SERUM AMYLOID A CONCENTRATIONS IN HORSES WITH ACUTE EQUINE PIROPLASMOSIS CAUSED BY BABESIA CABALLI OR THEILERIA EQUI

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Serum amyloid A concentrations in horses with acute equine piroplasmosis caused by Babesia *caballi* or Theileria *equi.*

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Running head: Serum amyloid A in horses with acute piroplasmosis

Abstract:

Equine piroplasmosis is a widespread tick-borne disease caused by Babesia *caballi* and Theileria *equi.* Clinical signs are non-specific and include fever, inappetence, icteric mucous membranes and lethargy; typical laboratory changes are hemolytic anemia and thrombocytopenia. Affected horses may become chronic carriers, which sometimes lead to unnecessary treatment in asymptomatic cases. Serum amyloid A (SAA) is a major equine acute-phase protein and a sensitive biomarker of inflammation. Well studied in bacterial and inflammatory conditions, its value in equine piroplasmosis remains unclear. Here, we investigated SAA concentrations in horses acutely infected with Babesia *caballi* or Theileria *equi* and compared to healthy horses. We included 25 horses: 10 healthy controls, 6 Babesia *caballi*-positive horses and 9 Theileria *equi*-positive horses. SAA concentrations were measured using a point-of-care immunoassay, and infection status (Babesia / Theileria) was determined using a loop-mediated isothermal amplification assay. We also performed ancillary diagnostic tests to exclude concurrent diseases. We found that SAA concentrations were significantly higher in horses positive for Babesia ($p = 0.001$) and Theileria ($p = 0.001$) compared to controls, with a respective increase of approximately 2312 µg/L (95% CI: 2234.0 to 2389.6 µg/L; $p < 0.001$) and 2024 µg/L (95% CI: 1955.2 to 2093.6 µg/L; $p < 0.001$) relative to controls. SAA value was not significantly different between Theileria and Babesia status. These results reflect the systemic inflammatory response associated with acute equine piroplasmosis; although nonspecific, SAA may represent a rapid and useful adjunctive marker for the diagnosis and monitoring of these cases.

Keywords: Babesia *caballi,* Horses, Piroplasmosis, Serum amyloid A, Theileria *equi*

Introduction:

Equine piroplasmosis is a tick-borne infectious disease caused by two hemoprotozoan parasites, Babesia *caballi* and Theileria *equi.*[17] This disease often results in severe systemic inflammation and illness. During the acute phase of infection, common clinical signs include fever, lethargy, petechiae, edema, pale mucous membranes, icterus, and pigmenturia. Hematologic findings, such as hemolytic anemia and thrombocytopenia, are very common in affected horses.[17]

Serum amyloid A (SAA) is a major acute-phase protein in horses, released in response to systemic inflammation. It is widely used as a biomarker for detecting inflammation in equine medicine.[15] In healthy horses, concentrations are very low or undetectable (< 100 mg/L), but they can increase more than 100-fold in response to acute inflammatory stimuli and tissue injury.[10-15] SAA levels typically begin to rise within 6–12 hours of an inflammatory event, peak at approximately 48 hours, and return to baseline within 5–7 days.**Erreur ! Source du renvoi introuvable.**-[10-15] While SAA concentrations have been extensively studied in various equine inflammatory diseases, after searching Google, PubMed, Web of science, and Scopus, no study has specifically evaluated SAA concentrations in naturally occurring equine piroplasmosis caused by Babesia *caballi* and Theileria *equi.*

Given that Babesia *caballi* and Theileria *equi* infections cause significant systemic inflammation, we decided to determine SAA concentrations in cases of acute infection with Babesia *caballi* and Theileria *equi*. We hypothesized that SAA concentrations would significantly increase during acute Babesia and Theileria associated infections, reflecting the severity of inflammation.

Materials and Methods:

Study Design and Population:

This study was conducted as a prospective cross-sectional observational study. Cases were enrolled consecutively over a 12-month period in order to obtain a sufficient number of horses diagnosed with acute equine piroplasmosis and suitable control horses.

