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# REVISED Risk factors, hematological and biochemical profile associated with colic in Delman horses in Gresik, Indonesia [version 2; peer review: 2 approved]

Previous title: Risk factors and hematological profile associated with colic in Delman horses in Gresik, Indonesia

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## Abstract
**Background:** Horses are herd animals that have been domesticated in the last century. In several countries, an overview of risk factors and clinical evaluation in horses with colic has not been well-described. This study aimed to evaluate risk factors and hematological profiles in horses associated with colic in Gresik, East Java, Indonesia.
**Methods:** A cross-sectional study was performed during April - October 2019. A total of 115 horses were diagnosed based on physical examination, clinical symptoms, and rectal examination. A questionnaire was asked to the horse-owners to analyze the risk factors while the clinical examination was performed and blood samples were collected for pre-treatment and 14 days post-treatment. Hematological profile was evaluated from a whole blood sample. Serum cortisol, plasma epinephrine, and norepinephrine concentrations were also evaluated after separating the aliquots.
**Results:** Of the 115 horses, 96 were diagnosed with colic. The horses with colic showed a significant association between cases with gender (p<0.021), breed (p<0.000), wheat bran feeding (p<0.015), concentrate feeding (p<0.003), anthelmintics administration (p<0.000), gastrointestinal parasites (p<0.000), dental diseases (p<0.024), previous exposure to colic (p<0.000), body condition score (p<0.000), and access to water per day (p<0.000). Based on whole blood and

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serum evaluation, there were ameliorated significantly on the hematological profile (p<0.01), serum cortisol (p<0.05), and plasma epinephrine (p<0.01) at 14 days post-treatment.
**Conclusion:** This study has identified factors associated with colic in Delman horses. The study provides crucial information to investigate cases of colic and to contribute the development of healthcare strategies during treatment and clinical evaluation.

**Keywords**
colic, Delman horses, domesticated animals, Gresik, hematological profiles, risk factors

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Any reports and responses or comments on the article can be found at the end of the article.

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> **REVISED** **Amendments from Version 1**
>
> This study has been revised according to the reviewers' suggestions. Revisions to the title and Table 3 were performed to specifically show the hematological and biochemical profiles of serum. The risk factor and prevalence information gaps reported in previous studies were inserted in the Introduction. Additional information on the total protein profile in the Discussion section and suggestions for further studies in the Conclusion were included. References have been adjusted according to the order and additional information in each section.
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## Introduction

Colic is defined as abdominal pain which is the most common cause of death in horses. Colic is categorized into two types: true colic caused by disorders of the digestive tract, and pseudo-colic due to disorders of organs other than the digestive tract.[1] True colic can be constipation colic (colon impaction), spasmodic colic (catarrhal enteralgia), tympanic colic (flatulent colic), and gastric colic (gastric distension) which are acute and implied to decrease horse performance and change habits.[2] Pseudo-colic can be caused by the presence of urolithiasis, uterine torsion, hepatitis, nephritis, myositis or tying up disease.[3] The severity of colic appears to vary from mild to severe based on its cause and treatment. The most common symptoms of colic are anorexia, sweating, restlessness, looking at the belly, kicking or biting the belly, spinning in the stable, scratching the legs, and rolling over. The appearance of common clinical symptoms usually cannot be distinguished between pseudo-colic and true colic.[4]

There is a limited overview of the prevalence and cause of horses associated with colic in respective countries, particularly in areas that use the horse for transportation. Colic in horses was reported as a welfare issue and a crucial concern among horse-owners.[5] In previous studies, the incidence of colic in the working horse population was reported to be 30.4% in Ireland,[6] 36.8% in England,[7] 83% in Egypt,[8] 54% in Albania,[9] and 20% in Sweden.[10] Low quality hay feed, poor hygiene are increased risk factors for colic. The study found that 46.15% of hay samples contaminated with yeast and 30.76% contaminated with bacteria.[11] In addition, gastrointestinal parasites, high activity of horses and low access to water are the highest risk causes of colic.[15] Many horses with colic are associated with hemostatic disorders, both as a primary effect and as a complication of cardiovascular disorders.[12] Diagnostic procedures and pathophysiological mechanisms were studied in-depth to investigate coagulation disorders in horses.[13]

In addition to identifying the history of the disease and performing a physical examination, evaluation of the hematological profile and blood chemistry of a horse can be done to trace the cause of the disease. The results of the examination can localize the cause of the disease based on the liver and spleen function. This method can evaluate hydration status, disease severity, inflammation, specific organ damage, endotoxemia probability, and determine disease prognosis.[14] This study was expected to provide an overview of colic risk factors and hematological profiles in horses with colic to monitor the progress of therapy during colic episodes and the possibility of recurrence.

## Methods

### Animals and ethical approval

Physical examination was performed according to standard operating procedures issued by the Indonesian Horse Veterinarian Association. This study was reported according to the Animal Research: Reporting of in vivo Experiments (ARRIVE) guidelines 2.0: author checklist.[51] All efforts were made to ameliorate any suffering of animals. The owner handles the horse by providing a sense of comfort to prevent stress, while the blood was collected according to standard examinations. Thus, a certificate of ethical clearance was not necessary for this study as the study did not affect normal animal behavior. Meanwhile, a permission letter (No.131/ADG/2019) was approved by Gresik Delman Association and informed consent was received by the horses' owner.

