Original Article

A retrospective study of the frequency and factors associated with elevated serum aspartate aminotransferase activity in domestic rabbits

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Abstract

This study aimed to identify factors associated with elevated aspartate aminotransferase (AST) activity and to evaluate the frequency of elevated AST and its association with mortality in domestic rabbits. A retrospective cohort study was conducted between 2021 and 2022, including 1,435 rabbits that underwent blood testing for AST. A significant association was observed between health status and elevated AST activity ($P < 0.001$). Elevated AST activity was observed in 14.64% (95/649) of healthy rabbits and 33.97% (267/786) of diseased rabbits. Among the 138 rabbits that died during hospitalization, 79 (57.24%) had elevated AST activity. Several hematological and biochemical parameters were associated with elevated AST activity. Leukocytosis, leukopenia, neutropenia, lymphopenia, decreased RBC count, decreased hemoglobin, decreased hematocrit, and thrombocytopenia, elevated ALT, elevated BUN, and elevated creatinine showed positive associations, whereas erythrocytosis, elevated total protein, and elevated albumin showed negative associations. Among diseased rabbits, elevated AST activity was positively associated with several clinical signs, including abnormal mucous membranes, depressed mentation, anorexia, emaciation, hyperthermia, unconsciousness, weakness, decreased fecal output, abdominal pain, and dyspnea; hypersalivation showed an inverse association. Disease conditions positively associated with elevated AST activity included gastrointestinal hypomotility, allergic rhinitis, pneumonia, heat stroke, anaphylactic shock, renal disease, cardiovascular disease, hepatic disease, toxin ingestion, malnutrition, and hypoglycemia, whereas malocclusion showed an inverse association. Multivariable logistic regression showed that the presence of clinical illness (defined as clinical abnormalities or a documented diagnosis of any disease), underweight body condition score, and anemia (hematocrit <25%) were independently associated with elevated serum AST activity in the overall population; among diseased rabbits, renal disease, hepatic disease, and underweight BCS remained independently associated with elevated AST activity. Increasing AST categories were associated with progressively higher odds of in-hospital mortality.

Keywords: aspartate aminotransferase, associated factors, AST, clinical pathology, mortality, rabbit

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Received March 12, 2026
Accepted October 18, 2026
https://doi.org/10.56808/2985-1130.4035

Introduction

Rabbits have become increasingly popular as companion animals, and a wide range of gastrointestinal, respiratory, reproductive, and other disorders are encountered in clinical practice. Based on historical records of rabbits admitted to the hospital from 2014 to 2015, a total of 7,425 cases (27.5%) involved rabbits with gastrointestinal tract diseases. Additionally, 6,886 cases (25.5%) were attributed to respiratory diseases (Woralar *et al.*, 2020). Furthermore, reproductive diseases accounted for 20% of the cases, while miscellaneous conditions constituted 7% of the total. Blood chemistry testing is an important component of clinical assessment. AST may increase with hepatic injury, but it is not liver-specific and is also present in myocardium, skeletal muscle, erythrocytes, kidneys, gastrointestinal tissues, pancreas, and other tissues (Murakami, 2013; Ali, 2016; Zhang, 2017; Cho, 2020; Ndrepepa, 2021). Therefore, elevated serum AST activity should be interpreted in conjunction with clinical findings and other laboratory or diagnostic information.

To our knowledge, veterinary studies evaluating the frequency and factors associated with elevated AST activity in rabbits remain limited. A clearer understanding of the clinical conditions associated with elevated AST activity may improve interpretation of this nonspecific biochemical marker. Therefore, the objective of this study was to describe the frequency of elevated serum AST activity and identify factors associated with elevated AST activity in domestic rabbits.

Materials and Methods

Data collection: This retrospective study was conducted to estimate the frequency and identify factors associated with elevated serum aspartate aminotransferase (AST) activity in domestic rabbits between 2021 and 2022. Clinical records of 1,435 rabbits that underwent blood chemistry analysis, including AST measurement, were reviewed.

Data collection was divided into three parts: (1) signalment and husbandry information, (2) physical examination findings and diagnostic assessments, and (3) laboratory data, including hematological and blood biochemical parameters. Data in the first and second parts were obtained from medical records and questionnaires.

Classification of AST status and clinical groups: Rabbits were classified into normal and elevated AST groups based on the reference range of 10–98 U/L, according to the Textbook of Rabbit Medicine (3rd ed., CABI). Rabbits were defined as healthy if they showed no clinical signs of disease and had normal physical examination findings, whereas affected rabbits were defined as those presenting clinical abnormalities or having documented disease conditions in medical records.

