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ACUTE-PHASE RESPONSES IN HEALTHY, MALNOURISHED, AND *OTOSTRONGYLUS*-INFECTED JUVENILE NORTHERN ELEPHANT SEALS (*MIROUNGA ANGUSTIROSTRIS*)

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Abstract: Acute-phase proteins (APPs) are utilized to detect early inflammation in many domestic and nondomestic species, but variability exists between species and inflammatory diseases as to which APPs are most useful. Stranded juvenile northern elephant seals (NESs; *Mirounga angustirostris*) undergoing rehabilitation at the Marine Mammal Center experience high mortality rates due to severe arteritis caused by the lungworm, *Otostrongylus circumlitis* (OC), and there are currently no effective antemortem diagnostic tools for this disease. To characterize patterns of the acute-phase response in the NES, two APPs—serum amyloid A (SAA) and C-reactive protein (CRP)—were measured, and serum protein electrophoresis was performed to measure albumin and globulin fractions in 81 serum samples from 58 NESs in four different health states: healthy, malnourished, preclinical for OC infection, or clinical for OC infection. Compared to healthy NESs (median, 11.2 mg/L), SAA concentrations were significantly increased in malnourished (33.9 mg/L), preclinical (247 mg/L), and clinical OC–infected NESs (328 mg/L) ($P < 0.05$). CRP concentrations were increased only in clinical OC–infected NESs (median, 53.9 mg/L) and were below detectable limits in the other three groups (<0.01 mg/L). These results show that SAA and CRP are positive APPs in NESs with OC infection, and that SAA may serve as the major APP for this species. Albumin : globulin ratios were significantly increased in malnourished NESs (median, 1.26) and decreased in clinical OC–infected NESs (0.53). As a result, albumin is a negative APP in the NES, similar to other mammalian species. APP monitoring can be helpful in detecting and monitoring inflammation in rehabilitating juvenile NESs.

Key words: Acute-phase proteins, *Mirounga angustirostris*, northern elephant seal, *Otostrongylus circumlitis*, protein electrophoresis, serum amyloid A.

INTRODUCTION

Acute-phase proteins (APPs) are integral to the acute-phase response of innate immunity and are expressed earlier during inflammatory processes when compared to changes in a complete blood count.[12,23,32] APPs, along with serum protein electrophoresis (SPE), have served as methods for detecting and characterizing early inflammation in domestic and nondomestic species.[4,5,8–11,13,15,20,22,24,27,36]

Serum amyloid A (SAA) and C-reactive protein (CRP) are positive APPs that are produced mainly in the liver and increase in peripheral circulation in less than 1 day in response to interleukins 1 and 6 after an inflammatory stimulus, as found in humans and dogs.[5] CRP has been shown to increase with inflammation in harbor seals (*Phoca vitulina*), but these proteins have not been reported further in other pinniped species.[8,14] SAA is involved in recruiting leukocytes to sites of inflammation, whereas CRP aids in complement binding to bacteria and opsonization, with subsequent production of cytokines through the complement system.[32] In contrast, albumin decreases in response to inflammation and is thus considered a negative APP in studied mammalian species.[3,8,12,32] APP concentrations should increase and the albumin : globulin (AG) ratio should decrease in the presence of inflammatory disease.[23,32] Variability exists as to which APPs are considered major (10–1,000-fold increase between healthy and diseased animals within 24–48 hr of the stimulus) or moderate (2–10-fold increase between healthy and diseased animals and usually occurring $\geq$3 days after the stimulus) in each species.[5,8] As a result, validation of multiple APPs for each individual species is utilized to determine which APP is considered major for that species.[8,24]

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APP assays and SPE to measure albumin and globulin fractions have not been optimized or evaluated for detection of early inflammation in the northern elephant seal (NES; *Mirounga angustirostris*).