The piroplasmosis group included horses or ponies older than one year of age that presented with clinical signs suggestive of equine piroplasmosis and tested positive for Babesia *caballi* and/or Theileria *equi* using a loop-mediated isothermal amplification (LAMP) assay (Enalees, France). Inclusion criteria required at least one of the following clinical signs: fever (>38.5°C), lethargy, or dysorexia. Additional clinical findings, including edema and icterus, were also recorded.

All horses in the piroplasmosis group underwent blood analysis including hematology and serum biochemistry, together with complete thoracic and abdominal ultrasonographic examinations to rule out any concurrentdiseases. Horses were also tested for Anaplasma *phagocytophilum* using the same LAMP platform (Enalees, France).

The control group included clinically healthy horses or ponies older than one year of age admitted for elective surgical or medical procedures. Eligibility criteria required normal clinical examination findings, normal hematology and serum biochemistry results, and negative LAMP assay results for Babesia *caballi,* Theileria *equi*, and Anaplasma *phagocytophilum*. Historical data regarding previous carrier status, prior exposure, or duration of clinical signs were not consistently available.

For statistical analysis, the piroplasmosis group is further divided into two subgroups: a Babesia *caballi*–positive group, including horses positive for Babesia *caballi* and negative for Theileria *equi*, and a Theileria *equi*–positive group, includinghorses positive for Theileria *equi* and negative for Babesia *caballi*.

Horses in the control and the piroplasmosis group did not receive antibiotics, anti-inflammatory drugs, or imidocarb dipropionate within the previous month. All blood samples were collected at admission, prior to administration of any medication.

Sample Collection and Analysis:

For both groups, blood samples were collected into EDTA and heparinized tubes and analyzed on the day of admission. All samples used in this study were obtained as part of routine clinical diagnostic, pre-anesthetic or medical procedures performed at hospital. No additional sampling was performed for research purposes, and no horses were enrolled specifically for this study.

Serum amyloid A (SAA) concentrations were measured using a point-of-care immunoassay with the EQ-1 quantitative reader (StableLab; Epona Biotech, Ireland) using whole blood collected in heparinized tubes, according to the manufacturer's instructions.

Detection of Babesia *caballi* and Theileria *equi* was performed using EDTA blood samples with the Epona® molecular diagnostic system (Enalees, France), using the EP-TBA assay based on LAMP technology. The LAMP method amplifies target DNA at a constant temperature (65°C) using a strand-displacing DNA polymerase. Each sample was processed according to the manufacturer's instructions. Amplification and detection were performed automatically by the Enalees reader, and results were available within approximately 40 minutes.

Statistical Analysis:

We performed all statistical analyses using R version 4.5.2 (2025-10-31).[13]

The Shapiro–Wilk test was used to assess normality of SAA concentrations and demonstrated a non-normal distribution for the Babesia *caballi*–positive group ($p = 0.008$) and the control group

($p < 0.001$). Consequently, non-parametric methods were used for group comparisons. Differences in SAA concentrations between groups were assessed using the Kruskal–Wallis test, followed by Dunn's post-hoc test with Holm correction for pairwise comparisons. A p-value < 0.05 was considered statistically significant (Figure 1).

Because residuals from an ordinary least-squares linear model of SAA concentration were not normally distributed (Shapiro–Wilk $p < 0.001$), and normality was not restored after log-transformation of the dependent variable (Shapiro–Wilk $p = 0.009$; non-normality also evident on QQ-plots), a robust linear regression was used instead. The model was fitted with the Huber M-estimator (MASS::rlm() in R), which down-weights atypical observations and is therefore less sensitive to non-normal residuals and extreme SAA values, while still allowing the coefficients to be interpreted on the original concentration scale (µg/L). Infection status was entered as a single three-level factor (LAMP-negative controls, Theileria *equi*-positive, Babesia *caballi*-positive), with the LAMP-negative control group as the reference category.