Diagnostic criteria for disease classification: Disease classifications were retrospectively obtained from the medical records and were based on the attending veterinarian's diagnosis together with available clinical, laboratory, and diagnostic imaging findings. Because of the retrospective nature of the study, diagnostic procedures were not standardized for all rabbits.

Pneumonia was diagnosed primarily based on compatible thoracic radiographic findings. Renal disease was diagnosed primarily based on abnormalities in renal biochemical parameters, including blood urea nitrogen (BUN) and creatinine concentrations, with ultrasonographic findings used as additional supporting evidence when available. Hepatic disease was diagnosed primarily based on blood biochemical findings, including alanine aminotransferase (ALT), together with ultrasonographic findings when available.

Heat stroke was diagnosed based on a compatible history of heat exposure together with clinical findings recorded at presentation. Cardiovascular disease was diagnosed based on available thoracic radiographic and/or echocardiographic findings, together with clinical findings and response to treatment when applicable. Toxin ingestion was classified primarily based on a documented history of known or suspected exposure to a toxic substance. Malnutrition was classified based on the recorded dietary history together with body condition assessment. Hypoglycemia was assessed using an AlphaTrak blood glucose monitoring system (Zoetis). Rabbits were classified as hypoglycemic when the glucose measurement was displayed as “LOW” by the device.

Blood chemistry analysis: Blood samples were analyzed using a fully automated biochemistry analyzer (DS-161 model, Sinnowa, China). The instrument operates based on the principle of the Lambert–Beer law, measuring absorbance within a wavelength range of 340-810 nm. The required sample volume ranges from 1 to 100 µL. According to the manufacturer’s recommendation, a minimum sample volume of 2 mL was used to reduce analytical error (Triup International, n.d.).

Serum aspartate aminotransferase (AST) activity was measured using the manufacturer's enzymatic kinetic assay and reported in U/L. Rabbits were classified into normal and elevated AST groups based on the reference interval of 10–98 U/L, according to the Textbook of Rabbit Medicine (3rd ed., CABI). Serum AST activity >98 U/L was considered elevated. Hemolyzed samples were not specifically excluded because hemolysis status was not routinely recorded.

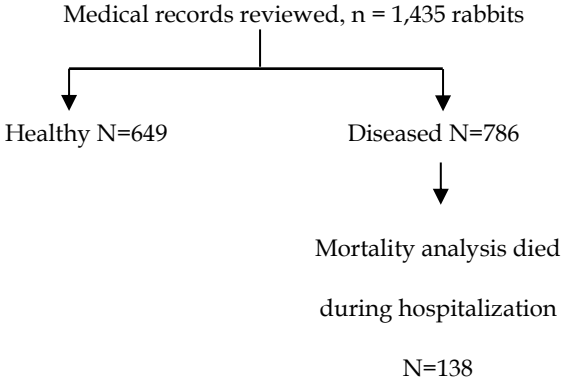
The potential effect of hemolysis on AST activity could not be evaluated and was considered a limitation of the study.

Study population: A total of 1,435 rabbits were included in this retrospective study, comprising 649 healthy rabbits and 786 diseased rabbits. Healthy rabbits were defined as those with no clinical signs of disease and normal physical examination findings, whereas diseased rabbits were defined as those presenting clinical abnormalities or having

documented disease conditions in their medical records. Among the diseased rabbits, 138 died during hospitalization and were included in the mortality analysis. These mortality cases represented a subset of the diseased rabbit population rather than a separate study cohort.

Mortality assessment: Mortality was defined as spontaneous death occurring during hospitalization. No rabbits included in the study underwent euthanasia. Mortality analyses were restricted to the 786 diseased rabbits and compared rabbits that died during hospitalization (n=138) with those that survived to hospital discharge (n=648). Only the first blood chemistry result obtained from each rabbit was used; therefore, serum AST activity was measured before the mortality outcome. Mortality occurring after hospital discharge was not assessed. For dose-response analysis, AST activity was categorized as normal (10–98 U/L), mild elevation (98.1–294 U/L), moderate elevation (294.1–490 U/L), or severe elevation (>490 U/L). A logistic regression model treating AST category as an ordinal variable was used to evaluate the linear trend in the odds of in-hospital mortality across increasing AST categories.

Flow diagram for study population:



Exclusion criteria and data handling: Rabbits with incomplete medical records or missing laboratory data were excluded from the analysis. To ensure independence of observations, each rabbit was included only once in the study. If multiple blood chemistry records were available for the same rabbit, only the first recorded visit was included in the analysis, and all subsequent records were excluded.

