Efficacy of Albendazole Against Strongylus sp. and Hematology Changes on Equine in Yogyakarta Special Region

Efikasi Albendazole Terhadap Cacing Strongylus sp. dan Gambaran Darah Pada Kuda di Daerah Istimewa Yogyakarta

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Abstrak


Kata kunci: albendazole; EPG; kuda; status hematologi; Strongylus sp.

Abstract

The infestation of Strongylus sp. in horses can cause losses to horse breeders, including anorexia, anemia, gastrointestinal diseases and can cause death and decrease the horse population in Yogyakarta Special Region (DIY). Albendazole was a Benzimidazole preparation that is often used to treat worms in ruminants. This study also aims to determine the effect of Albendazole on blood images before and after treatment. The material used in this study were 10 horses with male and female sex, over 3 years old, and infected with Strongylus sp. with an infestation rate of 200 EPG in faeces. Before treatment of drug was carried out, the faeces was examined with Mc Master method. After treatment with Albendazole, the worm eggs were examined three times at intervals of three days. Routine examination of worm eggs and blood was carried out at the Laboratory of the Department of Internal Medicine, Faculty of Veterinary Medicine, Universitas Gadjah Mada. The results showed that the EPG number decreased from the 0th, 3rd, 6th and 9th day of examinations. The average number
Introduction

Horses are animals that are commonly used as transportation animals, generally as a horse cart or cart. These animals have a significant share in the Yogyakarta area as a popular tourism facility. However, there are many obstacles faced by horse breeders, one of which is the case of gastrointestinal worms (Kristiyani et al., 2019). The main problem in most developing countries is worm infection which causes a decline in the health and performance of the horse. Mild to moderate worm infections do not always show obvious clinical symptoms, while severe adult worm infections can cause digestive disorders and stunted growth in horses (Subekti et al., 2010). Worm infection in adult horses has a lower prevalence, this is because the immune system in adult horses is well developed compared to young horses (Love, 2003).

The decline in horse health itself can be influenced by various factors, including poor environmental conditions, a favorable climate for the development of worms or bacteria, and feed contaminated by infective larvae (Koesdarto et al., 2007). Strongyloids in horses are generally caused by the nematodes Parascaris equorum and Strongylus sp. A disease caused by these two worms is an important disease for horses and can cause significant losses for horse cart breeders in Yogyakarta (Yuriadi, 2007). According to Apriliawati et al. (2019) and Ratnawati (2014), reported that worm infestations in the gastrointestinal tract in wagon horses in Bogor City were found to have Strongyloidi infestations with a prevalence of 70%, Ascarid 46.6% and Oxyurid as much as 30%. Meanwhile, Pradana (2012) reported that the sampling in Pohsarang, Kediri showed the prevalence of Strongylus sp. amounted to 34.78% and Trichonema sp. 8.7%. Siregar (2016) reported that horses in Batu City had a prevalence of Strongylus sp. by 32%, Trichonema sp. 2% and Parascaris equorum by 2%. Yogyakarta Special Region is a tourist city that is visited by many tourists, both domestic and foreign. One of its attractions is the horse-drawn carriage which is the hallmark of Yogyakarta. Horses are pets as well as transportation animals as carriage towers which have a big share in attracting tourists which also means increased income for the government of the Special Region of Yogyakarta (Yuriadi, 2007).

The use of a broad spectrum anthelmintic (worm medicine) Albendazole is very commonly used in Indonesia. This type of drug that is included in the Benzimidazole group is widely used because of its effectiveness in eliminating gastrointestinal worms (Kristiyani et al., 2019). The use of Albendazole is not common in horses and donkeys, and there is insufficient data available on the toxicity and side effects of the drug in horses and donkeys (Gokbulut et al., 2005). The higher metabolic capacity, the presence of the first pass-effect, and lower absorption of benzimidazole (including Albendazole) in the Equine group compared to ruminants cause the use of albendazole to be rarely used in horses (Imam et al., 2010). The use of albendazole which has been widely used is also feared to cause resistance, Cernea et al. (2007) reported that the widespread use of benzimidazole derivatives in Romania has led to reports of worm resistance. Furthermore, it was explained that the use of Albendazole in comparison with pyrantel resulted in an action that was three times higher than giving Albendazole alone. Research on the efficacy effect of Albendazole against Strongylus sp. and the hematological features of the drug administration have never been carried out in Yogyakarta, so this study aims to determine the effect of Albendazole’s killing power against Strongylus sp. on horses in the Yogyakarta area and knowing the effect of albendazole on hematology result before and after treatment.