Results:

Study population:

Twenty-seven horses were initially enrolled in the study, including 11 horses in the control group and 16 horses in the piroplasmosis group.

One horse was co-infected with both Babesia *caballi* and Theileria *equi* and was excluded from the statistical analysis because of the limited number of mixed infections. In addition, one horse initially included in the control group was excluded after testing positive for Theileria *equi*, despite showing no clinical signs or hematological evidence of infection.

The final study population therefore consisted of 25 horses: 15 horses in the piroplasmosis group and 10 horses in the control group. Within the piroplasmosis group, 6 horses were included into the Babesia *caballi*–positive group and 9 horses were assigned to the Theileria *equi*–positive group. Comprehensive clinical and hematological data, including complete blood count (CBC) parameters, clinical signs, and relevant laboratory findings, were recorded for all horses (Tables 1 and 2).

The study population consisted of 11 mares and 15 geldings, ranging from 2 to 29 years (median: 13 years). A variety of breeds were represented, including Thoroughbreds, Warmbloods (Selle Français), Arabians, ponies, and Clydesdales.

Statistical analysis:

The Kruskal–Wallis test demonstrated a highly significant difference in SAA concentrations across the three groups ($\chi^2(2) = 17.635$, $p < 0.001$), confirming that at least one group differed significantly from the others.

Dunn's post-hoc test with Holm correction revealed that both Babesia *caballi*–positive group and Theileria *equi*–positive group had significantly higher SAA concentrations than the control group ($p = 0.001$ for both comparisons). No significant difference in SAA concentrations was found between the Babesia *caballi*–positive group ($p = 0.666$) and the Theileria *equi*–positive group (Figure 1).

In the robust linear regression model, SAA concentrations were, on average, 2311.8 µg/L higher in Babesia *caballi*-positive horses (95% CI: 2234.0 to 2389.6 µg/L; $p < 0.001$) and 2024.4 µg/L higher in Theileria *equi*-positive horses (95% CI: 1955.2 to 2093.6 µg/L; $p < 0.001$) than in LAMP-negative control horses (Table 3). These estimates are consistent with the Kruskal–Wallis

and Dunn post-hoc results and quantify the between-group differences in a manner robust to extreme values; they should nonetheless be interpreted cautiously given the small sample size.

Discussion:

We investigated the value of serum amyloid A (SAA) in horses acutely infected with Babesia *caballi* or Theileria *equi*. Our results demonstrated that SAA concentrations were significantly higher in infected horses than in control horses. The robust linear regression model confirmed that both Babesia *caballi* and Theileria *equi* infection were associated with markedly increased SAA concentrations, with mean increases of 2311.8 µg/L ($p < 0.001$) and 2024.4 µg/L ($p < 0.001$), respectively, relative to LAMP-negative controls. These findings suggest that SAA reflects the inflammatory status associated with acute equine piroplasmosis. Similar findings have been reported in buffaloes infected with Theileria *annulata*, in which SAA and haptoglobin concentrations increased in association with parasitemia.[5]

Serum amyloid A is a major acute-phase protein synthesized by hepatocytes in response to pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). In horses, SAA concentrations may increase up to 1000-fold within 12 to 24 hours following an inflammatory stimulus, making it one of the most sensitive markers of systemic inflammation.[10,18] The elevated SAA concentrations observed in the present study are consistent with a systemic inflammatory response triggered by piroplasm infection. Babesia *caballi* and Theileria *equi* are obligate intraerythrocytic protozoa that parasitize red blood cells and induce erythrocyte destruction and hemolytic anaemia.[7,17] The release of heme, hemoglobin, and erythrocyte debris during infection acts as a potent inflammatory stimulus, activating macrophages and promoting the production of pro-inflammatory cytokines, particularly IL-6.[6]

This cytokine cascade subsequently induces hepatic synthesis of acute-phase proteins including SAA, fibrinogen, and haptoglobin, reflecting the magnitude of the host inflammatory response.[9,12] Such mechanisms support the marked increase in SAA observed in horses infected with Babesia *caballi* and Theileria *equi*.