Ethical considerations: This study was a retrospective review of existing clinical records from rabbits presented to the veterinary hospital for routine clinical care. All clinical examinations, diagnostic procedures, blood sampling, and laboratory testing were performed as part of routine patient management and were not conducted specifically for research purposes. No additional procedures, interventions, or sample collection were performed for the purpose of this study. Formal institutional animal ethics review or exemption was not obtained for this retrospective study.

Statistical analysis: Data were analyzed using IBM SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA). Elevated serum AST activity (>98 U/L) was defined as the primary binary outcome. Associations between categorical variables and elevated AST activity were initially evaluated using Pearson's chi-square test; Fisher's exact test was used when expected cell frequencies were <5. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for binary comparisons. For laboratory variables with normal, high, and low categories, each specified abnormal category was compared with the corresponding normal category; rabbits in the opposite abnormal category and rabbits with missing values were excluded from that specific comparison. For binary clinical signs and disease conditions, rabbits with the condition present were compared with rabbits in whom the condition was absent, using available recorded data, and these analyses were restricted to diseased rabbits. Continuous laboratory variables were categorized according to the study reference intervals; therefore, normality testing was not required for the categorical analyses presented here. Multivariable logistic regression was then used to identify factors independently associated with elevated AST activity. The model for the overall study population included disease status, sex, age category, body condition score (BCS) category, and anemia status, with anemia defined as hematocrit <25%. Because renal and hepatic disease classifications were applicable only to diseased rabbits, a second multivariable model restricted to diseased rabbits included sex, age category, BCS category, anemia (hematocrit <25%), renal disease, and hepatic disease. Adult rabbits, normal BCS, male sex, absence of anemia, and absence of the specified disease condition were used as reference categories. Rabbits with missing data for any variable included in a multivariable model were excluded from that model (complete-case analysis). Mortality was not included as an exposure variable in the elevated-AST models because mortality occurred after AST measurement and was analyzed separately. For mortality, analyses were restricted to diseased rabbits with known in-hospital outcome. A logistic regression model with ordered AST category was used for the mortality trend analysis. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. Secondary univariable comparisons were exploratory, and no formal multiple-comparison adjustment was applied; therefore, these findings were interpreted cautiously with emphasis on effect estimates and 95% CIs.

Results

Diseased rabbits had higher odds of elevated serum AST activity than healthy rabbits (OR, 3.00; 95% CI, 2.31–3.90; $P < 0.001$). Age, BCS, and diet were significantly associated with AST status, whereas sex, habitat, and medication use were not significantly associated (Table 1). Among hematological variables, leukocytosis, leukopenia, neutropenia, lymphopenia, decreased RBC count, decreased hemoglobin, decreased hematocrit, and thrombocytopenia were

positively associated with elevated AST activity, whereas erythrocytosis showed a negative association. Neutrophilia, lymphocytosis, elevated hemoglobin, elevated hematocrit, and thrombocytosis were not significantly associated. Among biochemical variables, elevated ALT, BUN, and creatinine were positively associated with elevated AST activity, whereas elevated total protein and albumin showed negative associations; elevated ALP was not significantly associated (Table 2).

Among the 786 diseased rabbits, abnormal mucous membranes, depressed mentation, anorexia, emaciation, hyperthermia, unconsciousness, weakness, decreased fecal output, abdominal pain, and dyspnea were positively associated with elevated AST activity. Hypersalivation showed a negative association. Weight loss, hypothermia, and abnormal feces were not significantly associated with elevated AST activity (Table 3).

In disease-specific analyses, gastrointestinal hypomotility, allergic rhinitis, pneumonia, heat stroke, anaphylactic shock, renal disease, cardiovascular disease, hepatic disease, toxin ingestion, malnutrition, and hypoglycemia were positively associated with elevated AST activity. Malocclusion showed a negative association. Neurological and ophthalmologic problems were not significantly associated with elevated AST activity (Table 4).

In the overall multivariable logistic regression model (n = 907 complete cases), clinical illness, underweight body condition score (BCS), and anemia (defined as hematocrit <25%) were associated with higher adjusted odds of elevated serum AST activity. In contrast, senior rabbits had lower adjusted odds of elevated AST activity than adult rabbits (aOR, 0.52; 95% CI, 0.34–0.79; $P = 0.002$).