Keywords: albendazole; EPG; hematological status; horse; Strongylus sp.
Material and Methods

This study used 10 adult horses from Gamping District, Sleman, 3 horses, 3 horses in Kotagede, Yogyakarta, and 4 Potorono horses, Bantul Regency. The sex of the horse is taken randomly, the mares are taken from those who are not pregnant. The collection was carried out on horses that had a Body Condition Score of 2-3 and were more than 3 years old. Before sampling, a health check was carried out and the selection of horses was prioritized on horses with diarrhea disorders. According to Howell et al. (2008) before giving Albendazole, ± 10 grams of feces were taken from each horse through the rectum and 10 cc of blood was taken through the jugular vein then placed in a vaccum tube that had been given the anticoagulant Ethylenediaminetetraacetic acid (EDTA) and a small portion of the blood was made smear preparations.

Stool and blood samples were examined at the Laboratory of Internal Medicine, Faculty of Veterinary Medicine, Gadjah Mada University. Examination of feces using the Mc Master method according to Nofyan et al. (2010), the level of infection in animals is divided into three levels based on the number of eggs per gram of feces, namely mild infection if the number of eggs is 1-499 per gram of feces, moderate infection if the number of eggs 500-5,000 eggs per gram and severe infection if the number of eggs> 5,000 eggs per gram of animal feces. Hematological examination based on smear preparations and blood mixed with anticoagulants was performed under an Olympus® microscope at a magnification of 40 x. Albendazole anthelminthic administration with a total dose of 5 mg/kg BW (Imam et al., 2010) with one administration on day 0. Stool collection for repeated testing with McMaster was carried out on days 3, 6, 9, and 12. Meanwhile, the second blood collection after drug administration was carried out on the 12th day. Data from laboratory examination, both feces, and blood tests, were analyzed using a paired T-test and Repeated Measure ANOVA Test and described descriptively.

Results and Discussion

Examination of worm eggs using fecal media is the main thing in determining the level of worm infestation in an animal’s body. The examination used in this research is to use the Mc Master method which is useful for knowing the results quantitatively. Feces examination were carried out repeatedly on day 0 (before treatment) and day 3, day 6, and day 9 (after treatment). The results of quantitative examination of worm eggs are shown in Table 1.

The number of worm eggs per gram of feces/egg per gram (EPG) from each of the tests showed different results. Observations on day 0 and day 3 showed significant differences (P <0.05), an examination on day 3 to day 6 also showed a significant difference (P <0.05), while examination on day 6 and the 9th day showed no difference (P> 0.05). Albendazole treatment was carried out on day 1, based on these results showed a decrease in the amount of EPG from before the treatment (990.00 ± 148.69) on day 0 and after the occurrence of treatment (250.00 ± 113.04) on day the 3rd. The results of the examination showed a reduction in the number of EPG on the 3rd day after giving albendazole by 74.74%, then increased again on the 6th day by 97.47% and finally reached 100% on the 9th day post anthelmintic administration. Amin et al. (2008) stated that there was a reduction in the number of worm eggs in donkeys given the 3rd day of Albendazole to 90.44% of the number of worm eggs compared to the time before being given anthelmintics. Islam et al. (2015) stated

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample (n)</th>
<th>0 day (EPG)</th>
<th>Post treatment examination</th>
<th>6th day (EPG)</th>
<th>9th day (EPG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of worm eggs</td>
<td>10</td>
<td>990.00 ± 148.69*</td>
<td>250.00 ± 113.04*</td>
<td>25.00 ± 26.35*</td>
<td>0.00 ± 0.00*</td>
</tr>
<tr>
<td>EPG decrease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values of different superscript on the same line are significantly different (P<0.05).
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that in cows given Albendazole on day 0 the average amount of EPG was 810 ± 33.17 eggs/gram on the 7th day post-treatment the number of eggs decreased by 46.91% (430 ± 20.00 eggs/gram), then on the 14th day the number of eggs decreased to 72.84% (220 ± 9.49 eggs/gram), on the 21st day it decreased again to reach 84.88% of the number of eggs at the start of the study (126 ± 9.80 eggs/gram).

