 cattle had 4.30 ($10^6/\mu l$) erythrocytes, which is less than 5.0 ($10^6/\mu l$) and categorized as anemia (indicating normochromic and normocytic anemia). The results for RBC are similar

abnormal iron metabolism due to chronic invasion, migration of immature flukes inside the liver parenchyma and the hepatocyte may unable to produce normal number of red blood cells. The severity of anemia depends on the number of flukes inside the liver (Egbu *et al*., 2013). Kramer (2000) and Lotfy et al. (2003) also reported that severe anemia may be due to a chronic liver inflammation, which causes depression of erythrogenesis.

Table 3. Examination red blood cells (means ± SD) of fasciolosis cattle (n=20).

No	Red blood cells	Means ± SD	Min- max	Kahn et al, 2010
1	Erythrocytes ($10^6/\mu l$)	6,12 ±1,02	4,30 -7,84	5,0-10,0
2	Hemoglobin (gr/dl)	12,13 ±1,71	9,50 – 12,80	8-15
3	Hematocrit (%)	33,84 ±4,82	26,30 – 37,70	24-46
4	MCV (fl)	55,39±4,84	47,30-61,80	40-60
5	MCH (pg)	19,90±2,15	18,20-21,50	11-17
6	MCHC (gr/dl)	36,00± 0,93	35-36	30-36

Normal standard (Kahn et al, 2010).

Evaluating hemoglobin and hematocrit showed that all cattle were under normal levels. Normal levels of hemoglobin indicated the ability of the erythrocyte to carry oxygen from the lungs and without signs of respiratory rates and heart rates. Hematocrit value indicated that percentage of corpuscular volume as part of blood, normal hematocrit showed corpuscular portion to blood plasma volume. It may indicate a low infestation of fasciolosis in the present study without hypoproteinemia.

The evaluation of mean corpuscular hemoglobin (MCH) showed that all cattle had higher than normal standard 17 (pg), indicating hyperchromic of erythrocyte or higher hemoglobin. While the mean corpuscular hemoglobin concentration (MCHC) of all cattle was at normal levels. The evaluation of the mean corpuscular volume (MCV) showed that 3 out of 20 cattle had higher MCV than normal conditions or 60 (fl). This indicated as macrocytic, in which the erythrocyte was bigger than their normal size. It may be the compensatory of erythrocytes to increase their capacity to carry oxygen. Egbu *et al*. (2013) also observed higher MCV and MCH in fasciola-infected sheep. Whereas, Hashem and Mohamed (2017) reported that no significant differences occurred between the erythrocytic indices (MCV, MCH & MCHC) of the infected and non-infected cattle indicating normocytic normochromic anemia. In severe fasciolosis due to blood suckling and blood leakage from the bile duct into the intestines may resulting in anemia normocytic hypochromic (Florence et al. 2013). The high number of infestations of Fasciolosis showed

chronic blood loss (Lotfollahzdeh et al 2008).

Table 4. Examination of white blood cells (means ± SD) of Fasciolosis cattle (n=20).

No	White blood cells	Means ± SD	Min- max	Kahn et al, 2010
1	Leukocytes ($10^3/\mu l$)	8,20±3,69	4,8-15,3	4,0-12,0
2	Eosinophils ($10^3/\mu l$)	2,50±0,76	2-4	0-2,4
3	Band neutrophils ($10^3/\mu l$)	0,88±0,83	0-2	0-0,1
4	Seg. neutrophils ($10^3/\mu l$)	2,99±0,92	1,7-4,3	0,6-4,0
5	Lymphocyte ($10^3/\mu l$)	5.04 ±1.47	3,2-7,1	2,5-7,5
6	Monocyte ($10^3/\mu l$)	1,00 ±0,00	0-2	0-0,9
7	Platelet ($103/\mu l$)	142.8±115,6	42-350	100-800

Normal standard (Kahn et al, 2010).

Evaluation of white blood cells (WBC) showed that 8 out of 20 cattle had leukocyte concentrations higher than 12 ($10^3/\mu l$), which may be indicated that there were responses WBC to protect again liver flukes. A high concentration of leukocyte may be related to 6 cattle that had eosinophils, band neutrophils, segmented neutrophils and monocyte higher than normal level. As further results showed that lymphocytes were in normal condition in all cattle and platelet concentration less than 100 ($103/\mu l$) in 6 cattle. These results were consistent with those obtained in sheep and bovine. Hashem and Samy (2017) reported that there was an increase in leukocyte, eosinophils and neutrophils in Fasciola infected cattle. Meeusen et al. (1995) reported that the infection of fasciolosis affected the increase of the inflammation cells, in which the number of eosinophils increased, and infiltrated mainly in the area of the parasite migration. Egbu *et al*. (2013) also reported that the result of the differential counts against Fasciola worm load in sheep is shown that neutrophils, eosinophils, monocytes and lymphocytes increased progressively as the worm load increased, only basophils showed no change.

CONCLUSIONS

The fluke eggs examination in this study found positive fasciolosis with epg varied from 1 to 30 and the prevalence rate 82% on Bali cattle. The physiology and liver function such as heart rates, respiratory rates and rectal temperature, bilirubin total, alanine amino transferase (ALT) and alkaline phosphatase (AP) were still under normal condition, except γ-glutamyl transferase (GGT) higher than the normal standard that indicated long term liver damage. The hematological assay in this study showed 3 out of 20 cattle a notable reduction in erythrocyte and categorized as anemia. All cattle had MCH higher than normal standard 17 (pg), while MCHC was still at normal levels. Three out of 20 cattle had higher MCV than normal and can be categorized as macrocytic erythrocyte. In evaluating WBC showed that 8

out of 20 cattle showed leukocytosis may indicate response WBC to protect against disease. May be associated with a high concentration of leukocyte, 6 cattle had eosinophils, band neutrophils, segmented neutrophils and monocyte higher than normal level. As further results showed that lymphocyte and Platelet under normal condition. Finally, it can be concluded that from asymptomatic fasciolosis, laboratory examination showed anemias, leukocytosis, eosinophilia and chronic liver damage.

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