In the analysis restricted to diseased rabbits (n = 535 complete cases), renal disease (aOR, 8.83; 95% CI, 4.22–18.46; $P < 0.001$), hepatic disease (aOR, 5.71; 95% CI, 1.48–21.96; $P = 0.011$), and underweight BCS (aOR, 1.83; 95% CI, 1.15–2.89; $P = 0.010$) were associated with higher adjusted odds of elevated serum AST activity. Senior rabbits had lower adjusted odds than adult rabbits (aOR, 0.49; 95% CI, 0.30–0.80; $P = 0.004$). Other covariates were not statistically significant in this model (Table 5).

Among the 786 diseased rabbits, 138 died during hospitalization, whereas 648 survived to hospital discharge. Elevated serum AST activity was associated with higher odds of in-hospital mortality compared with normal AST activity (OR, 3.28; 95% CI, 2.25–4.78; $P < 0.001$; Table 6).

When AST activity was categorized according to the degree of elevation, the odds of in-hospital mortality were higher in rabbits with mild (OR, 2.29; 95% CI, 1.43–3.65; $P < 0.001$; Table 7), moderate (OR, 4.16; 95% CI, 1.69–10.23; $P = 0.004$; Table 8), and severe elevations (OR, 5.06; 95% CI, 3.09–8.30; $P < 0.001$; Table 9), compared with rabbits with normal AST activity. A significant increasing trend in the odds of in-hospital mortality was observed across ordered AST categories (OR per category increase, 1.74; 95% CI, 1.48–2.04; P for trend < 0.001).

Table 1 Association between general characteristics and elevated serum AST activity in rabbits.

Factor	Category	N analyzed	Normal AST, n (%)	Elevated AST, n (%)	Odds ratio (95% CI)	*P*-value
Health status	Healthy	649	554 (85.4%)	95 (14.6%)	3.00 (2.31–3.90)	<0.001
	Diseased	786	519 (66.0%)	267 (34.0%)		
Sex	Male	692	511 (73.8%)	181 (26.2%)	0.88 (0.68–1.13)	0.311
	Female	562	429 (76.3%)	133 (23.7%)		
Age	Young	178	141 (79.2%)	37 (20.8%)	0.68 (0.46–1.00)	0.021
	Adult	786	566 (72.0%)	220 (28.0%)		
	Senior	269	213 (79.2%)	56 (20.8%)	0.68 (0.48–0.94)	
BCS	Underweight	432	288 (66.7%)	144 (33.3%)	3.01 (2.14–4.23)	<0.001
	Normal	414	355 (85.7%)	59 (14.3%)		
	Overweight	74	58 (78.4%)	16 (21.6%)	1.66 (0.89–3.08)	
Habitat	Indoor	1191	883 (74.1%)	308 (25.9%)	0.64 (0.14–2.96)	0.738
	Outdoor	11	9 (81.8%)	2 (18.2%)		
Diet	Proper	1086	817 (75.2%)	269 (24.8%)	1.60 (1.07–2.39)	0.022
	Improper	119	78 (65.5%)	41 (34.5%)		
Medication use	No	1149	854 (74.3%)	295 (25.7%)	0.99 (0.54–1.80)	0.966
	Yes	59	44 (74.6%)	15 (25.4%)		

Abbreviations: AST, aspartate aminotransferase; BCS, body condition score. Percentages were calculated within each category. Odds ratios (ORs) are unadjusted. Male sex, adult age, normal BCS, indoor habitat, proper diet, and no medication use were used as the reference categories for the corresponding comparisons. P-values for age and BCS were obtained using omnibus Pearson's chi-square tests; Fisher's exact test was used for habitat because of sparse expected cell counts.

Table 2 Associations between hematological and blood chemistry parameters and elevated serum AST activity in rabbits.

