

Coagulation Profiles of Healthy Andalusian Donkeys are Different than Those of Healthy Horses

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Background: Coagulation disorders are frequently diagnosed, especially in hospitalized equidae, and result in increased morbidity and mortality. However, hemostatic reference intervals have not been established for donkeys yet.

Objectives: To determine whether the most common coagulation parameters used in equine practice are different between healthy donkeys and horses.

Animals: Thirty-eight healthy donkeys and 29 healthy horses.

Methods: Blood samples were collected to assess both coagulation and fibrinolytic systems by determination of platelet count, fibrinogen concentration, clotting times (prothrombin time [PT] and activated partial thromboplastin time [aPTT]), fibrin degradation products (FDP) and D-Dimer concentrations.

Results: PT and aPTT in donkeys were significantly ($P < .05$) shorter than those of horses. In contrast, FDP and D-Dimer concentrations were significantly ($P < .05$) higher in donkeys than in horses.

Conclusions and Clinical Importance: The coagulation parameters most commonly determined in equine practice are different in donkeys compared with horses. Thus, the use of normal reference ranges reported previously for healthy horses in donkeys might lead to a misdiagnosis of coagulopathy in healthy donkeys, and unnecessary treatments in sick donkeys. This is the first report of normal coagulation profile results in donkeys, and further studies are warranted to elucidate the physiological mechanisms of the differences observed between donkeys and horses.

Key words: Clotting times; D-Dimers; Donkeys; Equid; Fibrinogen degradation products.

Coagulation abnormalities may be diagnosed in many hospitalized equidae, especially in those under intensive care and monitoring, contributing to poor prognosis and increased mortality.¹ In donkeys, reference ranges for the most commonly determined coagulation parameters in equine practice have not been reported and consequently reference ranges from horses are used. However, the extrapolation of these reference ranges to donkeys can lead to the misdiagnosis of hemostatic disorders, and equine clinicians must be aware of differences among species. In view of the importance of an accurate diagnosis for hemostatic disorders, the establishment of reference intervals among different equidae species is compelling.

Many differences have been reported between horses and donkeys, such as the ones reported on anatomical, hematological, and biochemical variables.² Additional precautions must be taken when a donkey is under surgery, because even minor surgeries (eg, castration) may be complicated by a high risk of bleeding. Despite conjecture, no studies regarding this presumption have been reported yet. For this reason our hypothesis was that reference intervals for the most common coagulation

Abbreviations:

aPTT	activated partial thromboplastin time
FDP	fibrin degradation products
PT	prothrombin time

parameters in healthy donkeys may be different from those reported in horses. The aim of the present study was to compare the most frequently used coagulation tests in equine practice for the diagnosis of hemostatic disorders between healthy donkeys and horses.

Materials and Methods

Animals

Blood samples were collected from 38 healthy Andalusian donkeys living in a sanctuary and from 29 healthy Andalusian horses living on a breeding farm. Horses were housed in premises very similar to those where the donkeys were housed and had the same management as the donkeys. Donkeys and horses had free access to water and they were fed the same diet (hay, oats, and a vitamin-mineral supplement) for at least 2 months before blood sampling. Donkeys and horses were currently on deworming and vaccination programs, and neither donkeys nor horses had received any treatment for at least 2 months before blood sampling, and no mare or jennet was pregnant. Animals were considered healthy based on normal clinical history, physical examination findings, and normal results of a CBC, total protein concentration, and fibrinogen concentration. Neither donkeys nor horses were used for sport or work.

Sample Handling and Measurement

Blood samples were obtained by jugular venipuncture into tubes containing 3.8% buffered sodium citrate^a for analysis of platelet count, fibrinogen concentration, prothrombin time (PT), activated partial thromboplastin time (aPTT), and plasma D-Dimer concentration. Blood samples for fibrin degradation products (FDP) determination were simultaneously collected and transferred to

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Submitted January 13, 2011; Revised April 26, 2011; Accepted May 5, 2011.

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10.1111/j.1939-1676.2011.0748.x

tubes containing *Bothrops atrox* venom with soybean trypsin inhibitor.^b An additional 10 mL of blood were withdrawn for CBC and total protein concentration.