### Study period and locations

This study was conducted for seven months (April - October 2019). The sample distribution was collected from Dukun (6°59′54.1′S 112°30′32.2′E) (n = 8), Panceng (6°55′42.0′S 112°27′58.0′E) (n = 21), Ujung Pangkah (6°55′10.2′S 112°32′46.7′E) (n = 19), Sidayu (6°59′33.1′S 112°33′44.6′E) (n = 15), Manyar (7°07′21.2′S 112°36′14.5′E) (n = 8), Kebomas (7°09′59.1′S 112°38′17.5′E) (n = 15), Menganti (7°17′34.9′S 112°35′07.9′E) (n = 8), Kedamean (7°19′20.5′S 112°33′57.6′E) (n = 10), and Driyorejo (7°21′11.1′S 112°37′43.9′E) (n = 11) (Figure 1). The questionnaire was collected based on the owners' reporting. Serum and whole blood were evaluated at Gamma Scientific Biolab, Malang, East Java.

### Data collection

A cross-sectional study was designed to enroll Delman horses in Gresik, East Java, Indonesia. A total of 115 Delman horses at 3-11 years old and 300-400 kg weight were studied. The Delman horse as a riding animal was found in
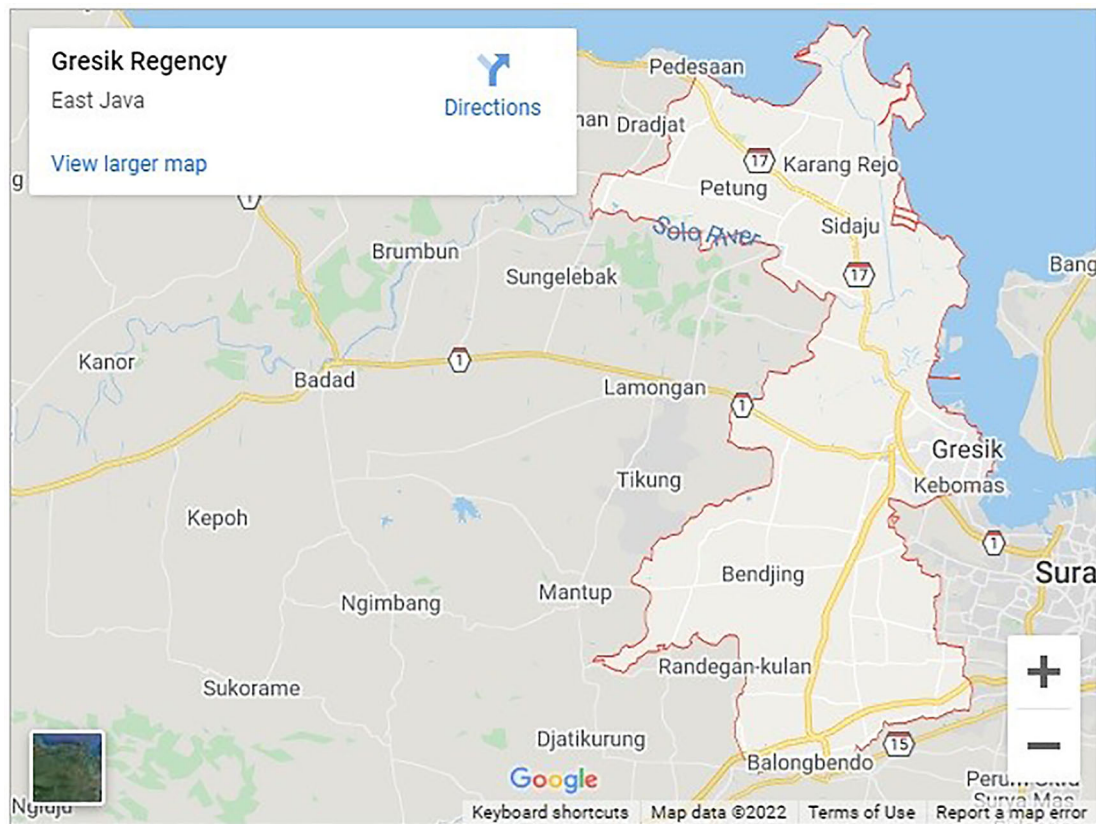
**Figure 1. Map of Gresik Regency.** [Powered by Google Map Source: https://goo.gl/maps/nZ5Aa1mhHqJkdGwe8].

the owners' farm. The diagnosis was performed based on animal history, physical examination, clinical symptoms, and rectal examination. A questionnaire was asked to the owner to analyze the risk factors for colic horses. Questions were grouped into the following sections: age, season influence, gender, breed, wheat bran feeding, feeding on green fodder, concentrate feeding, anthelmintics, gastrointestinal parasites, dental disease, previous exposure to colic, body condition score, water source, access to water per day, musculoskeletal disease and bad vices.[15] Horse-owners completed the inquiries with one of the study team while the clinical examination was performed and blood samples were collected. In an attempt to validate owner-reported recurrent colic episodes, owners were asked to describe behavioral alterations and clinical signs that horses demonstrated during the colic episodes.[16]

## Horse treatment
Colic therapy was performed according to current standard methods in the Indonesian Horse Veterinary Association. Lactated Ringers' solution (Ringer Lactate, Widatra, Indonesia) was administered as initial treatment via jugular vein in all cases. Concurrently, Flunixin Meglumine (Flumine, Jaapharm, Mano, Singapore) was administered routinely in a dosage of 0.5 mg/kg, q 12 h, for three days.[17] The time before therapy (admission) was considered as pre-treatment. The next observation period was performed at 14 days as a post-treatment evaluation.

