American Journal of Animal and Veterinary Sciences

Original Research Paper

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

[1]Maskur, [2]Adji Santoso Drajat, [3]Sulaiman Ngongu Depamede, [3]Made Sriasih and [4]Djoko Kisworo

[1]Department of Animal Genetics and Breeding, Faculty of Animal Science, University of Mataram, Indonesia
[2]Department of Animal Reproduction, Faculty of Animal Science, University of Mataram, Indonesia
[3]Department of Animal Biotechnology, Faculty of Animal Science, University of Mataram, Indonesia
[4]Department of Animal Production, Faculty of Animal Science, University of Mataram, Indonesia

Article history
Received: 02-03-2022
Revised: 25-07-2022
Accepted: 27-07-2022

Corresponding authors
Maskur
Department of Animal Genetics and Breeding, Faculty of Animal Science, University of Mataram, Indonesia
Email: maskur@unram.ac.id

Abstract: The research was to examine the hematological characteristic of fascioliasis in Bali cattle in year-round irrigated rice fields in tropical areas. Fifty cattle raised in rice field areas were examined by hematology, coproscopy, physiology, and liver function tests. The results showed that 50 cattle had heartbeat, respiratory and rectal temperature in normal standard, but, 41 (82%) out of 50 cattle were found positive for *Fasciola* eggs with egg per gram (epg) varying from 1 to 30. Indicators of liver function such as total bilirubin, alanine Amino Transferase (ALT), and Alkaline Phosphatase (AP) were under the normal conditions, except ɣ-Glutamyl Transferase (GGT) which was higher than the normal standard, indicating 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 an erythrocyte of 4.30 (10^6/µL) which can be categorized as anemia; 3 out of 20 cattle had higher Mean Corpuscular Volume (MCV) than normal, which indicated as a macrocytic condition. Mean Corpuscular Hemoglobin (MCH) in all the cattle was higher than 17 (p.g.,) which indicated hyperchromic erythrocyte, while MCHC was still at the normal levels. In terms of WBC, 8 out of 20 cattle showed leukocytes concentration higher than 12 (10^3/µL), and 6 cattle had eosinophils, band neutrophils, segmented neutrophils, and monocytes higher than the normal level and 6 cattle showed platelet concentration less than 100 (10^3/µL).

Keywords: Fascioliasis, Coproscopy, Hematology, Liver Function, Cattle

Introduction

The liver flukes *Fasciola sp*. are parasitic trematodes that affect the productivity of cattle, sheep, and goats, causing significant economic losses to agriculture (Beesley *et al*., 2018). In addition to reducing animal productivity, fascioliasis also causes moderate icterus, metabolic disorders, and secondary infections due to decreased immunity by chronic fascioliasis and liver condemnation during postmortem inspection in slaughterhouses while acute fascioliasis may lead to mortalities (Eman *et al*., 2016).

Fascioliasis is a liver fluke disease, that spreads worldwide that can infect not only livestock but also humans, with most cases of fascioliasis in humans concentrated in South America, Africa, and Asia (Hotez *et al*., 2008), although human fascioliasis cases have also been reported in other areas such as Turkey (Boşnak *et al*., 2016), Serbia (Pavlović *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. Miracidia can swim if there is water around the barn, (2) snails 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 leave 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).

Fascioliasis is one of the most important neglected parasitic diseases in humans and animals (Lalor *et al*., 2021). Although the impacts of fascioliasis have been

Science Publications

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recognized by researchers and investigators, this disease may still be neglected by farmers as they do not know that their cattle suffer from fascioliasis (Mas-Coma *et al.,* 2009). The fascioliasis in this study was shown to have no physiological effect on the cattle. It was reported previously that the chronic form is more common and without any clinical signs (Adrien *et al.,* 2013). The effect on the liver is when at least 52 flukes (Alemu and Abebe, 2015) or 56±28 flukes (Marcos *et al.,* 2007) and cirrhosis liver when infested with at least 116±30 flukes.

Most of the cattle raised on Lombok Island are Bali cattle (*Bos javanicus*). These cattle are an indigenous breed of Indonesia, originally from banteng of Java island. These cattle have been raised as part of agricultural activities. As a complement to rice producers, cattle are raised around rice fields, around wet areas, or in wet agriculture areas which are endemic to fascioliasis. A previous study showed that more than 76.9% of cattle suffered from fascioliasis in Central Lombok and though most of them did not show any visible symptoms, some cattle suffered a considerable weight loss (Astiti *et al*., 2015). Consequently, cattle suffering from fascioliasis may reduce the farmer's income by decreasing their body weight over time, as an infestation of *Fasciola sp*. can continuously for life. It was reported that most cattle suffer from fascioliasis without showing any signs of disease (Adrien *et al*., 2013).