Parameter	N analyzed (condition present)	Normal AST, n (%)	Elevated AST, n (%)	Odds ratio (95% CI)	*P*-value
Leukocytosis	1343 (92)	62 (67.4%)	30 (32.6%)	1.65 (1.04–2.60)	0.030
Leukopenia	1336 (85)	39 (45.9%)	46 (54.1%)	4.02 (2.57–6.28)	<0.001
Neutrophilia	1416 (215)	159 (74.0%)	56 (26.0%)	1.08 (0.78–1.51)	0.643
Neutropenia	1213 (12)	3 (25.0%)	9 (75.0%)	9.21 (2.48–34.26)	<0.001
Lymphocytosis	992 (28)	22 (78.6%)	6 (21.4%)	1.06 (0.42–2.65)	0.898
Lymphopenia	1400 (436)	279 (64.0%)	157 (36.0%)	2.19 (1.71–2.81)	<0.001
Erythrocytosis	1385 (262)	216 (82.4%)	46 (17.6%)	0.61 (0.43–0.86)	0.005
Decreased RBC count	1166 (43)	19 (44.2%)	24 (55.8%)	3.63 (1.96–6.72)	<0.001
Hemoglobin elevated	1360 (193)	157 (81.3%)	36 (18.7%)	0.69 (0.47–1.01)	0.055
Hemoglobin decreased	1235 (68)	36 (52.9%)	32 (47.1%)	2.66 (1.62–4.37)	<0.001
Hematocrit elevated	1356 (202)	156 (77.2%)	46 (22.8%)	0.93 (0.65–1.33)	0.685
Hematocrit decreased	1227 (73)	36 (49.3%)	37 (50.7%)	3.24 (2.01–5.22)	<0.001
Thrombocytosis	1412 (20)	18 (90.0%)	2 (10.0%)	0.33 (0.08–1.44)	0.189
Thrombocytopenia	1407 (15)	7 (46.7%)	8 (53.3%)	3.42 (1.23–9.49)	0.030
ALT elevated	1362 (955)	616 (64.5%)	339 (35.5%)	18.11 (10.05–32.66)	<0.001
ALP elevated	116 (91)	61 (67.0%)	30 (33.0%)	2.58 (0.81–8.20)	0.099
BUN elevated	1423 (603)	379 (62.9%)	224 (37.1%)	3.03 (2.36–3.88)	<0.001
Creatinine elevated	1343 (190)	100 (52.6%)	90 (47.4%)	3.32 (2.42–4.56)	<0.001
Total protein elevated	1114 (1010)	768 (76.0%)	242 (24.0%)	0.40 (0.26–0.60)	<0.001
Albumin elevated	1120 (983)	756 (76.9%)	227 (23.1%)	0.32 (0.22–0.47)	<0.001

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen. N analyzed is the number of rabbits included in the specified abnormal-versus-normal comparison; the number in parentheses is the number with the indicated abnormality. Normal and elevated AST counts and percentages are shown among rabbits with the indicated abnormality. For laboratory variables with high, normal, and low categories, each abnormal category was compared with the corresponding normal category; the opposite abnormal category and missing values were excluded. Fisher's exact test was used when expected cell frequencies were <5; otherwise Pearson's chi-square test was used.

Table 3 Associations between clinical signs and elevated serum AST activity in diseased rabbits.

Clinical sign	N analyzed (condition present)	Normal AST, n (%)	Elevated AST, n (%)	Odds ratio (95% CI)	*P*-value
Abnormal mucous membrane	543 (24)	8 (33.3%)	16 (66.7%)	4.45 (1.87–10.60)	<0.001
Depressed	786 (212)	111 (52.4%)	101 (47.6%)	2.24 (1.62–3.09)	<0.001
Anorexia	786 (346)	201 (58.1%)	145 (41.9%)	1.88 (1.39–2.53)	<0.001
Emaciation	786 (134)	73 (54.5%)	61 (45.5%)	1.81 (1.24–2.64)	0.002
Weight loss	786 (117)	72 (61.5%)	45 (38.5%)	1.26 (0.84–1.89)	0.266
Hyperthermia	786 (31)	9 (29.0%)	22 (71.0%)	5.09 (2.31–11.22)	<0.001
Hypothermia	786 (24)	13 (54.2%)	11 (45.8%)	1.67 (0.74–3.79)	0.213
Unconsciousness	786 (15)	5 (33.3%)	10 (66.7%)	4.00 (1.35–11.83)	0.007
Weakness	786 (88)	37 (42.0%)	51 (58.0%)	3.08 (1.96–4.84)	<0.001
Abnormal feces	786 (90)	62 (68.9%)	28 (31.1%)	0.86 (0.54–1.39)	0.543
Decreased fecal output	786 (307)	175 (57.0%)	132 (43.0%)	1.92 (1.42–2.60)	<0.001
Abdominal pain	786 (259)	139 (53.7%)	120 (46.3%)	2.23 (1.64–3.04)	<0.001
Hypersalivation	786 (108)	91 (84.3%)	17 (15.7%)	0.32 (0.19–0.55)	<0.001
Dyspnea	786 (42)	20 (47.6%)	22 (52.4%)	2.24 (1.20–4.18)	0.010

Abbreviations: AST, aspartate aminotransferase. N analyzed is the number of diseased rabbits with recorded data for the sign; the number in parentheses is the number with the sign present. Normal and elevated AST counts and percentages are shown among rabbits with the sign present. ORs compare presence versus absence of the indicated sign. For mucous membrane status, rabbits with missing status were excluded. Fisher's exact test was used when expected cell frequencies were <5; otherwise Pearson's chi-square test was used.