## Blood evaluation
For pre-treatment and 14 days post-treatment evaluation, blood from the jugular vein (10 ml) was collected into Vacutainer (BD®, USA) with EDTA for measurement of hematological profile and plain tubes for serum isolation, respectively. All plain tubes allowed to clot for 10-20 minutes then centrifuged in a centrifuge machine (Hettich EBA 200®, GER), at a speed of 4000 rpm for 15 minutes.[18] The obtained serum was aspirated using a Pasteur pipette into a microtube and stored at $-4°C$. Serum cortisol was determined using ELISA method (My-Bio-Source®, San Diego, CA, USA).[19] Meanwhile, for total protein, albumin, globulin and calcium were determined spectrophotometrically using Biuret method (Biolabo®, France).[20]

For hematological profile, whole blood samples were determined using the clinical chemistry analyzer (Hitachi 902®, Roche Diagnostics, USA). Each blood sample for evaluation of plasma epinephrine and norepinephrine concentrations was centrifuged immediately at 2500 rpm for 10 minutes, and plasma aliquots were analyzed using high performance liquid chromatography.[21]

## Statistical analysis

All variables in the questionnaire were transformed into nominal criteria. A Chi-square test was performed to analyze independent data for each criterion associated with colic. The final analysis was done using logistic regression analysis with selected variables depending on p-values. Associations derived from conditional logistic regression were marked by odds ratio (OR) and relative risk (RR). A RR >1 reveals increased risk and a RR <1 reveals decreased risk.

Data of hematological and hormone evaluation were expressed as mean ± standard deviation (SD) then analyzed using one-way analysis of variance (ANOVA) followed by post hoc Tukey multiple comparisons test. Values were considered significantly different at $p < 0.05$. Statistical analysis was performed using SPSS v.25 software (IBM, Armonk, NY, USA).

**Table 1. Distribution of colic clinical signs in Delman horses.**

| Variables | Colic (*n* = 96) | % |
|---|---|---|
| Abdominal pain | | |
| ▪ Curling upper lips up | 90 | 93.75 |
| ▪ Kicking at the belly | 67 | 69.79 |
| ▪ Looking at the belly | 81 | 84.38 |
| ▪ Paw at the ground | 95 | 98.96 |
| ▪ Rolling | 74 | 77.08 |
| Abdominal distention | | |
| ▪ Absent | 83 | 86.46 |
| ▪ Present | 13 | 13.54 |
| Dehydration | | |
| ▪ Mild | 31 | 32.29 |
| ▪ Moderate | 52 | 54.17 |
| ▪ Severe | 13 | 13.54 |
| Intestinal movement | | |
| ▪ Absent | 19 | 19.79 |
| ▪ Constipation | 56 | 58.33 |
| ▪ Diarrhea | 21 | 21.88 |
| Intestinal sound | | |
| ▪ Absent | 54 | 56.25 |
| ▪ Present | 42 | 43.75 |
| Appetite | | |
| ▪ Off food | 49 | 51.04 |
| ▪ Poor | 34 | 35.42 |
| ▪ Good | 13 | 13.54 |
| Heart rate | | |
| ▪ <80 beat/min | 41 | 42.71 |
| ▪ >80 beat/min | 55 | 57.29 |
| Profuse sweating | 95 | 98.96 |
| Frequent urination | 82 | 85.42 |
| Congested mucous membrane | 89 | 92.71 |
| Elevated body temperature | 81 | 84.38 |

## Results
### Clinical findings and risk factors

During the study period, 115 horses were examined and of these, 96 (83.48%) were diagnosed with colic. The colic diagnosis was based on the following typical clinical signs: abdominal pain, abdominal distention, dehydration, intestinal movement, intestinal sound, sense of appetite, heart rate per minute, profuse sweating, frequent urination, congested mucous membrane, and elevated body temperature (Table 1).

At the initial examination in the present study, all suspected horses were not classified based on the colic severity. Furthermore, according to the 96 questionnaires from colic horses and 19 questionnaires from normal horses, colic was associated with gender ($p < 0.021$; OR = 0.317), breed ($p < 0.000$; OR = n/a), wheat bran feeding ($p < 0.015$; OR = 0.180), concentrate feeding ($p < 0.003$; OR = n/a), anthelmintics administration ($p < 0.000$; OR = n/a), gastrointestinal parasites ($p < 0.000$; OR = n/a), dental diseases ($p < 0.024$; OR = n/a), previous exposure to colic ($p < 0.000$; OR = 32.250), body condition score ($p < 0.000$; OR = 0.022), and access to water per day ($p < 0.000$; OR = n/a) (Table 2).

In addition, based on RR score, an increased risk for colic was found for male horse (RR = 2.021), those administered wheat bran feeding (RR = 1.481), >5 kg of concentrate feeding (RR = 1.500), not performed anthelmintic administration (RR = 4.571), present of gastrointestinal parasites (RR = 4.364), present of dental diseases (RR = 1.280), colic recurrence (RR = 7.579), and poor body condition score (RR = 5.726) (Table 2).

### Hematological profile, serum cortisol, and plasma catecholamine evaluation

Comparative results regarding the hematological profile of the normal and horse with colic during pre and 14 days post-treatment are presented in Table 3. In general, at 14 days post-treatment, the horses with colic improved significantly compared to pre-treatment according to blood parameters [TRBCs ($p < 0.001$), HB ($p < 0.001$), PCV ($p < 0.001$), MCV ($p < 0.001$), MCH ($p < 0.001$), MCHC ($p < 0.01$), neutrophil ($p < 0.001$), basophil ($p < 0.001$), monocyte ($p < 0.01$), and lymphocyte ($p < 0.001$)], clotting factors [Plt ($p < 0.001$), MPV ($p < 0.001$), PDW ($p < 0.001$), CT ($p < 0.001$), Prt ($p < 0.001$), Appt ($p < 0.001$), and fibrinogen ($p < 0.001$)], and other biochemical profiles [total protein ($p < 0.001$), albumin ($p < 0.001$), globulin ($p < 0.001$), and calcium ($p < 0.001$)]. The result was also revealed that all variables at 14 days post-treatment improved gradually similar to the normal group (Table 3). The reference range[22] was also provided to add dynamic information on clinical evaluation during treatment of the colic episodes.