The NES is a phocid species inhabiting the California coast that was almost driven to extinction because of commercial hunting in the 19th century.[2] Despite a currently growing population, now estimated at 200,000 seals, an average of 148 juvenile NESs are admitted to the Marine Mammal Center (TMMC) in Sausalito, California, each year for treatment and rehabilitation because of malnutrition, trauma, human interaction, and infectious disease.[1,7,21]

Otostrongylus circumlitis (OC), a nematode lungworm, is a significant cause of morbidity and mortality among stranded and rehabilitating juvenile NESs.[7,18,19,30] OC infection has been identified as responsible for 12% of NES strandings and 37% of NES mortalities at TMMC.[7] Fecal examination is not effective for antemortem diagnosis of OC infection in NESs, as seals die from arteritis and disseminated intravascular coagulation before the nematode sheds ova into the gastrointestinal tract. Unfortunately, treatment is usually unsuccessful by the time clinical signs develop, with an 89% mortality rate for NESs exhibiting clinical signs.[18] Clinicopathologic changes such as leukocytosis, elevation in liver enzymes, and prolonged coagulation parameters have been found in NESs with OC infection.[18] These changes usually occur after or in concert with development of overt clinical signs that are observed on visual or physical examination of the animal and that frequently do not become evident before an advanced stage of infection; thus, an earlier method of diagnosis of inflammation is warranted. Detecting inflammation prior to development of severe clinical signs would allow earlier appropriate treatment and might reduce mortality rates. In addition, monitoring APPs during treatment will allow surveillance of the individual patient and its response to treatment.

The primary objective of this study was to identify the major detectable APP in NESs by comparing SAA and CRP concentrations and measuring SPE patterns between healthy NESs and those in three diseased states: malnourished, preclinical for OC infection, and clinical for OC infection. The secondary objective of this study was to identify changes in APP concentrations and albumin and globulin fractions in animals that underwent rehabilitation from being malnourished upon admission to healthy upon release, and in animals that ultimately died because of OC infection before and after development of clinical signs.

MATERIALS AND METHODS

Eighty-one banked blood serum samples stored at −80°C from 58 weanling NESs admitted to TMMC for rehabilitation during 2012–2014 were included in this study. Study NESs were 1–3 mo of age. Samples were retrospectively selected and classified into one of four groups: 1) healthy NESs ($n = 23$); 2) malnourished NESs ($n = 23$); 3) preclinical OC–infected NESs ($n = 12$); or 4) clinical OC–infected NESs ($n = 23$). Healthy NESs exhibited no abnormalities on physical examination or clinical pathology (hematologic and biochemical analyses); they were sampled during prerelease screenings ≤3 days prior to release back into the wild. Malnourished NESs were emaciated, but had no signs of infection, trauma, or disease on physical exam and clinical pathology; they were sampled during admission examinations ≤4 days after admission to TMMC. Preclinical OC–infected NESs exhibited no abnormalities on physical exam or clinical pathology at the time of sampling, but subsequently developed clinical signs of OC infection, which was later confirmed at necropsy (with no evidence of other underlying disease); they were sampled between 2 and 28 days prior to death or euthanasia. Clinical OC–infected NESs exhibited clinical signs of OC infection at time of sampling, and they were subsequently diagnosed with OC infection upon necropsy (with no evidence of other underlying disease); they were sampled between 0 and 17 days prior to death or euthanasia. Clinical signs of OC infection included at least three of the following physical exam and/or clinical pathologic findings: anorexia, lethargy, dehydration, mucus membrane congestion, respiratory difficulty, epistaxis, leukocytosis ($>29.6 \times 10^3$ white blood cells/μl), elevated number of immature neutrophils (>5%), elevated aspartate aminotransferase (>69 U/L) and/or γ-glutamyltransferase (>74 U/L) activities, elevated or decreased fibrinogen (>400 mg/dl), and/or elevated or decreased platelets ($<171 \times 10^3$ or $>561 \times 10^3$ cells/μl). In-house reference intervals for NESs at TMMC were used to evaluate complete blood counts and biochemical analyses.