Although the robust model estimated SAA concentrations to be, on average, approximately 287 µg/L higher in Babesia *caballi*-positive than in Theileria *equi*-positive horses, this difference was not statistically significant (Dunn's post-hoc test, $p = 0.666$), likely owing to the small sample size. The more pronounced increase observed in Babesia *caballi* cases may nevertheless reflect the acute and hemolytic nature of this infection. Babesia *caballi* typically causes a shorter but often severe clinical course characterized by fever, icterus, anaemia, and hemoglobinuria, all of which may intensify the systemic inflammatory response.[7,17] In contrast, Theileria *equi* infections are more frequently chronic, persistent, or subclinical, and may be associated with lower parasitemia and a milder inflammatory response.[3] This biological difference in disease kinetics may explain the numerical, albeit non-significant, trend toward higher SAA concentrations in Babesia *caballi*–positive horses, suggesting that the acute-phase response may be proportional to the intensity and duration of parasitemia. Larger studies are needed to confirm whether this difference is clinically meaningful.

The results of this study highlight the potential of SAA measurement as a complementary diagnostic tool in equine piroplasmosis. Although SAA is a non-specific biomarker, its rapid response and wide dynamic range make it a valuable indicator of active inflammation.

In endemic regions, the prevalence of chronic carriers of Theileria *equi* is relatively high, and positive LAMP assay results frequently reflect persistent low-grade infection rather than

clinically active disease.[11,17] This was illustrated in the present study, where one horse initially considered for inclusion in the control group tested positive for Theileria *equi* despite showing no clinical abnormalities, normal hematological findings, and SAA concentration of 10 mg/L. Although this horse was excluded from the final analysis, its status highlights a common diagnostic challenge in endemic areas, where treatment decisions may be based solely on a positive LAMP assay result. In such cases, administration of imidocarb dipropionate may occur in horses without evidence of active disease. The combination of an increased SAA concentration together with compatible clinical findings and exclusion of alternative causes of inflammation may therefore help identify horses more likely to benefit from treatment. Conversely, normal SAA concentrations in clinically healthy horses testing positive for Theileria *equi* may support a carrier status rather than active inflammatory infection.

In France, equine piroplasmosis is considered endemic, with marked regional variations in prevalence. A recent epidemiological study reported that approximately 38.3% of horses are carriers, with prevalence ranging from 18.7% in northern regions to more than 56% in southern areas.[11] These findings are consistent with field observations indicating that a large proportion of horses are asymptomatic carriers, particularly for Theileria *equi*.[14] Importantly, Theileria *equi* is known to establish lifelong infections, whereas Babesia *caballi* may occasionally be cleared spontaneously, which may explain the higher prevalence of Theileria *equi* in endemic regions.[17] As a result, many horses may test positive without exhibiting clinical signs or hematological abnormalities. Such cases are well described in endemic regions, where positive test results often reflect chronic carrier status rather than active disease.[16] These observations highlight the limitations of relying solely on LAMP assay results to guide treatment decisions, as a positive

result does not necessarily indicate clinically relevant infection. In endemic countries such as France, this may lead to overdiagnosis and unnecessary treatment with imidocarb dipropionate.

In this context, the integration of clinical examination, hematological findings, and biomarkers of systemic inflammation such as SAA appears particularly relevant. Increased SAA concentrations, in the absence of alternative causes of inflammation, may help distinguish between inactive carrier states and active disease, thereby supporting more rational therapeutic decision-making in endemic settings. Elevated SAA values, in conjunction with compatible clinical signs such as fever, anemia, or icterus, may also guide clinicians toward a presumptive diagnosis of piroplasmosis pending confirmatory test results.