As shown in Figure 2, serum cortisol ($p < 0.05$) and plasma epinephrine ($p < 0.01$) concentrations were decreased significantly at 14 days post-treatment compared to pre-treatment. However, plasma norepinephrine showed no significant difference ($p > 0.05$) at whole colic episodes. These findings indicate amelioration of the degree of sympathetic activation in horses associated with colic at 14 days post-treatment.

## Discussion

The current study presents the incidence and risk factors for the recurrence of colic in horses. Colic is still the main concern in horse management worldwide. In this study, the incidence of colic was higher in 71 male horses than 25 female horses (Table 2). The incidence in Sumba horse was higher than other breeds, where 89 cases (92.7%) with colic were Sumba, five cases of Thoroughbred and two cases of mixed breeds (Table 2). Male horses are widely used for traditional transportation. Meanwhile, female horses are only kept in the breeding area and are rarely used as working horses.[23] Sumba horses originated from the Sumbawa island and its known for having high movement and mobility. Sumba horses are commonly used as traditional transportation due to their ability to explore and survive in tropical environments.[24]

The administration of wheat bran (58 cases) and >5 kg of concentrate feeding (64 cases) can increase the risk of colic especially if it does not appropriate the normal horse feed ratio (Table 2). The procedure for feeding foals and five year old horses was equal. Horses in the growth period require quality raw materials for feeding, including protein with balanced amino acids for muscle development, contributing energy to the metabolic processes. Feed formula ratio consists of 60-70% concentrate.[25] Weaning horses are able to consume up to 3.5 kg of concentrate with 14-16% crude protein. The ratio of green fodder and concentrate is 30: 70 based on the dry matter. When a year old, horses need 13.5% crude protein with a green fodder ratio and a 40:60 concentrate of dry matter in the total ratio. Crude protein intake decreases to 11.5% at 18 months with a ratio of green fodder and 55:45 concentrate of dry matter ratio. Meanwhile, at the age of 24 months, crude protein needs reach up to 10% with a ratio of green fodder and 65:35 concentrate based on dry matter ratio.[26]

In our study, 75 cases of colic horses without anthelmintic administration and 74 horses with administration were found to have gastrointestinal parasitic infections (Table 2). Worm infections can occur through a single or mixed infection. Different types of infections occur in each animal due to differences in immunity to the worm infection.[27] Deworming leads a major role in controlling mixed infections. Horses infected with more than one type of worm may have a weak

**Table 2. Distribution of risk factors associated with colic in Delman horses.**

| Variables | Normal (*n* = 19) | Colic (*n* = 96) | SE | p-value | OR | RR |
|---|---|---|---|---|---|---|
| Age | | | | | | |
| ■ <5 years | 6 | 17 | 0.065 | 0.222 | n/a | n/a |
| ■ 5-10 years | 10 | 48 | | | | n/a |
| ■ >10 years | 3 | 31 | | | | n/a |
| Season | | | | | | |
| ■ Winter | 9 | 53 | 0.093 | 0.531 | 0.730 | 1.175 |
| ■ Summer | 10 | 43 | | | | 0.858 |
| Gender | | | | | | |
| ■ Male | 9 | 71 | 0.043 | 0.021* | 0.317 | 2.021 |
| ■ Female | 10 | 25 | | | | 0.640 |
| Breed | | | | | | |
| ■ Sumba | 9 | 89 | 0.041 | 0.000* | n/a | n/a |
| ■ Thoroughbred | 9 | 5 | | | | n/a |
| ■ Mixed | 1 | 2 | | | | n/a |
| Wheat bran feeding | | | | | | |
| ■ No | 2 | 38 | 0.045 | 0.015* | 0.180 | 0.266 |
| ■ Yes | 17 | 58 | | | | 1.481 |
| Feeding on green fodders | | | | | | |
| ■ No | 16 | 89 | 0.026 | 0.230 | 0.419 | 2.165 |
| ■ Yes | 3 | 7 | | | | 0.908 |
| Concentrate feeding | | | | | | |
| ■ <5 kg | 0 | 32 | 0.042 | 0.003* | n/a | n/a |
| ■ >5 kg | 19 | 64 | | | | 1.500 |
| Anthelmintics administration | | | | | | |
| ■ No | 0 | 75 | 0.045 | 0.000* | n/a | 4.571 |
| ■ Yes | 19 | 21 | | | | n/a |
| Gastrointestinal parasites | | | | | | |
| ■ No | 19 | 22 | 0.045 | 0.000* | n/a | n/a |
| ■ Yes | 0 | 74 | | | | 4.364 |
| Dental diseases | | | | | | |
| ■ Absent | 19 | 21 | 0.036 | 0.024* | n/a | n/a |
| ■ Present | 0 | 75 | | | | 1.280 |
| Previous exposure to colic | | | | | | |
| ■ Absent | 15 | 10 | 0.039 | 0.000* | 32.250 | 0.235 |
| ■ Present | 4 | 86 | | | | 7.579 |
| Body condition score | | | | | | |
| ■ Poor | 2 | 81 | 0.042 | 0.000* | 0.022 | 5.726 |
| ■ Good | 17 | 15 | | | | 0.125 |
| Water source | | | | | | |
| ■ Soft water | 6 | 38 | 0.046 | 0.512 | 0.704 | 0.798 |
| ■ Well | 13 | 58 | | | | 1.132 |

**Table 2.** *Continued*

| Variables | Normal (*n* = 19) | Colic (*n* = 96) | SE | p-value | OR | RR |
|---|---|---|---|---|---|---|
| Access to water/day | | | | | | |
| ▪ Once | 0 | 25 | 0.078 | 0.000* | n/a | n/a |
| ▪ Twice | 1 | 60 | | | | n/a |
| ▪ Three times | 12 | 8 | | | | n/a |
| ▪ More than three times | 6 | 3 | | | | n/a |
| Musculoskeletal diseases | | | | | | |
| ▪ Absent | 19 | 88 | 0.024 | 0.192 | n/a | 1.091 |
| ▪ Present | 0 | 8 | | | | n/a |
| Bad vices | | | | | | |
| ▪ Absent | 15 | 85 | 0.032 | 0.257 | 0.485 | 1.837 |
| ▪ Present | 4 | 11 | | | | 0.892 |

Abbreviations: *n* = number of samples; SE = standard error; OR = odds ratio; RR = relative risk; n/a = not applicable.