Finally, paired blood serum samples were included in this set of 81 samples collected from NESs. These samples were obtained from the same animal at two different time points—either malnourished upon admission followed by

healthy upon release ($n = 9$), or preclinical OC infected followed by clinical OC infected after the individual developed clinical signs ($n = 12$).

Blood samples were collected using vacutainer serum separator tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey 07417, USA), using 20-ga × 1½-inch Monoject needles (Covidien, Mansfield, Massachusetts 02048, USA) from the extradural intravertebral sinus of NESs under physical restraint.[19] Blood was collected as part of the routine entrance physical examinations, for monitoring treatments, prior to euthanasia, or during an exam prior to return back into the wild. Blood tubes were centrifuged at 3,000 rpm for 10 min and serum was collected and stored at −80°C until analyzed. All serum samples were free of lipemia and hemolysis.

All serum samples were shipped overnight on dry ice to the Acute Phase Protein Laboratory at the University of Miami Miller School of Medicine for analyses. SAA was quantitated via immunoturbidimetric assay performed on the Randox RX Daytona analyzer (Randox, Kearneysville, West Virginia 25430, USA). The assay uses human monoclonal antibodies (SAA-1, Eiken Chemical Company, Tokyo 110-8408, Japan) and has been previously described for use in cats, dogs, and horses.[6] CRP was quantitated using the Randox RX Daytona analyzer with a immunoturbidimetric assay for measuring human CRP concentration as previously described for use in dogs (Canine CRP, Randox).[24] Preliminary studies were conducted using samples from four clinically normal and five clinically abnormal NESs (three with clinical OC infection, one with septicemia, and one with severe facial trauma) to validate the SAA and CRP assays. None of these nine validation samples were included in the 81 samples used for the main portion of this study. A sample pool from the clinically abnormal NESs was diluted 0–100% in 10% steps using saline to test for linearity. SAA and CRP assays were successfully validated: the 95% confidence intervals for the slope included 1 and for the y intercept included 0. The r value for both assays was 0.99. A secondary statistical test for linearity, the runs test, did not indicate a deviation for CRP ($P = 0.74$) or SAA ($P = 0.20$). All testing was conducted in one batch to minimize intra-assay variation. SAA was detected in both clinically normal and abnormal validation samples, with the median concentration of the normal samples (6.3 mg/L) being 50-fold lower than that of the abnormal samples (317 mg/L). CRP was not detected (<0.1 mg/L) in the clinically normal validation samples, but was detected in the abnormal samples with a median concentration of 25 mg/L.

SAA and CRP assays, in addition to SPE and biuret method for total protein analysis, were performed on all serum samples. Total protein was determined using the Ortho Vitros 250 analyzer (Ortho Clinical Diagnostics, Rochester, New York 14626, USA). Electrophoresis was performed on the Helena SPIFE 3000 system using split β gels (Helena Laboratories, Beaumont, Texas 77707, USA).

Descriptive statistics (median and first and third quartiles) were calculated for the outcome (dependent) variables of APPs and protein fractions in study NESs. The null hypotheses that the distributions of APP concentrations and albumin and globulin fractions were not different between study samples classified as healthy ($n = 23$), malnourished ($n = 23$), preclinical OC–infected ($n = 12$), or clinical OC–infected ($n = 23$) were tested by using the nonparametric Kruskal-Wallis test because the data were not normally distributed. The Dunn method was used to compare mean ranks between study groups. Normal probability plots and the Shapiro-Wilk normality analysis were performed to examine whether study variables conformed to a normal distribution.