Although the point-of-care SAA analyzer offers rapid results, its analytical precision may be slightly lower than that of laboratory-based immunoturbidimetric assays, which could contribute to inter-sample variability. Persistently high SAA concentrations after treatment could reflect ongoing inflammation, treatment failure, or co-existing disease. This dynamic response makes SAA concentration a practical tool for monitoring recovery in equine medicine. Therefore, SAA should not be used as a stand-alone diagnostic tool, but rather as a complementary indicator interpreted alongside molecular testing and hematological findings.

While SAA has been extensively studied in bacterial diseases such as colitis, pleuropneumonia, and septicemia.[2, 9] its role in protozoal infections such as piroplasmosis has been largely unexplored. Thisstudy is the first to quantify SAA concentrations in horses with confirmed Babesia *caballi* and Theileria *equi* infections. Previous research has shown that SAA concentrations correlate closely with disease severity and respond more rapidly than other acute-phase markers such as fibrinogen or haptoglobin.[8] The magnitude of SAA elevation observed in

this study—often exceeding 1000 µg/L in Babesia *caballi* cases is consistent with levels reported in other severe systemic inflammatory diseases, confirming that piroplasmosis induces a substantial inflammatory response.

These findings are also consistent with observations in other species, where *Babesia* spp. infections trigger marked acute-phase responses secondary to hemolysis and pro-inflammatory cytokine release.[4] The observed difference in SAA magnitude between Babesia *caballi* and Theileria *equi* infections is also coherent with previously described pathophysiological differences between the two parasites.

Several limitations should be considered when interpreting the present findings. Firstly, the relatively small sample size restricts statistical power and may limit results generalizability. Secondly, the cross-sectional design does not capture the temporal dynamics of SAA during infection or following treatment. Serial measurements would provide valuable information regarding the kinetics of SAA as an indicator of clinical improvement or relapse. Thirdly, although horses with concurrent inflammatory diseases were excluded based on clinical examination, ultrasonography, and laboratory findings, subclinical conditions or parasitic co-infections cannot be completely ruled out. In addition, historical data regarding previous carrier status, prior treatments, or duration of clinical signs, were not consistently available and may have influenced the variability observed in SAA concentrations, particularly among Theileria *equi*-positive horses. Finally, as SAA is a non-specific marker, its diagnostic use should always be interpreted in the context of clinical signs, hematology, and molecular testing.

A binary logistic regression using SAA as a continuous predictor of infection status (infected vs. non-infected) could represent an interesting approach in future studies. Such analyses would

allow estimation of odds ratios and assessment of the discriminatory performance of SAA using receiver operating characteristic (ROC) curves and area under the curve (AUC) analysis. However, the limited sample size of the present study (n = 25; 6 Babesia *caballi*–positive, 9 Theileria *equi*–positive, and 10 control horses) did not provide sufficient statistical power to reliably perform these analyses. Larger studies are therefore warranted to determine whether SAA thresholds, interpreted in combination with clinical findings and molecular testing, could help distinguish active piroplasmosis from chronic carrier states rather than serve as a stand-alone diagnostic test.

Future studies should aim to establish reference intervals for SAA in different equine populations, including healthy horses, subclinically infected carriers, and acutely infected horses, in order to refine its diagnostic utility. Combining SAA with other acute-phase proteins such as haptoglobin, fibrinogen, or serum iron, may improve diagnostic specificity and provide a more comprehensive assessment of inflammation. Longitudinal studies following clinically normal horses testing positive for Theileria *equi* would also be valuable to determine whether persistently normal SAA concentrations are associated with chronic carrier states, whereas increasing SAA concentrations may precede clinical disease. Quantitative PCR or other quantitative molecular approaches could additionally be used to correlate parasite burden with SAA concentration, potentially allowing SAA to serve as a surrogate marker of infection intensity.

Such studies would help define the role of SAA not only as a diagnostic marker, but also as a prognostic and monitoring tool in equine protozoal infections.

These findings highlight the potential value of SAA as a supportive marker in the diagnosis and clinical assessment of equine piroplasmosis. Owing to its rapid kinetics and sensitivity to

systemic inflammation, SAA may represent a useful adjunct to molecular testing and clinical evaluation, particularly when differentiating active disease from asymptomatic carrier states in endemic regions.