Table 4 Associations between selected diseases or conditions and elevated serum AST activity in diseased rabbits.

Disease/condition	N analyzed (condition present)	Normal AST, n (%)	Elevated AST, n (%)	Odds ratio (95% CI)	*P*-value
Gastrointestinal hypomotility	786 (392)	219 (55.9%)	173 (44.1%)	2.52 (1.86–3.42)	<0.001
Malocclusion	786 (211)	165 (78.2%)	46 (21.8%)	0.45 (0.31–0.64)	<0.001
Allergic rhinitis	786 (5)	1 (20.0%)	4 (80.0%)	7.88 (0.88–70.84)	0.048
Pneumonia	786 (90)	51 (56.7%)	39 (43.3%)	1.57 (1.00–2.45)	0.046
Heat stroke	786 (20)	7 (35.0%)	13 (65.0%)	3.74 (1.48–9.50)	0.003
Anaphylactic shock	786 (18)	5 (27.8%)	13 (72.2%)	5.26 (1.86–14.92)	<0.001
Renal disease	786 (86)	15 (17.4%)	71 (82.6%)	12.17 (6.81–21.76)	<0.001
Cardiovascular disease	786 (6)	1 (16.7%)	5 (83.3%)	9.89 (1.15–85.05)	0.019
Hepatic disease	786 (22)	3 (13.6%)	19 (86.4%)	13.18 (3.86–44.95)	<0.001
Toxin ingestion	786 (5)	1 (20.0%)	4 (80.0%)	7.88 (0.88–70.84)	0.048
Malnutrition	786 (61)	23 (37.7%)	38 (62.3%)	3.58 (2.08–6.15)	<0.001
Hypoglycemia	786 (14)	4 (28.6%)	10 (71.4%)	5.01 (1.56–16.13)	0.007
Neurological problems	786 (12)	10 (83.3%)	2 (16.7%)	0.38 (0.08–1.77)	0.356
Ophthalmologic problems	786 (14)	12 (85.7%)	2 (14.3%)	0.32 (0.07–1.44)	0.157

Abbreviations: AST, aspartate aminotransferase. Analyses were restricted to 786 diseased rabbits. The number in parentheses is the number with the indicated disease or condition present. Normal and elevated AST counts and percentages are shown among rabbits with the condition present. ORs compare presence versus absence of the indicated disease or condition. Fisher's exact test was used when expected cell frequencies were <5; otherwise Pearson's chi-square test was used.

Table 5 Multivariable logistic regression analyses of factors associated with elevated serum AST activity.

Factor	Comparison	Adjusted OR	95% CI	*P*-value
Overall population (n=907 complete cases)				
Disease status	Diseased vs healthy	3.06	2.05–4.56	<0.001
Sex	Female vs male	0.90	0.65–1.25	0.541
Age	Young vs adult	0.67	0.41–1.09	0.106
Age	Senior vs adult	0.52	0.34–0.79	0.002
BCS	Underweight vs normal	2.10	1.45–3.05	<0.001
BCS	Overweight vs normal	1.34	0.70–2.55	0.375
Anemia (hematocrit <25%)	Present vs absent	2.53	1.31–4.89	0.006
Diseased rabbits only (n=535 complete cases)				
Sex	Female vs male	0.84	0.57–1.25	0.387
Age	Young vs adult	0.90	0.50–1.62	0.725
Age	Senior vs adult	0.49	0.30–0.80	0.004
BCS	Underweight vs normal	1.83	1.15–2.89	0.010
BCS	Overweight vs normal	1.21	0.54–2.67	0.643
Anemia (hematocrit <25%)	Present vs absent	1.95	0.92–4.13	0.081
Renal disease	Present vs absent	8.83	4.22–18.46	<0.001
Hepatic disease	Present vs absent	5.71	1.48–21.96	0.011

Abbreviations: BCS, body condition score; aOR, adjusted odds ratio; CI, confidence interval. Elevated AST activity (>98 U/L) was the binary outcome. Complete-case analysis was used for each model.

Table 6 Association between elevated AST activity and in-hospital mortality in diseased rabbits.

In-hospital outcome	N analyzed	Normal AST, n (%)	Elevated AST, n (%)	Odds ratio (95% CI)	*P*-value
Died during hospitalization	138	59 (42.8%)	79 (57.2%)	3.28 (2.25–4.78)	<0.001
Survived to discharge	648	460 (71.0%)	188 (29.0%)		—

Abbreviations: AST, aspartate aminotransferase; OR, odds ratio; CI, confidence interval.