After blood collection, whole blood samples were chilled immediately on ice. Samples for plasma were centrifuged at $1,000 \times g$ for 15 minutes immediately at the farm and chilled on ice until measurements were performed, except those samples used for platelet count that were kept on ice. FDP tubes were processed according to the manufacturer's instructions.^c To avoid bias, both horse and donkey blood samples were simultaneously collected, processed, and blindly analyzed.

Platelet count was performed with an automated analyzer.^d Fibrinogen concentration was determined by the heat denaturation method.³ Clotting times were measured with an automated analyzer^e and commercial available kits.^{f,g} Serum FDP concentration was measured in 20 donkeys and 20 horses by a commercial semi-quantitative slide latex agglutination test^e validated previously for horses⁴ (detection limit, $>2 \mu\text{g/mL}$). Results of this test are reported as a concentration score of $<10 \mu\text{g/mL}$ (score 1), $10\text{--}40 \mu\text{g/mL}$ (score 2), or $>40 \mu\text{g/mL}$ (score 3). Plasma D-Dimer concentration was measured in 25 donkeys and 25 horses with a slide latex agglutination kit^h validated for horses⁵ (specificity, 95.9%; detection limit, $1\text{--}256 \mu\text{g/L}$). Results also were reported as a concentration score of $<1 \mu\text{g/mL}$ (score 1), $1\text{--}2 \mu\text{g/mL}$ (score 2), or $>2 \mu\text{g/mL}$ (score 3).

Data Analysis

Quantitative results are reported as means \pm standard deviation. Normality distribution was corroborated by Kolmogorov-Smirnov's test. An unpaired *t*-test was used to compare results between species, and Fisher's LSD was performed to study differences among >2 groups. A Kruskal-Wallis test was performed to identify any associations between categorical variables. Outlier values were determined by Huber's test, but they were not excluded from statistical analysis. $P < .05$ was considered significant. SPSS 15.0 software package was used.

Results

No differences were observed in PCV or total protein concentration between donkeys (PCV, $37.5 \pm 6.3\%$; total protein, $6.5 \pm 0.4 \text{ g/dL}$) and horses (PCV, $35.8 \pm 4.2\%$; total protein, $6.4 \pm 0.4 \text{ g/dL}$). No differences were seen in platelet count and fibrinogen concentration between donkeys and horses (Table 1). In donkeys, PT and aPTT were significantly ($P < .05$) shorter than in horses (Table 1). There was a slight overlapping in the 95% confidence interval for the upper and lower limits of the reference interval for PT results between both species (donkeys, $8.56\text{--}8.88$ seconds; horses, $8.76\text{--}9.43$ seconds; lower-upper limit, respectively). In contrast, the upper and lower limits for aPTT did not overlap (donkeys, $30.95\text{--}32.85$ seconds; horses, $43.25\text{--}45.82$ seconds; lower-upper limit, respectively).

On the other hand, FDP concentrations were significantly ($P < .05$) higher in donkeys than in horses. No donkeys had FDP concentrations $<10 \mu\text{g/mL}$, 10/20 donkeys had FDP concentrations between 10 and $40 \mu\text{g/mL}$, and the remaining 10 donkeys had FDP concentrations $>40 \mu\text{g/mL}$. In contrast, 7 horses had FDP concentrations $<10 \mu\text{g/mL}$, and the remaining horses (13/20) had FDP concentrations between 10 and $40 \mu\text{g/mL}$. Mirroring the FDP results, the D-Dimer score in

Table 1. Coagulation parameters from healthy donkeys and horses.

	Donkeys	Horses
Platelet count ($\times 10^3/\mu\text{L}$)	190.8 (105–458) 195 (143.5–268.0) n = 38	193.6 (112–376) 190.5 (148.5–213.0) n = 29
Fibrinogen (mg/dL)	462.1 (100–600) 400 (200–550) n = 38	389.6 (100–700) 400 (400–650) n = 29
PT (seconds)	8.71 (7.4–9.7) 8.7 (8.4–8.8) n = 38	9.09 (7.9–11.7) ^a 8.8 (8.3–9.2) n = 29
aPTT (seconds)	31.90 (23.2–37.7) 31.9 (30.1–34.2) n = 38	44.53 (37–51.9) ^b 44.7 (43.1–46.3) n = 29

Data are expressed as mean and range, below the median and 25th–75th percentiles, and number of samples for each test. All parameters were normally distributed.