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References:

1. Aitken, M. R., Stefanovski, D., & Southwood, L. L. (2018). Serum amyloid A concentration in postoperative colic horses and its association with postoperative complications. *Veterinary Surgery*, *48*(2), 143-151. https://doi.org/10.1111/vsu.13133
2. Belgrave, R. L., Dickey, M. M., Arheart, K. L., & Cray, C. (2013). Assessment of serum amyloid A testing of horses and its clinical application in a specialized equine practice. *Journal Of The American Veterinary Medical Association*, *243*(1), 113-119. https://doi.org/10.2460/javma.243.1.113

3. Davitkov, D., Vucicevic, M., Stevanovic, J., Krstic, V., Slijepcevic, D., Glavinic, U., & Stanimirovic, Z. (2016). Molecular detection and prevalence of Theileria equi and Babesia caballi in horses of central Balkan. *Acta Parasitologica*, *61*(2). https://doi.org/10.1515/ap-2016-0044
4. De Waal, D. (1992). Equine piroplasmosis : A review. *British Veterinary Journal*, *148*(1), 6-14. https://doi.org/10.1016/0007-1935(92)90061-5
5. El-Deeb, W. M., & Iacob, O. C. (2012). Serum acute phase proteins in control and Theileria annulata infected water buffaloes (Bubalus bubalis). *Veterinary Parasitology*, *190*(1-2), 12-18. https://doi.org/10.1016/j.vetpar.2012.06.019
6. Gopalakrishnan, A., Maji, C., Dahiya, R. K., Suthar, A., Kumar, R., & Kumar, S. (2015). Oxidative Damage Inflicted by Theileria equi on Horse Erythrocytes When Cultured In Vitro by Microaerophilous Stationary Phase Technique. *Journal Of Equine Veterinary Science*, *35*(9), 763-767. https://doi.org/10.1016/j.jevs.2015.07.020
7. Hermans, L., Tortereau, A., Riccio, B., & Desjardins, I. (2024). Fatal acute clinical babesiosis in an adult gelding pony living in an endemic area. *Equine Veterinary Education*, *36*(11). https://doi.org/10.1111/eve.14009
8. Hobo, S., Niwa, H., & Anzai, T. (2007). Evaluation of Serum Amyloid A and Surfactant Protein D in Sera for Identification of the Clinical Condition of Horses with Bacterial Pneumonia. *Journal Of Veterinary Medical Science*, *69*(8), 827-830. https://doi.org/10.1292/jvms.69.827
9. Jacobsen, S. (2022). Use of serum amyloid A in equine medicine and surgery. *Veterinary Clinical Pathology*, *52*(S1), 8-18. https://doi.org/10.1111/vcp.13195

10. Jacobsen, S., & Andersen, P. H. (2007). The acute phase protein serum amyloid A (SAA) as a marker of inflammation in horses. *Equine Veterinary Education*, *19*(1), 38-46. https://doi.org/10.1111/j.2042-3292.2007.tb00550.x

11. Jouglin, M., Bonsergent, C., De la Cotte, N., Mège, M., Bizon, C., Couroucé, A., Lallemand, É., Leblond, A., Lemonnier, L. C., Leroux, A., Marano, I., Muzard, A., Quéré, É., Toussaint, M., Agoulon, A., & Malandrin, L. (2025). Equine piroplasmosis in different geographical areas in France : Prevalence heterogeneity of asymptomatic carriers and low genetic diversity of Theileria equi and Babesia caballi. *Ticks And Tick-borne Diseases*, *16*(1), 102434. https://doi.org/10.1016/j.ttbdis.2024.102434

12. Long, A., & Nolen-Walston, R. (2020). Equine Inflammatory Markers in the Twenty-First Century. *Veterinary Clinics Of North America Equine Practice*, *36*(1), 147-160. https://doi.org/10.1016/j.cveq.2019.12.005