Table 7 Association between mild AST elevation and in-hospital mortality.

AST category	N analyzed	Survived, n (%)	Died, n (%)	Odds ratio (95% CI)	*P*-value
Mild (98.1–294 U/L)	150	116 (77.3%)	34 (22.7%)	2.29 (1.43–3.65)	<0.001
Normal (10–98 U/L)	519	460 (88.6%)	59 (11.4%)		—

Abbreviations: AST, aspartate aminotransferase; OR, odds ratio; CI, confidence interval.

Table 8 Association between moderate AST elevation and in-hospital mortality.

AST category	N analyzed	Survived, n (%)	Died, n (%)	Odds ratio (95% CI)	*P*-value
Moderate (294.1–490 U/L)	23	15 (65.2%)	8 (34.8%)	4.16 (1.69–10.23)	0.004
Normal (10–98 U/L)	519	460 (88.6%)	59 (11.4%)	Reference	—

Abbreviations: AST, aspartate aminotransferase; OR, odds ratio; CI, confidence interval.

Table 9 Association between severe AST elevation and in-hospital mortality.

AST category	N analyzed	Survived, n (%)	Died, n (%)	Odds ratio (95% CI)	*P*-value
Severe (>490 U/L)	94	57 (60.6%)	37 (39.4%)	5.06 (3.09–8.30)	<0.001
Normal (10–98 U/L)	519	460 (88.6%)	59 (11.4%)		—

Abbreviations: AST, aspartate aminotransferase; OR, odds ratio; CI, confidence interval.

Discussion

After adjustment for sex, age category, BCS, and anemia (hematocrit <25%), diseased rabbits had approximately threefold higher odds of elevated serum AST activity than healthy rabbits (aOR, 3.06; 95% CI, 2.05–4.56; $P < 0.001$). Underweight BCS and anemia (hematocrit <25%) also remained independently associated with elevated AST activity in the overall model. In the diseased-only model, renal disease, hepatic disease, and underweight BCS remained independently associated with elevated AST activity. These findings represent statistical associations within this retrospective dataset and should not be interpreted as evidence of causation.

AST is not liver-specific in rabbits, and increased serum AST activity should not be interpreted as evidence of hepatopathy in isolation. AST may increase with injury to hepatic and non-hepatic tissues, and ALT and other biochemical findings may provide additional context when hepatic disease is suspected. Previous reports have described relationships between AST and other biochemical abnormalities; however, evidence from other species or experimental settings cannot be assumed to apply directly to naturally occurring disease in domestic rabbits. Therefore, the present findings are interpreted primarily as associations rather than as evidence of a specific pathophysiological mechanism.

Age and body condition were associated with AST activity in some analyses, although the direction and magnitude of these relationships were not uniform. Evidence concerning age-related changes in AST is limited in rabbits; reports from humans should therefore be regarded only as contextual information rather than direct evidence for rabbits (Bussler, 2018). Similarly, hepatic lipidosis is recognized as a clinical concern in rabbits with anorexia or altered nutritional status (Meredith, 2000), but hepatic lipidosis was not systematically confirmed in all rabbits in this dataset. The association between underweight BCS and elevated AST may consequently reflect underlying illness, reduced nutritional intake, tissue injury, or other unmeasured factors rather than a single mechanism.

Because standardized measures of disease severity were not consistently available in the retrospective medical records, a multivariable mortality model adjusted for disease severity could not be reliably constructed. Therefore, residual confounding by the severity of underlying illness cannot be excluded, and elevated AST activity should be interpreted as a marker associated with in-hospital mortality rather than as an independent predictor of death.

Several clinical signs, including abnormal mucous membrane findings, depressed mentation, anorexia, emaciation, hyperthermia, unconsciousness, weakness, reduced fecal output, abdominal pain, and dyspnea, were positively associated with elevated AST activity, whereas hypersalivation showed an inverse association. These findings may reflect systemic illness or tissue injury, but the present study was not designed to determine the biological mechanism linking individual clinical signs with AST activity. Experimental and non-rabbit studies have reported changes in AST in association with hyperthermia, tissue injury, ischemia, and other systemic disturbances (Abdelatif, 1994; Ali, 2016; Zhang, 2017). Such evidence is cited only as possible biological context and should not be interpreted as confirmation that the same mechanisms occurred in the rabbits in this study.