Economic losses and the impact of fascioliasis (Adrien *et al.,* 2013) could firstly be identified by body weight loss (Ardo *et al.,* 2013) which may reach 157 gr/fluke/years (Wamae, 1996), or daily body weight gain may decrease to 1.96±0.8 kg (Genicot *et al.,* 1991). Secondly, milk production may reduce, ranging from 8 to 17% of their total production (Chakraborty and Podhan 2015). Thirdly, the flukes may destroy the liver. It was reported that 25% of fascioliasis was condemned totally, while 75% was condemned partially (Ssimbwa *et al.,* 2014), resulting in the loss of 4000 USD per year (Abunna *et al*., 2010). These indicated that fascioliasis significantly decreases farmers' income, especially traditional farmers in Lombok Island who raise cattle in endemic areas of fascioliasis and feed cattle by cut and carry. As this disease is asymptomatic, consequently farmers neglect the economic losses.

Based on the abovementioned description, this study was performed to evaluate fascioliasis of Bali cattle raised under rice field conditions in Central Lombok of Indonesia. This study aimed to identify fascioliasis by fecal coproscopy, examination of hematology, and liver function test to evaluate the characteristic of fascioliasis in Bali cattle under rice field conditions. Based on the results of this study, the farmers could anticipate that their cattle should be treated with medication for fascioliasis.

Materials and Methods

All procedures carried out involving animals had been approved by the ethics committee of the Faculty of Animal Science, University of Mataram, Indonesia, with registration number: 08/UN18.F2/EC/2020.

Detection of Fasciola sp. Eggs in Feces

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

Fifty mature cattle (consisting of bulls and cows) were used in the study, by examination of fluke eggs by a sedimentation technique (Avcioglu *et al.,* 2014). A fecal sample was collected by insertion of a finger covered with a plastic glove into the rectum, approximately 10 gr of feces was preserved by 3 drops of 1% formaldehyde. The worm eggs were identified 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 min, then the solution was decanted, and the sediment was collected and stained with two drops of methylene blue. The stained filtrate was then mixed using a pipette and dropped into an object glass and examined under binoculars with 100 x magnification and *Fasciola sp*. eggs would show yellowish color.

Physiological Examination and Liver Function Test

The physiological examination of the 50 cattle consisted of rectal temperature (ºC), heart rates, and respiratory rates. The examination of rectal temperature was performed by thermometer; when the temperature had reached 35°C, it was then inserted into the rectum and after in the rectum for 5 min, it was read. The examination of heart rate was performed by listening using a stethoscope at the left front breast of the cattle and counting the number of systolic sounds for one minute. The respiratory examination was performed by putting the right hand in front of the 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 and 39.5°C, while heart rates and respiratory rates were between 100 and 140 beats, 24 and 26 expirations per minute.

The 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).

Hematological Analyses

Hematology examinations were performed on 20 cattle randomly by an automatic hematology machine (SYSMEX KX-21) from blood collected from the jugular vein. The blood sample was dispensed into a 10 mL vacuum tube containing Ethylene Diamine Tetra Acetic Acid (EDTA). The hematology examinations consisted of Red Blood Cells count (RBC), the concentration of Hemoglobin (HGB) and corpuscular parts of Hematocrit (HCT), White Blood Cells (WBC), basophils, eosinophils, neutrophils, lymphocytes, and monocytes.

Results and Discussion

Prevalence of Liver Flukes

The results of the coproscopy (Fig. 1) showed that 41 (82%) of 50 cattle were found positive for *fasciola* eggs, with epg varying from 1 to 30 eggs per gram of feces. This prevalence of liver flukes was higher than that of a previous study on Lombok Island, up to 67.5% (Astiti *et al*., 2015), and some areas in Indonesia, such as Lampung 26.72% (Aryandrie *et al*., 2015) and Malang 26.03% (Paramanandi *et al*., 2020). Adedokun *et al*. (2008) reported that the prevalence of fascioliasis in winter (52.3%) was higher than that in the dry season (21%) in Nigerian cattle. Elshraway and Mahmoud, 2017 also reported that the prevalence of seasonal fascioliasis was highest in winter, followed by autumn, spring, and summer, respectively. This finding may be associated with the rainy season where fascioliasis 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). The prevalence and distribution of liver flukes are influenced by climate change/seasons and livestock grazing conditions (Mas-Coma *et al*., 2019).