Saeed et al. (2008) reported that the use of Farbenda® containing Albendazole was able to fight <90% of naturally infected Strongyl infections. Efficacy in horses given Oxafax® (containing Oxfendazole) reached 100% after 28 days of drug administration. The efficacy of Ivermectin® (Ivermectin) and farbenda was 96% and 86%, respectively, on day 28 after administration of the drug. When compared with research conducted, the observed efficacy rate was higher where on the 9th day after drug administration had reached the level of 100%. Salas Romero et al. (2018) reported that the use of drugs from the benzimidazole group provided low levels of efficacy in horses of various breeds and races. Worm resistance from the use of benzimidazole in equine cyathostomin cases was reported in at least 14 countries (Peregrine et al., 2014). Meanwhile, Matthews (2014) reports that there is a high level of efficacy against the use of albendazole in natural Strongyl infections in horses and donkeys. The low level of efficacy in several other studies indicates that the prevalence of efficacy of albendazole and other drugs such as Ivermectin and Oxfendazole is due to frequent use, which causes resistance to worms (Islam et al., 2015).

The mechanism of action of albendazole is that it binds to beta-tubulin on the colchicine-sensitive side of the cell wall so that it inhibits polymerization and formation of worm tubules. This compound also inhibits spindle formation in the process of cell division, causing obstacles to egg formation and development. Furthermore, albendazole will inhibit glucose uptake by larvae and adult worms resulting in depletion of glycogen stores (Plumb, 2011). However, a longer period time from drug administration without re-administration of anthelmintics can lead to reinfection in horses. Treatment of drug administration for more than 6 weeks without a repeat is likely to lead to worm reinfection in the gastrointestinal tract of horse (Saeed et al., 2008). Imam et al. (2010) stated that giving albendazole both in single and repeated doses showed a reduction in the number of worm eggs by 100% after 7 days of drug administration. Kuzmina and Kharchenko (2008) also stated that giving albendazole at a dose of 5 mg/kg body weight showed a significant decrease in the eggs of Strongylus sp. after 10 days of drug administration. Güzel et al. (2014) stated that after a post mortem examination, administration of a single dose of Albendazole produced a low efficacy result of 49.48% for Habronema sp., 67.09% for Trichostrongylus axei worms 67.09%, and 67.74% for worms. Strongyloides westeri. However, Albendazole was found to produce a high level of efficacy in dealing with Parascaris equorum nematodes by 100%, in Strongylus sp. amounted to 98.40% and Cyathostomum sp. as much as 98.4%.

Table 2. Hematology test results on day-0 (before treatment) and day-12 (after treatment)

<table>
<thead>
<tr>
<th>Observation time</th>
<th>Samples (n)</th>
<th>Erythrocytes (10⁶/µL)</th>
<th>Hemoglobin (g/dL)</th>
<th>PCV (%)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>TPP (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal standart*</td>
<td></td>
<td>6.2-10.2</td>
<td>11.4-17.3</td>
<td>31-50</td>
<td>37-53</td>
<td>14-20</td>
<td>36-39</td>
<td>5.8-8.7</td>
</tr>
<tr>
<td>Examination D-0</td>
<td>10</td>
<td>6.49 ± 0.61a</td>
<td>9.55 ± 1.62a</td>
<td>35.40±</td>
<td>54.82±</td>
<td>14.84±</td>
<td>27.20±</td>
<td>6.76± 0.60</td>
</tr>
<tr>
<td>Examination D-12</td>
<td>10</td>
<td>7.93 ± 0.56b</td>
<td>11.34 ± 0.45b</td>
<td>36.30±</td>
<td>45.97±</td>
<td>14.35±</td>
<td>31.35±</td>
<td>7.08± 0.31</td>
</tr>
</tbody>
</table>

**Values of different superscript on the same column are significantly different (P<0.05)

*Normal standard of hematology refers to Louise (2013)

Explanation: PCV = Packed Cell Volume, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, TPP = Total Protein Plasma
The administration of albendazole is directly or indirectly related to changes that occur in the blood. Routine hematology results are shown in Table 2.

Hematological examination showed a significant difference in the number of erythrocytes which increased from 6.49 ± 0.61 million/µL to 7.93 ± 0.56 million/µL (P <0.05), the total amount of hemoglobin also increased, from 9.55 ± 1.62 g/dL to 11.34 ± 0.45 g/dL. Other measurement results that showed a significant difference were MCV from 54.82 ± 8.62 fL, which decreased to 45.97 ± 4.31 fL, and MCHC, which increased from 27.20 ± 4.67% to 31.35 ± 2.39% (P <0.05). Louise (2013) states that the normal range of erythrocyte counts in adult horses is 6.2 - 10.2 x106 cells/µL, normal hemoglobin in adult horses ranges from 11.4 - 17.3 g/dL. Hemoglobin showed deficiency on examination day 0, due to high infestation of Strongyl sp. in the horse’s digestive tract. However, after treatment with Albendazole administration, it seems that it has increased. Rob et al. (2014) stated that sheep given albendazole increased compared to before drug administration. Amin et al. (2008) stated that the increase in hemoglobin content in the blood is directly proportional to the decreasing number of worms in the cow’s body. Axon and Palmer (2008) reported that normal MCV levels in adult horses ranged from 37 - 53 fL, while normal MCHC values ranged from 36 - 39 g/dL. Based on these results, it can be seen that the MCV results on the examination experienced a slight increase on the 0th day but not too significant, the MCHC examination results on the 0th day also looked higher than the normal range. Agina (2017) states that the MCV and MCHC indicators on hematological examination are related to erythrons and can be used to determine whether the animal has anemia, polysetinemia or in normal conditions.