**Table 3. Hematology profile and blood biochemistry at pre and 14 days post-treatment in Delman horses with colic.**

| Parameters | Ref[22] | Normal (*n* = 19) | Colic (*n* = 96) | |
|---|---|---|---|---|
| | | | **Pre-treatment** | **14 d post-treatment** |
| TRBCs ($\times 10^6$) | 6.5-12.5 | $10.7 \pm 0.08^{a,***}$ | $9.6 \pm 0.23^b$ | $10.7 \pm 0.12^{a,***}$ |
| HB (mg dL$^{-1}$) | 11-19 | $13.4 \pm 0.08^{a,***}$ | $14.4 \pm 0.24^c$ | $13.6 \pm 0.06^{b,***}$ |
| PCV (%) | 32.0-37.4 | $31.1 \pm 0.59^{b,***}$ | $52.7 \pm 0.58^c$ | $27.8 \pm 0.45^{a,***}$ |
| MCV (fl) | 36-52 | $39.2 \pm 0.08^{a,***}$ | $43.7 \pm 0.59^b$ | $39.1 \pm 0.13^{a,***}$ |
| MCH (pg) | 12.3-19.7 | $15.2 \pm 0.14^{a,***}$ | $18.4 \pm 0.09^c$ | $15.6 \pm 0.19^{b,***}$ |
| MCHC (g dL$^{-1}$) | 34-39 | $335.5 \pm 0.91^{ab}$ | $334.9 \pm 0.63^b$ | $336.3 \pm 0.12^{a,**}$ |
| Eosinophil ($\times 10^3$ µL$^{-1}$) | 0.0-0.9 | $0.8 \pm 0.08^{a,*}$ | $0.9 \pm 0.04^b$ | $0.8 \pm 0.02^{ab}$ |
| Neutrophil ($\times 10^3$ µL$^{-1}$) | 2.7-6.7 | $6.4 \pm 0.30^{a,***}$ | $8.9 \pm 0.26^c$ | $7.1 \pm 0.08^{b,***}$ |
| Basophil ($\times 10^3$ µL$^{-1}$) | 0.0-0.2 | $0.1 \pm 0.01^{a,***}$ | $0.3 \pm 0.02^b$ | $0.1 \pm 0.01^{a,***}$ |
| Monocyte ($\times 10^3$ µL$^{-1}$) | 0.0-0.8 | $0.7 \pm 0.06^{ab}$ | $0.7 \pm 0.07^b$ | $0.6 \pm 0.01^{a,**}$ |
| Lymphocyte ($\times 10^3$ µL$^{-1}$) | 1.5-5.5 | $5.3 \pm 0.18^{a,***}$ | $3.4 \pm 0.09^b$ | $5.2 \pm 0.07^{a,***}$ |
| Plt ($\times 1$) | 100-600 | $568.4 \pm 5.05^{b,***}$ | $256.9 \pm 1.21^c$ | $590.6 \pm 3.94^{a,***}$ |
| MPV (fl) | 9.0-10.2 | $8.9 \pm 0.11^{a,***}$ | $13.3 \pm 0.81^b$ | $8.4 \pm 0.10^{a,***}$ |
| PDW (%) | 14.8-18.4 | $15.7 \pm 0.34^{b,***}$ | $19.5 \pm 1.19^c$ | $14.4 \pm 0.12^{a,***}$ |
| CT (min) | 2.9-3.9 | $3.2 \pm 0.05^{a,***}$ | $5.4 \pm 0.08^b$ | $3.2 \pm 0.04^{a,***}$ |
| Prt (sec) | 9.5-12.1 | $10.4 \pm 0.29^{a,***}$ | $24.9 \pm 0.42^c$ | $11.1 \pm 0.46^{b,***}$ |
| Appt (sec) | 45.7-55.1 | $51.0 \pm 0.59^{b,***}$ | $68.1 \pm 0.73^c$ | $48.8 \pm 0.34^{a,***}$ |
| Fibrinogen (mg 100 mL$^{-1}$) | 100-400 | $364.6 \pm 5.31^{b,***}$ | $254.8 \pm 10.43^c$ | $382.6 \pm 0.87^{a,***}$ |
| Total Protein (gm 100 mL$^{-1}$) | n/a | $6.1 \pm 0.04^{a,***}$ | $7.3 \pm 0.17^b$ | $6.2 \pm 0.05^{a,***}$ |
| Albumin (gm 100 mL$^{-1}$) | n/a | $2.9 \pm 0.05^{b,***}$ | $3.8 \pm 0.04^c$ | $2.7 \pm 0.04^{a,***}$ |
| Globulin (gm 100 mL$^{-1}$) | n/a | $3.3 \pm 0.05^{b,**}$ | $3.5 \pm 0.05^c$ | $2.9 \pm 0.09^{a,***}$ |
| Calcium (gm 100 mL$^{-1}$) | n/a | $11.4 \pm 0.12^{a,***}$ | $7.8 \pm 0.12^c$ | $8.7 \pm 0.05^{b,***}$ |