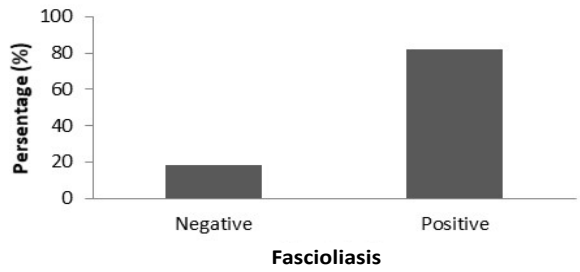

Fig. 1: The fecal examination results of *Fasciola sp*. eggs of Bali cattle

Furthermore, livestock grazing conditions with high water levels or frequent flooding are at a high risk of becoming infected with liver flukes. 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 miracidia to swim to find snails *Lymnaea sp.* and facilitate cercaria to swim to find grass or other vegetations (Admassu *et al*., 2015; Ardo *et al*., 2013), causing the surrounding grasslands to remain infected so livestock raised in that area can also become infected through contaminated grasses.

The increase in asymptomatic fascioliasis cases in the present study was caused by increased rainfall which increased water depth and an increased snail (*Lymnea sp*.), thus increasing fascioliasis cases. On the other hand, a decrease in rainfall during the dry season may reduce snails and infestation of fascioliasis on Bali cattle (Surana *et al*., 2019).

In addition to these factors, diagnostic techniques also greatly determine the level of accuracy of fascioliasis prevalence. There has been no accurate technique to diagnose fascioliasis. Only the sedimentation test is available to evaluate epg, but this test has a high specificity or true positive cases up to 100%, while the sensitivity or true negative cases is only 67.8% (Alemu and Abebe, 2015), depending on sample collection and eggs released by flukes.

Physiology and Liver Function of Fascioliasis Cattle

The results of the physiological examinations such as heart rates, respiratory rates and rectal temperature as seen in Table 1 were still under normal conditions (Kahn *et al*., 2010). Taimur *et al*. (1993) reported that in low infestation of liver flukes without showing any sign of disease, the symptoms of fascioliasis were detected when infestations were higher than 300 epg. In this present study, epg was found only between 1-30 with no symptoms of disease in the livestock, so it was neglected by the breeders. Another study showed that the prevalence of 31.14% and detected with the eggs of 133.03± 9.04 showed signs of disease, i.e., poor health (Affroze *et al*., 2013). The results of this study and several previous studies indicated that the symptoms of fascioliasis appear at a certain level of infestation and prevalence. Behm (1999) reported that the severity of fascioliasis is largely influenced by the infectious dose and the age and immune status/response of the host.

Another reason was that liver tissues can remove dead cells to regenerate and produce new hepatocytes to replace the damaged ones. The pathogenesis of fascioliasis depends on the ability to repair and regenerate tissues damaged by juvenile *F. hepatica* infection and the liver has a high capacity to not only repair but also regenerate following injury (Michalopoulos, 2007; Robinson *et al*., 2016;

Escamilla *et al*., 2016). In sheep, infiltration of the parasite into the liver stimulates a wound healing response, which is characterized by an influx of lymphocytes, macrophages, and eosinophils and the induction of fibrosis to repair the damage (Dorey *et al*., 2021). Consistent with that, Alvarez Rojas *et al*. (2015) also found a significant up-regulation of genes associated with the cell cycle and mitosis in infected livers. They also noted that tumor necrosis factor-alpha (tnfα) contributes to the restoration of functional liver mass by promoting hepatocyte proliferation and liver regeneration.

The liver function test was also evaluated using an auto-analyzer (ILab 300 Plus Chemistry System) for bilirubin Total (T), aspartate Amino Transferase (AST), alanine Amino Transferase (ALT), Alkaline Phosphatase (AP), and γ-Glutamyl Transpeptidase or transferase (GGT). The results of the liver function test of the fascioliasis cattle are shown in Table 2.

The data in Table 2 show that total bilirubin, alanine Amino Transferase (ALT), and Alkaline Phosphatase (AP) were under normal conditions. Two cattle out of seven showed aspartate Amino Transferase (AST) less than normal. All the seven cattle showed ɣ-Glutamyl Transferase (GGT) higher than the normal value, indicating long-term liver damage. Hepatic enzymes can be used to indicate the presence of diseases such as fascioliasis. Coppo *et al*. (2010) also reported significant increases in leukocytes, eosinophils, GGT, gamma globulin, and total protein in blood and serum samples of cattle infected with *F. hepatica*, which is indicative of cholestasis and liver inflammation, dysfunction, and necrosis. In this study, some liver function indicators showed average values under normal conditions. This condition indicates that in the case of liver fluke infections, animals may not be continually re-infected, thus neither causing any permanent damage nor compromising overall liver function (Dorey *et al*., 2021). Moreover, cattle have partial resistance to reinfection due to the more extensive fibrosis and less visible tracts associated with previous infection (Marcos *et al*., 2007). The liver also plays a pivotal 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 in terms of innate immunity (Arias *et al*., 2009).