Among disease categories, gastrointestinal hypomotility, allergic rhinitis, pneumonia, heat stroke, anaphylactic shock, renal disease, cardiovascular disease, hepatic disease, toxin ingestion, malnutrition, and hypoglycemia showed positive associations with elevated AST activity, whereas malocclusion showed an inverse association. These disease-specific findings should be interpreted cautiously because diagnoses and diagnostic workups were not standardized and several categories contained relatively small numbers

of rabbits. Published studies in rabbits or other species have proposed mechanisms involving ischemia, inflammation, impaired perfusion, muscle injury, or organ dysfunction (Meredith, 2000; Ali, 2016; Zhang, 2017). However, conditions such as sepsis, myocardial infarction, hepatorenal syndrome, viral infection or viral load, and bone marrow depletion were not systematically diagnosed in this study and therefore are not presented as explanations for the observed AST associations.

It is important to note that AST is present in skeletal and cardiac muscle, erythrocytes, kidneys, gastrointestinal tissues, liver, and other organs. Consequently, increased serum AST activity may reflect injury to multiple tissues rather than hepatic dysfunction alone. Muscle injury associated with handling, restraint, or trauma may contribute to increased AST activity, and hemolysis may also affect measured AST activity. Because hemolysis status was not routinely recorded, its contribution could not be evaluated in this retrospective dataset.

Data on CK, LDH, bilirubin, bile acids, GGT, and confirmatory diagnostic imaging or histopathological findings were not consistently available for all rabbits. Consequently, the tissue source of increased AST activity and the extent of hepatic involvement could not be determined reliably. This limitation is particularly important when interpreting associations between AST and disease categories that may involve several organ systems.

Elevated serum AST activity was associated with in-hospital mortality, and the odds of mortality increased across ordered AST categories. Studies in humans have also reported associations between abnormal AST activity and mortality (Lee, 2008; Xie, 2019; Oh, 2021); however, these human findings are not directly generalizable to rabbits and are included only as contextual evidence. In the present study, AST should therefore be considered a marker associated with in-hospital mortality and systemic illness rather than a validated independent prognostic biomarker.

To the authors' knowledge, this study is among the first large retrospective investigations to evaluate elevated serum AST activity across multiple health conditions in domestic rabbits. The findings provide preliminary clinical associations that may help guide future rabbit-specific research. Nevertheless, several associations require confirmation in prospective studies using standardized diagnostic criteria, disease-specific cohorts, complementary biomarkers, and more complete assessment of potential confounding factors.

This study has several limitations. First, its retrospective, hospital-based design may introduce selection bias because the study population consisted of rabbits for which blood chemistry testing, including AST measurement, was available and therefore may not represent the general domestic rabbit population. Second, some clinical and laboratory variables were missing; analyses involving these variables, including the multivariable models, were consequently based on complete cases. Third, diagnostic investigations were not standardized across all rabbits because disease classifications were derived from routine clinical records. Although repeated observations were avoided by including only the first recorded blood chemistry result for each rabbit, residual misclassification and unmeasured confounding remain possible. Fourth, serum AST activity is not tissue-specific. Contributions from skeletal or cardiac muscle injury, erythrocytes, gastrointestinal tissues, kidneys, or other organs could not be fully distinguished from hepatic sources. Hemolysis status was not routinely recorded, and complementary markers or diagnostic procedures such as CK, LDH, bilirubin, bile acids, GGT, imaging, and histopathology were not consistently available for all rabbits. Finally, standardized measures of disease severity were not consistently recorded, limiting adjustment of the mortality analysis for underlying illness severity. Accordingly, the observed relationships should be interpreted as associations and do not establish causality or demonstrate that elevated AST activity is an independent predictor of mortality.

In conclusion, elevated serum AST activity was more frequent in diseased than healthy rabbits and was independently associated with disease status, body condition, anemia (hematocrit <25%), renal disease, and hepatic disease in the multivariable analyses. Increasing AST categories were also associated with progressively higher odds of in-hospital mortality. Because AST is not tissue-specific and this study was retrospective, these findings should be interpreted as clinical associations rather than evidence of causation or of a liver-specific process. Serum AST activity may be useful as part of the overall clinical assessment of rabbits, but prospective rabbit-specific studies with standardized diagnostic criteria and complementary biomarkers are needed to determine its diagnostic and prognostic value.

Acknowledgements

This study was funded by the Faculty of Veterinary Medicine, Khon Kaen University.

Statement of author contributions: **Natakorn Siriwattanasatorn:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Software, Writing – original draft. **Sompoth Weerakhun:** Conceptualization, Methodology, Supervision, Validation, Resources, Project administration, Funding acquisition, Writing – review & editing.

Declaration of artificial intelligence use: During the preparation of this work, the authors used ChatGPT for language editing. The authors take full responsibility for the integrity, accuracy, and originality of the work.

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