Values are expressed in mean $\pm$ SD. One-way ANOVA was carried out followed by post hoc Tukey multiple comparisons test. Values are represented statistically when $^{a,b,c,}$ in comparison with normal group; *p < 0.05, **p < 0.01, ***p < 0.001, in comparison with pre-treatment group. TRBCs = total erythrocyte count; HB = hemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean cell hemoglobin concentration; Plt = platelets count; MPV = mean platelets volume; PDW = platelets distribution width; CT = clotting time; Prt = prothrombine time; Appt = activated partial thromboplastine time; n/a = not applicable (reference range not established).
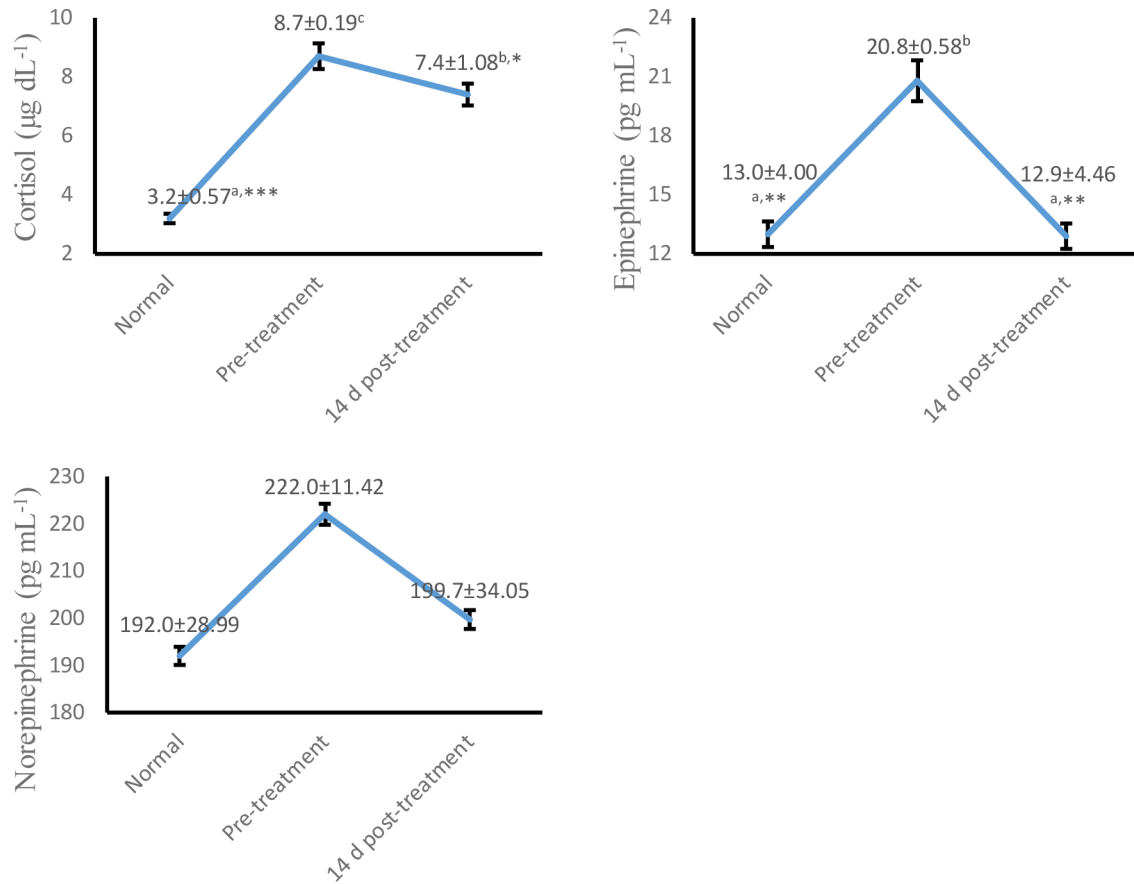
**Figure 2. Trend concentrations of serum cortisol, plasma epinephrine and norepinephrine at pre and 14 d post-treatment in Delman horses with colic.** Values are expressed in mean ± SD. One-way ANOVA was carried out followed by post hoc Tukey multiple comparisons test. Values are represented statistically when [a,b,c,] in comparison with normal group; * p < 0.05, ** p < 0.01, *** p < 0.001, in comparison with pre-treatment group.

immune condition. Body endurance can be influenced by various factors such as nutrient intake, enclosure conditions, weather, anthelmintic administration, and the development factor of larval life in grasslands, i.e. climatic conditions, soil type, geographical location, and types of plants. High rainfall can also increase soil moisture. Humid conditions support infective larvae to survive.[28]

There were 21 cases of colic horse with dental disease in our sample (Table 2). Special treatment is needed due to the condition of the horses' teeth, which are not suitable for chewing hard grass. Low lignin grass will be easily digested mechanically to prevent the risk of colic.[29] Lack of access to water per day can increase the risk of colic. In this study, horses received water access (per day) once in 25 cases, twice for 60 cases, three times for eight cases and more than three times for three cases (Table 2). Horses must regularly get access to water to prevent colic at least eight liters every 6 hours.[30]

In this study, 86 cases of horses had a history of colic, with 81 cases also having poor body scoring conditions (Table 2). Poor body score condition and previous history of colic are correlated with recurrent colic cases. The aspects of the body score are influenced by daily nutrition, horse training, movement intensity, and anthelmintic administration. Horses who get regular dental and nail checks can improve animal behaviour. Horses with a history of colic should receive special treatment after feeding. Mild activity and exercise after feeding can reduce the risk of horse colic.[31]