13. R Core Team (2025). *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

14. Respe. (2024, 26 avril). *PiroGo Tick : les premiers résultats du projet de recherche participative sont là !* Respe - Réseau D'Epidémio-Surveillance En Pathologie Équine. https://respe.net/pirogo-tick-les-premiers-resultats-du-projet-de-recherche-participative-sont-la/?utm_source=chatgpt.com

15. Runge, K. E., Bak, M., Vestergaard, A., Stærk-Østergaard, J., Jacobsen, S., & Pihl, T. H. (2023). Serum amyloid A does not predict non-survival in hospitalised adult horses with acute colitis. *Veterinary Record*, *192*(7). https://doi.org/10.1002/vetr.2644

16. Tirosh-Levy, S., Gottlieb, Y., Fry, L. M., Knowles, D. P., & Steinman, A. (2020). Twenty Years of Equine Piroplasmosis Research : Global Distribution, Molecular Diagnosis, and Phylogeny. *Pathogens*, *9*(11), 926. https://doi.org/10.3390/pathogens9110926

17. Wise, L. N., Pelzel-McCluskey, A. M., Mealey, R. H., & Knowles, D. P. (2014). Equine piroplasmosis. *Veterinary Clinics Of North America Equine Practice*, *30*(3), 677-693. https://doi.org/10.1016/j.cveq.2014.08.008

18. Witkowska-Piłaszewicz, O. D., Żmigrodzka, M., Winnicka, A., Miśkiewicz, A., Strzelec, K., & Cywińska, A. (2018). Serum amyloid A in equine health and disease. *Equine Veterinary Journal*, *51*(3), 293-298. https://doi.org/10.1111/evj.130

365 Tables:

366 Table 1. Clinical and laboratory findings in horses with positive LAMP results for *Babesia*
367 *caballi* or *Theileria equi*

Horse ID	Clinical signs	Hematology findings	*Babesia caballi* LAMP	*Theileria equi* LAMP	Serum amyloid A (µg/L)
1	Fever, edema	Anemia	-	+	2553
2	Fever, edema	Anemia	-	+	1700
3	Fever	Anemia	-	+	2030
4	Depression	Anemia, hyperbilirubinemia	+	-	2518
5	Fever, edema	Anemia	-	+	247
6	Fever, edema	Anemia, thrombocytopenia	+	-	2350
7	Fever	Anemia, neutropenia	+	-	2282
8	Fever	Anemia, hyperbilirubinemia	-	+	74

9	Depression, icteric mucosae	Hyperbilirubinemia (59 mg/L), anemia	-	+	2363
10	Depression, fever, anorexia	Hyperbilirubinemia (148 mg/L), anemia	+	-	1492
11	Fever, anorexia	Hyperbilirubinemia (32 mg/L)	-	+	3000
12	Fever, dull colic signs	Marginal anemia	+	-	2347
13	Fever, dull colic signs	Marginal anemia	+	-	2290
14	Fever	Anemia, hyperbilirubinemia	-	+	2179
15	Fever, depression, anorexia	Creatinine 18.8 mg/L; increased enzymes	-	+	1865
16	**Fever**	**Hypoalbuminemia**	+	+	**416**

368 **Note:** Horse #16 was the only horse testing positive for both Babesia *caballi* and Theileria *equi*
369 (co-infection). This horse was excluded from primary statistical analyses due to the small sample
370 size of mixed infections.

371 Table 2. Clinical and laboratory findings in LAMP negative control horses

Horse ID	Presenting complaint	Hematology findings	*Babesia caballi* LAMP	*Theileria equi* LAMP	Serum amyloid A (µg/L)
17	Pre-castration	Normal	-	-	0
18	Vaccination	Normal	-	-	1
19	Vaccination	Normal	-	-	5
20	Medical follow-up	Marginal anemia	-	-	0
21	Vaccination	CPK 364 U/L	-	-	5
22	Vaccination	Normal	-	-	19
23	Vaccination	CPK 381 U/L	-	-	0
24	Vaccination	CPK 552 U/L	-	-	0
25	Lameness evaluation	Normal	-	-	25
26	Vaccination	Hypoalbuminemia (26 g/L)	-	-	0

27	**Lameness evaluation**	**Normal**	**-**	**+**	**10**

Note: Horse #27 was the only horse testing positive for *Theileria equi*, with no other clinical signs. This horse was excluded from primary statistical analysis due to the small sample size.

