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Acute-phase proteins and blood parameters as potential additional markers for ante-mortem tuberculosis diagnosis in European bison (*Bison bonasus*)

ANNA DIDKOWSKA[1], BLANKA ORŁOWSKA[1], MONIKA KRAJEWSKA-WĘDZINA[2], JAN WIŚNIEWSKI[1], KRZYSZTOF ANUSZ[1]

[1]Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Nowoursynowska 159, 02-776 Warsaw, Poland

[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Institute of Preclinical Veterinary Sciences, University of Life Sciences, 12 Akademicka Street, 20-950 Lublin, Poland

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Didkowska A., Orłowska B., Krajewska-Wędzina M., Wiśniewski J., Anusz K.

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Summary

According to the International Union for Conservation of Nature, European bison (*Bison bonasus*) is a vulnerable species. European bison is susceptible to mycobacterial infections, as evidenced by numerous confirmed tuberculosis cases in this species. Diagnostic methods for tuberculosis have developed significantly in recent years, but performing several parallel tests (serological, microbiological, molecular, cell-mediated based tests) is still the most appropriate algorithm to confirm or exclude *Mycobacterium tuberculosis* complex infection in this species. This study primarily aimed to determine whether some additional biomarkers could be helpful in tuberculosis diagnosis in European bison. Whole blood and serum from eight European bison with known TB status were used for the study. Four of these bison were *Mycobacterium caprae*-positive and four were *M. caprae*-negative. The material was tested for basic hematological and biochemical parameters and three selected acute phase proteins: ceruloplasmin, haptoglobin, and serum amyloid A (SAA). The study found no significant changes in biochemical or hematological parameters in European bison with tuberculosis, but one European bison with the most advanced tuberculosis lesions showed increased concentrations of haptoglobin and SAA. These preliminary studies show that determining the concentrations of acute-phase proteins could be an additional supportive tool that can be used to diagnose tuberculosis or to determine its severity. Further research is needed with an increased number of individuals.

Keywords: acute phase proteins, bovine tuberculosis, European bison, haptoglobin, *Mycobacterium caprae*, serum amyloid A (SAA)

According to the International Union for Conservation of Nature (IUCN), the European bison (*Bison bonasus*) is a vulnerable species. The current European bison population is characterized by low genetic variability (10), leading to particular susceptibility to viral, bacterial, and parasitic pathogens. This, combined with the increasing density of free-living herds (2), increases the risk of the spread of infectious diseases. For these reasons, health monitoring should be an essential element of the ongoing reintroduction of European bison. A health threat for European bison is *Mycobacterium caprae* infection. Tuberculosis (TB) has been confirmed in this species in both free-ranging (12) and captive herds (7, 22).

The diagnosis of TB, both in European bison and in other wildlife animal species, is still a challenge (20). Therefore new biomarkers are still being sought. So far, several *ante-mortem* diagnostic methods have been tested in European bison, including culture tests (9), serological tests (6, 13), gamma-interferon tests, tuberculin skin test (8), as well as diagnostic imaging (5). However, for valuable individuals suspected of having TB, it is worth using as many biomarkers as possible, including non-specific biomarkers such as

acute-phase proteins (APPs) or hematological and biochemical blood parameters. The acute phase reaction is inflammation caused by various factors, including bacteria. As a result, the concentration of positive APPs (e.g., ceruloplasmin, haptoglobin (Hp), serum amyloid A (SAA) increases, and negative ones (e.g., transferrin, albulmin) decrease. The most important APPs in cattle are Hp and SAA, whose concentrations are low in the serum of healthy cattle but increase during inflammation (19). Preliminary studies that were carried out to determine the concentration of selected APPs in European bison suggested that they were higher in diseased animals compared to clinically healthy ones (17).

This study aimed to determine whether APPs and blood parameters can be a useful supplement to diagnosing TB in European bison.