Hematological Indices of Fascioliasis Cattle

The results of the hematological indices determined for *Fasciola*-infected and non-infected cattle are presented in Table 3. The results of the red blood cell evaluation showed that 3 out of 20 cattle had 4.30 (10^6/µL) erythrocytes, which is less than 5.0 (10^6/µL), thus being categorized as anemia (indicating normochromic and normocytic anemia). The results for RBC are similar to those found by Egbu *et al*. (2013) in bovine infected by *fasciola*, namely 3.86×10^6/µL. The anemia may be caused by the infestation of liver flukes in which young flukes may burrow and destroy hepatic cells and lead to hemorrhage (blood loss anemia) which is associated with abnormal iron metabolism due to chronic invasion, migration of immature flukes inside the liver parenchyma and the hepatocyte may be unable to produce the normal count of red blood cells. The severity of anemia depends on the number of flukes inside the liver (Egbu *et al*., 2013). Weiss and Wardrop (2011) and Lotfy *et al*. (2003) also reported that severe anemia may be due to chronic liver inflammation, which causes depression of erythrogenesis.

The hemoglobin and hematocrit evaluation showed that all the cattle were under normal levels. The normal hemoglobin levels indicate the ability of the erythrocytes to carry oxygen from the lungs without signs of respiratory rates and heart rates. Hematocrit value indicates the percentage of corpuscular volume as part of blood, while normal hematocrit shows corpuscular portion to blood plasma volume. This indicates a low infestation of fascioliasis in the present study without hypoproteinemia.

The evaluation of Mean Corpuscular Hemoglobin (MCH) showed that all the cattle had higher MCH than the normal standard 17 (p.g.,), indicating hyperchromic erythrocytes or higher hemoglobin. While the Mean Corpuscular Hemoglobin Concentration (MCHC) of all the cattle was at normal levels. The evaluation of the Mean Corpuscular Volume (MCV) showed that 3 out of 20 cattle had higher MCV than the normal conditions or 60 (fl), indicated as macrocytic, in which the erythrocytes were bigger than their normal size. It may be compensatory for erythrocytes to increase their capacity to carry oxygen. Egbu *et al*. (2013) also observed higher MCV and MCH in *fasciola*-infected sheep. Whereas, El-Aziem Hashem and Mohamed (2017) reported that no significant differences occurred between the erythrocytic indices (MCV, MCH, and MCHC) of the infected and non-infected cattle, indicating normocytic normochromic anemia. In severe fascioliasis, there are blood suckling and blood leakage from the bile duct into the intestines, resulting in normocytic hypochromic anemia (Egbu *et al*., 2013). A high number of fascioliasis infestations as shown by epg affects a decrease in erythrocytes, hemoglobin, hematocrit, and anemia because of chronic blood loss (Lotfollahzadeh *et al*., 2008).

Table 1: Heart rates, respiratory rates, and rectal temperature of fascioliasis cattle (n = 20)

No	Examination	Mean ± SD	Min-max	*Kahn *et al.*, 2010
1	Heart rates/minute	49.00±7.31	42.00-60.00	48.00-84.00
2	Respiratory rates/minutes	22.00±4.21	21.00-29.00	26.00-50.00
3	Rectal temperature (°C)	38.19±0.70	36.00-38.20	36.70-39.00

*Normal standard (Kahn *et al*., 2010)

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

No	Liver function test	Mean ± SD	Min-max	*Kahn *et al*., 2010
1	Bilirubin T (mg/100 mL)	0.25±0.10	0.20 - 0.40	0.00-1.60
2	AST (mg/100 mL)	75.67±7.09	51.00 - 84.00	60.00-125.00
3	ALT (mg/100 mL)	27.00±3.46	20.00-29.00	6.90-35.00
4	AP (mg/100 mL)	110.67±33.56	72.00-148.00	18.00-153.00
5	GGT (mg/100 mL)	33.00±14.11	18.00-46.00	6.00-17.40

*Normal standard (Kahn *et al.*, 2010)

Table 3: Examination of red blood cells of fascioliasis cattle (n = 20)