Table 3. Robust Linear Regression Model Results

Variable	**Coefficient (µg/L)**	**Standard Error**	**t-value**	**p-value**	**95% Confidence Interval**
LAMP− (reference)	5.50	24.31	0.23		(-42.14, 53.14)
Babesia+ (Effect on SAA)	2311.75	39.69	58.24	< 0.001	(2233.95, 2389.55)
Theileria+ (Effect on SAA)	2024.42	35.32	57.32	< 0.001	(1955.20, 2093.64)

Note: Coefficients estimated by robust linear regression (MASS::rlm(), Huber M-estimator). The reference category is the LAMP-negative control group; coefficients represent the mean increase in SAA concentration relative to controls. P-values for the slopes were obtained from robust F-tests (sfsmisc::f.robftest); no p-value is reported for the intercept.

381 Figure Legends:

382 Figure 1: Distribution of SAA levels by infection status, with statistical significance assessed by a

383 Dunn-test.

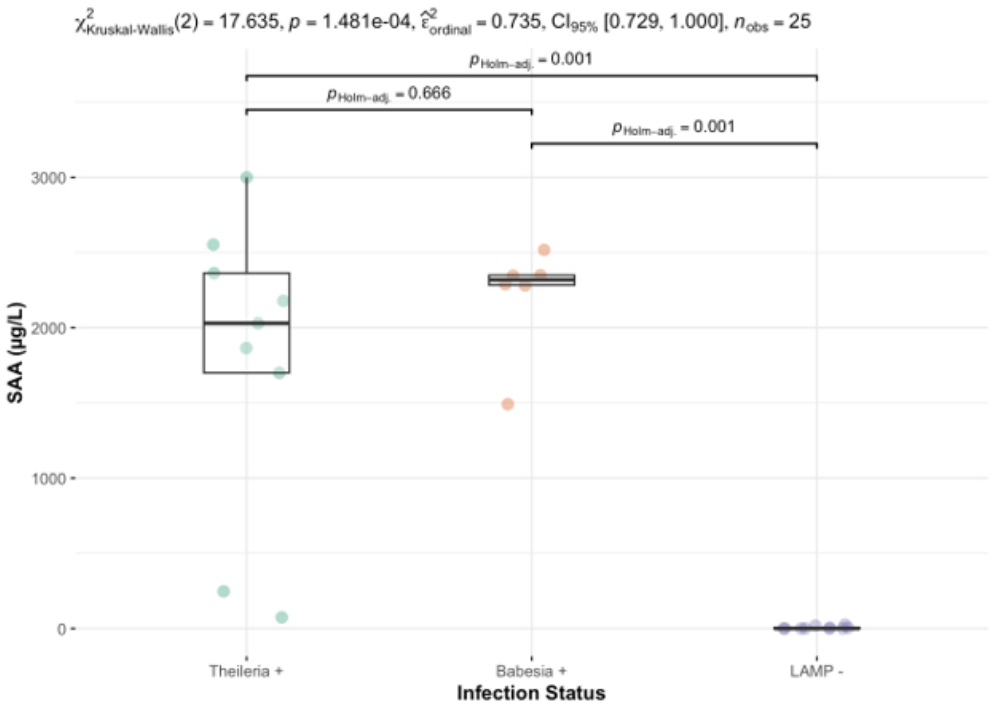

Figure 1: Distribution of SAA levels by infection status, with statistical significance assessed by a Dunn-test.

419x299mm (38 x 38 DPI)