PT, prothrombin time; aPTT, activated partial thromboplastin time.

Statistically significant when compared with donkeys:

^a $P < .05$,

^b $P < .01$.

donkeys was significantly ($P < .01$) greater than in horses. In donkeys, except for 2 animals that had a D-Dimer score of $1\text{--}2 \mu\text{g/mL}$, most of them (23/25) had a D-Dimer score $>2 \mu\text{g/mL}$. In contrast, 10/25 horses had a D-Dimer score $<1 \mu\text{g/mL}$, 9/25 horses ranged between 1 and $2 \mu\text{g/mL}$, and only 6/25 had a D-Dimer score $>2 \mu\text{g/mL}$. No overlap in the 95% confidence interval for upper and lower limits of the reference interval was observed for FDP (donkeys, $2.26\text{--}2.74$; horses, $1.49\text{--}2.01$; lower-upper limits, respectively) and D-Dimer scores (donkeys, $2.89\text{--}3.04$; horses, $1.51\text{--}2.17$; lower-upper limits, respectively).

When donkeys were grouped by sex (18 males, 20 females), no differences were found in any parameter. In order to study whether age affected the results, donkeys were grouped according to the frequencies of age (mean: 8.4 ± 5.1 years) in the following groups: Group 1: <5 years old ($n = 13$), Group 2: $5\text{--}10$ years old ($n = 12$), and Group 3: >10 years old ($n = 13$). The only statistical differences ($P < .05$) was found for FDP concentration, where donkeys of Group 2 had significantly ($P < .05$) lower FDP concentration than Group 1 and Group 3. When horses were grouped either by sex (13 males, 16 females) or age (6.1 ± 2.4 years), no differences were observed for any coagulation parameter.

Discussion

The aim of this study was to determine whether the hemostatic parameters commonly used to assess the coagulation and fibrinolytic systems in equine practice are different between healthy donkeys and horses. Our results showed that donkeys have shorter clotting times than horses, whereas FDP and D-Dimer concentrations were higher in donkeys than in horses. If clotting parameters from donkeys were evaluated using the reference

intervals reported for horses, sick donkeys with a hypo-coagulable state could be considered healthy.

This study is the first to report times and concentrations for hemostatic parameters in donkeys. PT was significantly shorter in donkeys compared with horses. The PT results in donkeys were within horse ranges according to other authors.^{6,7} aPTT also was significantly shorter in donkeys compared with horses, although falling into the reference ranges reported previously for horses.⁶ Both PT and aPTT tests have been validated previously in horses,⁸ and although they are widely accepted in veterinary medicine, it is possible that the existence of a species-dependent effect could have affected the results. Moreover, the coagulometer used for PT and aPTT determinations may not have recognized clot formation accurately in donkey blood, which could have influenced the results. In addition, aPTT is susceptible to calcium chloride concentration variations during the procedure, but all measurements were carried out using the same protocol in order to exclude this factor. Results of PT and aPTT in horses were within the reference ranges reported.^{9,10}

The most common coagulation abnormality is prolongation, normally as a consequence of coagulation factor deficiencies, primary or secondary to consumption, liver disease, the presence of inhibitors (such as anticoagulants), iron toxicosis, and infectious diseases (eg, equine viral arteritis, equine infectious disease).¹⁰ However, in this study clotting times were shorter, which might lead a clinician to inaccurately diagnose a hypercoagulable state, which rarely is diagnosed in equidae using the PT and aPTT. In our study, all of these underlying causes could be excluded because all animals were healthy and were not exercised. Another possible cause of the shorter times could be an increase in coagulation factor activity. In mares, a gradual increase in von Willebrand factor and factor VII:C occurs from midgestation to parturition,¹¹ changes in factor VII and IX activities or clotting times, however, were not found. Moreover, interspecies differences have been described. For example, rabbits have 50 times higher activity of factor V than do humans.¹² Whether donkeys have higher coagulation factor activity compared with horses remains unclear. In addition, as has been proven in human medicine, hemostatic tests may be influenced by plasma volume and hematocrit values. In this way, lower hematocrit values could result in a hypercoagulable state,¹³ whereas overhydration secondary to fluid therapy could lead dilutional coagulopathy.¹⁴ In our study, these effects can be excluded, because both donkeys and horses had similar PCVs and total plasma protein concentrations that were within the normal reference ranges, and fluids were not administered, excluding any dilutional effect.