Tab. 1. Characteristics of the tested European bison

ID	Sex	Age [year]	Necropsy (6)	Microbiological examination (9)
S10	M	5	no lesions	negative
S11	M	7	no lesions	negative
S12	F	7	caseous necrosis in retropharyngeal and mesenteric lymph nodes	*Mycobacterium caprae*
S13	F	10	generalized tuberculosis, pregnant	*Mycobacterium capare*
S14	F	6	caseous necrosis in retropharyngeal and mesenteric lymph nodes, enlarged duodenal and mesenteric lymph nodes	*Mycobacterium caprae*
S15	F	6	caseous necrosis in retropharyngeal and mesenteric lymph nodes, pregnant	*Mycobacterium capare*
Z1	M	10	not tested	negative
Z2	F	19	no lesions	negative

Material and methods

The material was collected from eight captive European bison. In four of them, natural *M. caprae* infection was confirmed; in the other four, microbiological examination and necropsy ruled out tuberculosis, as described previously (6, 9, Tab. 1).

Blood was collected from the jugular vein (*vena jugularis externa*) after immobilization using a Palmer Cap-Chur tranquilization gun (Palmer Cap-Chur Equipment, Inc., Powder Springs, GA, US). The clot activator tubes were transported to the laboratory, where the serum was separated and used for biochemical and APP tests after centrifugation (3000 g, 10 min). Blood was collected in tubes with EDTA-K3 (tripotassium edetate) to test its hematological parameters. Ante-mortem collecting of blood is part of normal health monitoring activities, therefore it does not require the consent of an ethics committee (Act of 15 January 2015 on the protection of animals used for scientific or educational purposes sets out the principles and conditions for the protection of animals used for scientific or educational purposes).

In a commercial laboratory, the levels of the following bovine proteins were determined in blood serum samples: haptoglobin (Hp) (photometric method), ceruloplasmin (Cp) (turbidimetric method), and serum amyloid A (SAA) (photometric method). No designated reference values exist for European bison for Hp, Cp, and SAA. The test results were compared to laboratory-provided reference values for cattle.

Blood biochemistry and hematology were performed at the Laboratory of Clinical Diagnostics and Science Department of the Faculty of Veterinary Medicine in Warsaw. The devices were set up as for bovine blood testing. The Abacus Junior Vet (Diatron, Budapest, Hungary) was used to test hematology parameters. The following parameters were determined: leukocyte count (WBC), erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean erythrocyte volume (MCV), mean mass of hemoglobin in red blood cells (MCH), mean red cell hemoglobin concentration (MCHC), platelet count (PLT). Blood smears were used to assess the white blood cell count (Schilling percentage formula), stained using the May-Grünwald-Giemsa method. Biochemical parameters were checked with the Minura One device (Pointe Scientific, Warsaw, Poland). The following parameters are specified by the guidelines of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC): aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), glucose (Glu), cholesterol (CHOL), triglycerides (TGL), creatinine (CREA), urea (UREA), total protein (TP), albumin (SA), bilirubin (TBIL), gamma-glutamyl transferase (GGTP), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl), creatine kinase (CPK), lactate dehydrogenase (LDH) and amylase (AMY).

Results and discussion

The results of APPs (haptoglobin, ceruloplasmin, serum amyloid A) for individual European bison are presented in Table 2. In 2 cows (S13, Z2), the haptoglobin level was higher than the reference values for cattle. In cow S13, an increased level of serum amyloid A was additionally noted (Tab. 2).

Most hematological and biochemical parameters in *M. capre*-positive and *M. caprae*-negative European bison were within the reference values range. Detailed results of the morphological and biochemical blood tests are presented in Table 3 and Table 4.