The decrease in MCHC values that occurred on day 0 on examination was closely related to the anemia status that occurred in horses. Amirullah et al. (2018) stated that cows infected with worms will suffer from hypochromic macrocytic anemia or anemia with the formation of erythrocytes larger than normal and decreased hemoglobin content. Egbu et al. (2013) reported that MCV and MCHC levels were indicators of anemia in fasciolosis cases. Wiedosari et al. (2006) also stated that adult worms can suck blood 0.2-0.5 ml/day and produce an excretory-secretory product substance that will suppress hematopoietic so that the total production of erythrocytes or hemoglobin decreases. Several studies have reported alterations in blood parameters and blood chemistry as a consequence of S. vulgaris infection, including a decrease in RBC, PCV, total serum proteins and an increase in WBC. Intestinal haemorrhages lead to reduced RBC survival loss of albumin in intestine leads to increased albumin catabolism (Khan et al, 2015). Islam et al. (2015) reported that cows given treatment with albendazole experienced an increase in hemoglobin counts 28 days after administration. The same thing was reported by Soutello et al. (2009) and Demeler et al. (2009) who stated that there was an increase in the total number of erythrocytes and hemoglobin in animals given albendazole treatment from day 14 to day 28. Barrelet and Rickets (2013) state that anemia caused by parasites is regenerative anemia. In the case of regenerative anemia, the bone marrow is still able to produce erythrocytes in a sustainable manner, but reticulocytes are rarely found in the peripheral blood tissue of horses. The macrocytic condition which shows an increase in the MCHC value at the beginning of the examination, this is due to the large number of young erythrocytes produced by the bone marrow.

Apart from the results of erythrocyte measurements, changes were also seen from the measurement of leukocyte levels as shown in Table 3.

Observation of leukocytes showed no significant differences in leucocytes, segmented neutrophils, and lymphocytes (P> 0.05), compared with the normal standard, the results showed also showed no difference. Observations on eosinophils and monocytes showed a difference (P <0.05), the percentage of monocytes also showed a difference with the normal standard where the examination on day 0 showed the number of monocytes that were above the normal limit. The level of eosinophils, which are specific leukocytes against parasitic
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...patients, did not show a percentage that exceeded the standard, but it was seen that on the 12th day of the examination, euosinophils had increased compared to the examination on day 0. According to Amirullah et al. (2018) several factors that determine the ups and downs of euosinophil levels include the number of worms in the animal’s body and the presence or absence of secondary infections that occur after treatment. Wiedosari et al. (2006) stated that after reinfection in animals, the immune process occurs at least 5 weeks after infection occurs, and the level of antibodies formed is also higher than that of the first infection. Zhang et al. (2006) stated in cases of infection from Fasciola sp. which continues will cause eosinophilia at week 3 to week 13 post-primary infection, after which the eosinophil content will decrease.

The high number of leukocytes found on the 0th day of examination can be caused by the high number of phagocytosis that occurs during inflammation, this can be caused by tissue necrosis. Besides, monocytosis is also related to the recovery phase after a viral infection (Barrelet and Rickets, 2013). Examination of total leukocytes indicated a decrease in the number of blood counts from day 0 of blood count to day 12 of blood count, although it did not look significant. Amin et al. (2008) stated that the results of treatment with albendazole resulted in a decrease in the total number of leukocytes compared to before being given the drug. Khalid et al. (2005) also stated that the total leukocytes in sheep given albendazole also decreased significantly compared to pre-treatment.

**Conclusion**

The results showed that giving albendazole to 10 guava horses gave a significant level of efficacy to the number of Strongyl sp. Worm eggs, in every gram of feces. Examination with Mc Master showed an efficacy rate of 100% on the 9th day after administration of albendazole took place. Hematological examination showed that the horse had symptoms of hyperchromic macrocytic anemia when albendazole treatment had not been performed, this was due to the presence of Strongyl sp. in high infection rates. Observation of the hematological parameters also showed some significant differences in the presence of albendazole administration compared to before treatment.

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