Cases of colic in horses can be associated as a cause of blood coagulation disorders.[32] It is triggered by an increase in blood concentration and a simultaneous decrease in coagulation factors.[33] Common symptoms of coagulopathy can be associated in colicky horses with gastrointestinal lesions, ischemia, inflammation, and peritonitis, which also depend on the severity of intravascular coagulation.[34] In the current study, the decrease in total platelet and fibrinogen at pre-treatment increased after 14 days post-treatment. In addition, there was an increase in coagulation time, activated partial

thromboplastine time and prothrombine time at pre-treatment followed by a decrease after 14 days post-treatment. Thrombocytopenia, hypofibrinogenemia and decreased blood clotting time are reflected in the length of capillary refill time and petechial bleeding. Moreover, if the disorder is followed by inflammation as an activator of platelets which is released as an endogenous mediator.[35]

Coagulation is disseminated in overlapping stages i.e. initiation, amplification, and propagation.[36] The initiation stage is characterized in cells that express tissue factor, which forms a complex with factor VIIa and activates factors IXa and Xa. Factor Xa binds to factor Va on the cell surface and produces a small amount of thrombin. Factor Xa is immediately inhibited so that it cannot move to other cells.[37] The amplification stage is characterized after exposure to colic, where platelets are released from the blood vessels, resulting in platelet attachment to the thrombin produced at the initiation stage. Thrombin activates platelets followed by surface changes and the release of partially active factor V. Thrombin also activates cofactors V and VIII, and activates factor XI to factor XIa.[38] The propagation step is performed on the surface of activated platelets. At this stage factor IXa binds to VIIIa, followed by an increase in the number of factor IXa as a result of platelet binding to factor XIa. The factor IXa/VIIIa complex activates factor Xa on platelets and immediately binds to factor Va thereby converting prothrombin to thrombin, followed by the thrombin complex converting fibrinogen to fibrin.[39] In another study, it has been reported that prolonged activated partial thromboplastine time and prothrombine time are the most frequently observed abnormalities in the coagulation profile of the colicky horse.[40]

This study showed an increase in total protein, albumin, and globulin in the pre-treatment period. Several colicky horses with symptoms of dehydration, hemoconcentration, and acute loss of consciousness showed similar results with an increase in total protein.[41] In addition, decreased calcium levels are associated with tremor and muscle paralysis during episodes of colic.[42] Calcium also plays a role in the coagulation stage, in particular being positively correlated with fibrin activity.[43] The duration of the disease is related to the increase in plasma fibrinogen, serum albumin concentration and WBC count. Plasma fibrinogen concentrations and total WBC were significantly increased when evaluated as a single variable. Serum amyloid A (SAA) concentration showed a better improvisation as a marker for the duration of colic. This is due to the long interval between pre and post colic investigations and the support of early symptom information from horse owners.[44]

Meanwhile, serum cortisol and plasma epinephrine levels were detected to be elevated in colic episodes in our study. This activity is predicted to be associated with an increase in plasma lactate concentration, blood pH, heart rate, and hemodynamic disorders.[45] The severity of septic shock following lactic acidosis has the potential to induce acute endotoxemia and deteriorating the condition of the horse during episodes of colic. High levels of plasma epinephrine can carry a risk of death.[46] In a previous study, horses did not survive if the plasma epinephrine concentration was <4 pg/mL at initial examination, whereas some colicky horses survived at a concentration of 222 pg/mL.[47] However, no definitive references have been reported for survivor horses in episodes of colic. The hemostatic response during tissue hypoxia and a decrease in mean arterial pressure are indicated to be the cause of the high plasma epinephrine concentration.[48] The increase in serum cortisol concentration during episodes of colic accumulates as a secondary result of increased secretion of the adrenal glands.[49] An indication of stress during colic episodes may be the common symptom of high serum cortisol concentrations, as shown in this study. In addition to our identified risk factors, the results of this study revealed improvements in hematological profiles, serum cortisol, and plasma epinephrine. We also emphasized information on the probability of coagulopathy and hemodynamic disorders during a colic episode.

## Conclusion

In conclusion, the main risk factors for colic in Delman horses in Gresik are gender, breed, wheat bran feeding, concentrate feeding, anthelmintics administration, gastrointestinal parasites, dental diseases, previous exposure to colic, body condition score, and access to water per day. Meanwhile, evidence of improved hematological profile, serum cortisol and plasma epinephrine were observed at 14 days post-treatment for colic. The results of this evaluation proved the improvement of the horses' condition after therapy due to colic. This study may be used to inform future prospective studies investigating colic in working horse populations and to contribute effective preventative measures. In particular, it is necessary to evaluate the association of colic risk factors with working horses and gastric abnormalities.

## Data availability
### Underlying data
Figshare: Risk factor in horse with colic. https://doi.org/10.6084/m9.figshare.15148851.v1.[50]

This project contains the following underlying data:

- Risk factor in horse with colic.xlsx

  - **Sheet 1.** Raw data for physical examination

  - **Sheet 2.** Raw data for risk factor questionnaires

  - **Sheet 3.** Raw data for hematological analysis

## Reporting guidelines

Figshare: ARRIVE checklist for 'Risk factors and hematological profile associated with colic in Delman horses in Gresik, Indonesia'.

https://doi.org/10.6084/m9.figshare.16617511.[51]

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

## Acknowledgments

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# Open Peer Review

## Current Peer Review Status:

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**Version 2**

Reviewer Report 24 January 2022

https://doi.org/10.5256/f1000research.119273.r119244

**Muhammad Fiaz Qamar**
Department of Pathobiology, "University of Veterinary and Animal Sciences sub Campus College of Veterinary and Animal Sciences", Jhang, Pakistan
**Aziz ur Rehman**
Department of Pathobiology, "University of Veterinary and Animal Sciences sub Campus College of Veterinary and Animal Sciences", Jhang, Pakistan

The Research article has been updated and improved by the authors. All the comments have been addressed and answered. Now it is suitable for indexing.