Tab. 2. Results of acute-phase proteins (Hp – haptoglobin, Cp – ceruloplasmin, SAA – serum amyloid A) in the serum from European bison

ID	Hp (g/l)	Cp (g/l)	SAA (mg/l)
Reference value for cattle	< 0.05	0.15-0.35	< 5
S10	< 0.05	0.19	< 5
S11	< 0.05	0.22	< 5
S12	< 0.05	0.21	< 5
S13	> 3.6 ↑	0.31	7.93 ↑
S14	< 0.05	0.19	< 5
S15	< 0.05	0.22	< 5
Z1	< 0.05	0.33	< 5
Z2	> 0.2 ↑	0.21	< 5

Explanation: ↑ – result exceeds the reference value for cattle

Tab. 3. Results of morphological blood tests of tested European bison (S10-S15 and Z1-Z2)

ID	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	PLT	BAND	SEG	MONO	EOS	LYM
Ref	2.1-10.3	3.7-9.5	6.4-14.7	19.7-41.9	37.7-59.8	13.5-19.3	31.1-36.7	9.8-330.5	0-3	32.1-77.6	0-2	0-20	17.9-53.2
S10	4.83	6.19	9.5	30.13	46	15.3	31.4	185	1	38	0	50 ↑	10 ↓
S11	6.62	5.59	9.9	32.46	58	17.7	30.5	172	1	49	0	22	27
S12	4.80	5.65	10.0	31.37	56	17.7	31.9	152	0	48	2	10	40
S13	5.40	5.81	8.9	26.43	45	15.3	36.6	324	0	56	1	10	31
S14	5.61	5.59	9.2	28.54	51	16.4	32.2	126	0	40	0	16	43
S15	6.02	5.76	9.9	30.65	53	17.3	32.4	183	2	60	–	12	26
Z1	8.59	5.75	9.8	29.13	51	17.0	33.5	222	0	74	1	1	24
Z2	7.05	5.14	9.6	30.37	59	18.8	31.8	200	3	71	3 ↑	3	17

Explanations: WBC – leukocyte count (thous/mm^3); RBC – erythrocyte count (mln/mm^3); Hb – hemoglobin concentration (g/dl); HCT – hematocrit (%); MCV – mean erythrocyte volume (fl); MCH – mean red cell hemoglobin mass (pg); MCHC – mean red cell hemoglobin concentration (g/dl); PLT – platelet count (thous/mm^3); BAND – band nucleus neutrophils (%); SEG – segmented nucleus neutrophils (%); MONO – monocytes (%); EOS – eosinophils (%); LYM – lymphocytes (%); Ref – reference interval for European bison according to Didkowska et al. (unpublished), ↑ – result exceeds the reference value, ↓ – result is below the reference value

Tab. 4. Results of biochemistry blood tests of tested European bison (S10-S15 and Z1-Z2)

ID	AST	ALT	AP	Glu	CREA	UREA	TP	Ca	P	Mg	Na	K	Cl
Ref	41.0-102.3	12.3-39.6	23.7-72.2	No data	0.9-2.8	13.5-62.1	47.3-72.7	7.4-11.2	2.0-7.1	1.1-3.0	129.5-143.3	3.0-7.0	93.3-109.2
S10	90.9	19.2	56.6	196.5	1.18	25.9	54	9.0	3.85	2.20	137.1	4.75	100.5
S11	78.3	36.3	43.6	193.1	1.33	22.0	50	8.9	1.50	2.31	136.7	4.33	97.7
S12	82.4	27.4	37.0	132.2	1.30	30.0	64	8.1	2.10	2.46	141.2	6.14	108.3
S13	93.8	16.1	50.6	162.0	0.73 ↓	20.0	67	7.0	3.17	1.40	146.6	6.06	101.4
S14	99.6	27.5	46.9	194.3	0.80 ↓	34.0	53	8.9	1.17	2.15	139.0	4.62	103.4
S15	98.4	29.7	42.2	146.4	1.25	23.0	60	7.1 ↓	4.33	1.79	145.4 ↑	5.39	99.9
Z1	142.7 ↑	44.6 ↑	45.6	66.8	2.03	96.1 ↑	66	4.0	11.97 ↑	1.52	152.2 ↑	5.95	107.2
Z2	91.6	36.6	44.5	144.7	2.27	49.8	54	7.4	5.29	1.46	149.1 ↑	4.11	105.5