No	Red blood cells	Mean ± SD	Min-max	*Kahn *et al*., 2010
1	Erythrocytes (10^6/µl)	6.12±1.02	4.30-7.84	5.00-10.00
2	Hemoglobin (gr/dl)	12.13±1.71	9.50-12.80	8.00-15.00
3	Hematocrit (%)	33.84±4.82	26.30-37.70	24.00-46.00
4	MCV (fl)	55.39±4.84	47.30-61.80	40.00-60.00
5	MCH (pg)	19.90±2.15	18.20-21.50	11.00-17.00
6	MCHC (gr/dl)	36.00±0.93	35.00-36.00	30.00-36.00

*Normal standard (Kahn *et al.*, 2010)

Table 4: Examination of white blood cells of fascioliasis cattle (n = 20)

No	White blood cells	Mean ± SD	Min-max	*Kahn *et al*., 2010
1	Leukocyte (10^3/µL)	8.20±3.690	4.80-15.300	4.00-12.000
2	Eosinophils (10^3/µL)	2.50±0. 760	2.00-4.0000	0.00-2.4000
3	Band neutrophils (10^3/µL)	0.88±0.830	0.00-2.0000	0.00-0.1000
4	Seg. neutrophils (10^3/µL)	2.99±0.920	1.70-4.3000	0.60-4.0000
5	Lymphocyte (10^3/µL)	5.04 ±1.470	3.20-7.1000	2.50-7.5000
6	Monocyte (10^3/µL)	1.00 ±0.000	0.00-2.0000	0.00-0.9000
7	Platelet (103/µL)	142.80±115.60	42.00-350.00	100.00-800.00

*Normal standard (Kahn *et al.*, 2010)

The evaluation of White Blood Cells (WBC) Table 4 showed that 8 out of 20 cattle had leukocyte concentrations higher than 12 (10^3/µL), indicating that there were WBC responses to protect against liver flukes. A high concentration of leukocytes may be related to 6 cattle that had eosinophils, band neutrophils, segmented neutrophils, and monocytes higher than the normal levels. Further, the results showed that the lymphocytes were in normal condition in all the cattle, while the platelet concentrations were less than 100 (103/µL) in 6 cattle. These results are consistent with those obtained in sheep and bovines. El-Aziem Hashem and Mohamed (2017) reported that there was an increase in leukocytes, eosinophils, and neutrophils in *Fasciola*-infected cattle. Meeusen *et al.* (1995) reported that the infection of fascioliasis increased 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 showed that neutrophils, eosinophils, monocytes, and lymphocytes increased progressively as the worm load increased, only basophils showed no change.

Conclusion

The fluke egg examination in this study found positive cases for fascioliasis with epg varying from 1 to 30 and the prevalence rate was 82% on Bali cattle. The physiology and liver functions such as heart rates, respiratory rates, rectal temperature, total bilirubin, alanine Amino Transferase (ALT), and Alkaline Phosphatase (AP) were still under the normal condition, except ɣ-Glutamyl Transferase (GGT) which was higher than the normal standard, indicating long-term liver damage. The results of this study indicated that the asymptomatic fascioliasis is due to the level of epg and the ability of liver tissue to regenerate and repair the damaged tissue. The hematological assay in this study showed that 3 out of 20 cattle experienced a notable

reduction in the erythrocytes, thus being categorized as anemia. All the cattle had MCH higher than the normal standard 17 (p.g.,), while MCHC was still at the normal levels. Three out of 20 cattle had higher MCV than normal and can be categorized as macrocytic erythrocytes. The WBC evaluation showed that 8 out of 20 cattle had leukocytosis, indicating a WBC response to protect against disease. Possibly associated with a high concentration of leukocytes, 6 cattle had eosinophils, band neutrophils, segmented neutrophils, and monocytes higher than the normal levels. In addition, the results also showed that lymphocytes and platelets were under normal conditions. Finally, it can be concluded that from the asymptomatic fascioliasis, the laboratory examination showed anemia, leukocytosis, eosinophilia, and chronic liver damage.

Acknowledgment

This research was funded by PNBP University of Mataram. The authors thank the Rector of the University of Mataram for the financial support through the University Research Grant with contract number: 167C/SP-BLU/UN18.12.2/PL.

Author's Contributions

Maskur: Responsible for all experiments, designed, analyzed, interpreted data, and preparation of the manuscript.

Adji Santoso Drajat: Responsible executor for laboratory research, collection, data analysis, and manuscript preparation.

Sulaiman Ngongu Depamede: Designed and supervised the experiment work, participated in data collection, and proofread the manuscript.

Made Sriasih: Designed and supervised the experiment work, participated in data analysis, and proofread the manuscript.

Joko Kisworo: Participated in data collection, analysis, and proofreading of the manuscript.

Ethics

This article is original and contains unpublished material before. The authors declare that there are no ethical issues may arise after the publication of this study.

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