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Molecular and diagnostic parasitology

**We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 18 January 2022

https://doi.org/10.5256/f1000research.119273.r119245

**Hamny Sofyan**
Laboratory of Anatomy, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

Generally, authors have been revised the manuscript according to reviewer's suggestions. The

discussion section has focused on the risk factors for the occurrence of colic in Delman horse. The relationship between the hematology profile and blood chemistry have been described in relation to the occurrence of colic. However, in some parts still need minor revision, although my decision is the manuscript was approved.

There is still reference that is found in the results section, especially in the subsection, "Hematology Profile, Serum Cortisol, and Plasma Cathecolamin Evaluation". The sentence is, "*the reference range[22] was also provided to add dynamic information on clinical evaluation during treatment of the colic episodes*". Please move this sentence to the discussion section.

Authors have been able to justify a non-stressful blood collection procedure in the revised manuscript. I think the statement "*a certificate ethical clearance is not necessary etc*" should be removed.

***Competing Interests:*** No competing interests were disclosed.

***Reviewer Expertise:*** Animal Anatomy and Physiology, immunohistochemistry

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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**Version 1**

Reviewer Report 03 December 2021

? **Muhammad Fiaz Qamar**
Department of Pathobiology, "University of Veterinary and Animal Sciences sub Campus College of Veterinary and Animal Sciences", Jhang, Pakistan
**Aziz ur Rehman**
Department of Pathobiology, "University of Veterinary and Animal Sciences sub Campus College of Veterinary and Animal Sciences", Jhang, Pakistan

I have reviewed the manuscript and found minor gaps in the manuscript. The manuscript is well-written and clear to understand. A small number of typos and grammatical errors are present in the manuscript.

Manuscript contains risk factors data associated with Colic in horses, some other risk factors can be added or addressed.

The introduction section only describes the colic and its prevalence, the introduction should also contain the risk factors already reported and hematological alterations reported by researchers. Similarly, the prevalence parameter is missing in the methods section.

The discussion section needs some improvements regarding objective and protein.

The conclusion section can be improved by adding missing links for future studies.

**Is the work clearly and accurately presented and does it cite the current literature?**
Yes

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Yes

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Molecular and diagnostic parasitology

**We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.**

Author Response 23 Dec 2021
**Muhammad Thohawi Elziyad Purnama**, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

Dear Prof. Fiaz.

I would like to thank you for your kind review of our manuscript.

Regarding your judgment on our manuscript, we have added to the risk factor information gap in previous studies and the association with colic-relevant disease. We have added additional information on total protein as part of the discussion because it relates to the duration of the examination during colic. In the conclusion section, we have emphasized

> further research and the most important risk factors to be evaluated during the colic period.
>
> All the best wishes.
> Thank you.
>
> **Competing Interests:** All authors declare no competing interest.

Reviewer Report 14 October 2021

https://doi.org/10.5256/f1000research.58877.r94978

? **Hamny Sofyan**
Laboratory of Anatomy, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

**General comments:**
This research analyzed the risk factors for colic in Delman horses in Gresik, Indonesia and showed the success of the treatment based on hematological and biochemical profile and also catecholamine hormone levels. The result of this study can be used to decrease the incidence of colic in Delman horses in Indonesia.

**Specific comments:**

**Title:**
Suggestion for title: Risk factors, hematological and biochemical profile associated with colic in Delman horses in Gresik, Indonesia

**Introduction**
Author should pay more attention in this section. Author needs to highlight the researchable problem with colic in Delman horses, especially in Indonesia. Add the information about incidence rate of colic is in horses in Indonesia (if data is available).

**Methods**
I think this study requires ethical clearance because there was a blood collection process and treatment was carried out in Delman horses with colic. Does your unit have ethics related to routine procedures performed on animals? If there is, I don't think ethical clearance is needed anymore.

**Results**
○ I found discussion statement in the result section, please revised it.

- ○ Suggestion: the title of Table 3: Hematology profile and blood biochemistry at pre and 14 days post-treatment in Delman horses with colic.

**Discussion**
- ○ I think the discussion should be focus on the risk factors that significantly influence the occurrence of colic in Delman horses according to the results of this study and then compare with previous study.

- ○ In this section, the relationship between hematology and chemical components of blood with the incidence of colic in Delman horse have not been clearly.

- ○ Why are working horse not included in the variables of risk factors?

**Conclusion**
- ○ "Meanwhile, evidence of improved hematological profile, serum cortisol and plasma epinephrine were observed at 14 days post-treatment for colic". What is the meaning of this statement? Does this mean that the colic treatment has been suitable?

- ○ Please, add the future improvements based on the research results in this section.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
I cannot comment. A qualified statistician is required.

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Animal Anatomy and Physiology, immunohistochemistry

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 23 Dec 2021

**Muhammad Thohawi Elziyad Purnama**, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

Dear Dr. Hamny

I would like to thank you for your kind review of our manuscript.
We have improved the title and Table 3 heading according to your suggestion. Regarding the introduction section, we only found a colic report based on a repository and not yet published properly.

We have attached the ethical review along with the document (ARRIVE) guidelines 2.0: author checklist.

We have also revised the results and discussion sections according to the findings of the investigated risk factors and compared them with previous study reports.

As emphasized in the conclusion section, this study showed improvement in the condition of horses after treatment. Further studies need to examine the association of working horse risk with colic which is a limitation in this study.

All the best wishes.
Thank you.

***Competing Interests:*** No competing interests were disclosed.

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