Explanations: AST – aspartate aminotransferase (U/I); ALT – alanine aminotransferase (U/I); AP – alkaline phosphatase (U/I); Glu – glucose (mg/dl); CREA – creatinine (mg/%); UREA – urea (mg/dl); TP – total protein (g/l); Ca –calcium (mg/dl); P – phosphorus (mg/dl); Mg – magnesium (mg/dl); Na – sodium (mmol/dl); K – potassium (mmol/dl); Cl – chlorine (mmol/dl); Ref – reference interval for European bison according to Didkowska et al. (unpublished), ↑ – result exceeds the reference value, ↓ – result is below the reference value

Our research has shown that biochemical and morphological parameters are not useful for diagnosing tuberculosis in European bison, but haptoglobin and SAA may be helpful additional parameters.

In the examined *M. caprae*-infected European bison (S12-S15), no significant deviations in blood morphological and biochemical parameters were found in relation to the reference values and TB-negative bison (S10-S11 and Z1-Z2) (Tab. 3, 4). It was determined that hematological and biochemical parameters are not appropriate additional diagnostic tools for TB in European bison. Other authors have also drawn similar conclusions (3), but our study is the first to compare the results to reference values for European bison instead of those for cattle or American bison (*Bison bison*). Our results contrast with research on TB-positive American bison in which monocytosis and lymphocytosis were found (16). It should be emphasized that in cattle the number of peripheral white blood cells has lower diagnostic value compared to other species. Even during diseases with an intense inflammatory process, leucocytosis is rarely found, and sometimes even leukopenia occurs (18). We assume that this could be similar in the case of European bison, therefore different inflammation biomarkers have been also checked, namely APPs.

APPs are useful as biomarkers, primarily to detect the initial phase of inflammation. This has been confirmed in cattle with acute and subacute inflammation in which SAA and Hp concentrations were increased (11). In cattle with chronic inflammation, concentrations of SAA and Hp did not differ from the reference values (11). In the case of TB, increased Cp, Hp, and SAA concentrations have been detected in *M. bovis*-positive cattle (15). Increased Hp concentration in the course of TB has been confirmed in red deer (*Cervus elaphus*) (21).

In our study, most of the examined European bison showed no deviations from the reference values of APPs for cattle (14). In a cow with generalized TB lesions (S13), increased concentrations of Hp and SAA were noted (Tab. 2). This may be related to the specificity of the course of TB. In so-called post-primary

TB, tuberculous foci are reactivated, therefore the inflammation is acute (4). It should be noted that cow S13 was pregnant and increased levels of APPs have previously been found during pregnancy in cows (1). However, the second pregnant cow (S15) showed no deviation from the reference values. The increased concentration of Hp in the TB-negative cow (Z2) could be associated with other inflammatory processes and the advanced age of the animal.

There are some limitations of our study. No statistical analysis has been conducted due to the limited sample size. However, we still believe that every piece of diagnostic information is very valuable for an endangered wildlife species as collecting samples is difficult.

To conclude, this is the first report to analyze the concentration of selected APPs in TB-positive European bison. Our preliminary study has shown that determining Hp and SAA concentrations could potentially be a supportive tool for TB diagnosis in European bison. Further research is needed to confirm this, but our preliminary research shows that it is a tool that could possibly be used to determine the stage of progression of this disease. We have shown that none of the biochemical and hematological parameters were significantly altered in TB-positive European bison.

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Corresponding author: Anna Didkowska, DVM, PhD, Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Nowoursynowska 166, 02-787 Warsaw, Poland; e-mail: anna_didkowska@sggw.edu.pl