Both FDP and D-Dimer concentrations were higher in donkeys than in horses. Despite the fact that both tests have been validated previously for horses,⁵ a species-dependent effect still could have influenced the results. Both FDP and D-Dimer concentrations were slightly higher than those reported in the veterinary literature, likely because of the lower specificity of the technique used compared with quantitative methods.^{1,15-17} Because

the values obtained for healthy donkeys were out of the reference ranges described for healthy horses, these results could be classified as abnormal according to the literature. In addition, the differences observed in FDP and D-Dimer concentrations between the 2 species could not be attributed to the use of the animal, because no animal was used either for sport or work purposes. Additional studies to elucidate reference ranges for D-Dimer and FDP concentrations should be carried out, because both reference ranges were higher compared with those reported in the literature for horses. Differences observed between both species may be attributed exclusively to interspecies characteristics, because other many differences have been reported between donkeys and horses.

Preliminary studies have demonstrated that newborn foals have prolonged clotting times, lower fibrinogen concentration, and higher FDP concentrations compared with adult horses.¹⁶ Our results showed unexpected variation among the 3 different age groups of donkeys for FDP concentration; the cause for this variation was not determined and should be investigated in future studies. A possible explanation could be the low number of animals included in each group, because differences were not found in other coagulation parameters.

In view of the findings reached in this study, the differences observed between donkeys and horses could be explained by 2 possible hypotheses: (a) higher fibronectin concentration or higher concentrations of coagulation factors; or (b) a decrease in coagulation system inhibitory activity because of low protein C concentration or antithrombin activity. The facility of clot formation in donkeys would lead to activation of the fibrinolytic system, increasing FDP and D-Dimer concentrations, as was observed in our results, and likely of other parameters such as tissue plasminogen activator or plasminogen concentration. The widely accepted assumption that donkeys undergoing minor surgical procedures bleed more than horses should be questioned, considering the results of this study. A more prolonged time of bleeding in donkeys could be because of lower platelet reactivity. Therefore, platelet function should be tested by aggregometry and PFA 100 techniques. In addition, more studies are necessary to investigate the concentrations of each parameter associated with the coagulation system in donkeys, especially clotting factor concentrations, concentration and activity of the antithrombin and plasminogen activator, or plasminogen concentrations.

One limitation of this study was the low number of samples. The 95% confidence intervals for the upper and lower limits of the reference intervals showed, however, that the results did not overlap. These findings reinforce the conclusion that the coagulation parameters between species truly are different. Another limitation is that only Andalusian donkeys and Andalusian horses were included in this study. Although no breed-related differences have been demonstrated for hemostatic parameters in the veterinary literature yet, it cannot be excluded that Andalusian donkeys are closer to other horses breeds such as Thoroughbred or Standardbred. Because no other donkey breeds were included in this

study, these results should not be extrapolated to all donkey breeds.

In conclusion, our results show that hemostatic parameters in healthy donkeys are different from those reported for horses, and donkeys have shorter clotting times than horses and increased D-Dimer and FDP concentrations. Additional studies are necessary to determine the underlying mechanisms of these differences and whether these coagulation profiles are reliable diagnostic tests in clinically ill donkeys.

Footnotes

^a Sodium citrate tubes, Aquisel S.L., Barcelona, Spain

^b FDP tubes, BD Vacutainer Systems, Plymouth, UK

^c Thrombo-Wellcotest, Remel, Kansas City, KS

^d Sysmex F-820, Sysmex Corporation, Kobe, Japan

^e Amelung Coagulometer KC1A, Amelung GMBH, Lemgo, Germany

^f Neoplastin Plus, Roche Diagnostic, Madrid, Spain

^g PTT reagent, Roche Diagnostic

^h D-Dimer, Remel

Acknowledgment

This work was supported with funding from Plan Andaluz de Investigacion (CTS-661 group) from Consejería de Salud de la Junta de Andalucía. The authors thank Dr Manuel Ruiz, Angela Pardo, and Susana Sevilla for their help during sample collection, and Pascual Rovira and the farm "El Diabolo" for providing the animals.

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