In addition, the null hypotheses that paired APP concentrations and albumin and globulin fractions were not different between study samples classified as malnourished at admission ($n = 9$) and as healthy at release ($n = 9$) were tested by using the Wilcoxon rank sign test. Finally, the null hypotheses that paired APP concentrations and albumin and globulin fractions were not different between study samples classified as preclinical OC infected ($n = 12$) and as clinical OC infected ($n = 12$) were also tested using the Wilcoxon rank sign test. In all analyses, values of $P < 0.05$ were considered significant. The commercial software Statistix 10 (Tallahassee, Florida 32312, USA) was used for data analysis.

RESULTS

Overall, larger differences in SAA concentrations were observed between samples collected from malnourished (median, 33.87 mg/L), preclinical OC–infected (246.63 mg/L), and clinical OC–infected (327.98 mg/L) NESs, compared to samples from healthy NESs (11.20 mg/L) ($P < 0.05$) (Table 1). In addition, CRP concentrations were significantly higher in samples from clinical OC–infected NESs (53.92 mg/L) compared to

Table 1. Acute-phase protein concentrations and albumin and globulin fractions in serum samples collected from northern elephant seals classified as healthy, malnourished, preclinical *Otostrongylus circumlitis* (OC)–infected, or clinical OC–infected.[a]

Variable	Healthy, $n = 23$	Malnourished, $n = 23$	Preclinical OC–infected, $n = 12$	Clinical OC–infected, $n = 23$
Serum amyloid A (mg/L)	11.20 (3.09, 14.38)^{A}	33.87 (27.09, 61.09)^{B}	246.63 (63.60, 401.69)^{B,C}	327.98 (314.00, 352.12)^{C}
C-reactive protein (mg/L)	<0.1^{A}	<0.1^{A}	0.1 (0.1, 1.78)^{A}	53.92 (31.53, 80.59)^{B}
Total protein (g/dl)	6.50 (6.30, 6.60)^{A,B}	6.20 (6.00, 6.50)^{A}	6.05 (5.82, 6.40)^{A}	6.80 (6.30, 7.20)^{B}
Albumin : globulin ratio	1.03 (0.96, 1.06)^{A}	1.26 (1.18, 1.43)^{B}	0.94 (0.81, 1.03)^{A}	0.53 (0.48, 0.64)^{C}
Albumin (%)	50.60 (48.90, 51.50)^{A}	55.70 (54.20, 58.80)^{B}	48.40 (44.67, 50.80)^{A}	34.60 (32.30, 38.90)^{C}
Albumin (g/dl)	3.27 (3.18, 3.33)^{A,B}	3.47 (3.37, 3.59)^{A}	2.91 (2.68, 3.19)^{B,C}	2.36 (2.18, 2.64)^{C}
α1 (%)	4.20 (3.70, 5.10)^{A,B}	5.10 (4.30, 6.70)^{A,C}	6.00 (4.87, 7.50)^{C}	4.00 (3.20, 4.70)^{B}
α1 (g/dl)	0.29 (0.24, 0.33)^{A,B}	0.30 (0.27, 0.45)^{A,B}	0.40 (0.28, 0.46)^{A}	0.26 (0.22, 0.32)^{B}
α2 (%)	20.90 (19.80, 22.00)^{A}	13.00 (10.90, 13.90)^{B}	18.50 (17.70, 19.37)^{A,B}	25.80 (23.20, 29.30)^{C}
α2 (g/dl)	1.35 (1.22, 1.42)^{A,B}	0.78 (0.68, 0.86)^{C}	1.09 (0.99, 1.28)^{B,C}	1.80 (1,52, 1.98)^{A}
β (%)	15.70 (15.10, 16.00)^{A}	13.50 (12.90, 14.70)^{B}	15.10 (13.75, 16.07)^{A,B}	16.10 (14.70, 16.90)^{A}
β (g/dl)	1.03 (0.93, 1.07)^{A}	0.84 (0.79, 0.90)^{B}	0.93 (0.79, 1.05)^{A,B}	1.06 (0.93, 1.22)^{A}
γ (%)	8.70 (7.30, 10.20)^{A}	11.80 (9.80, 12.50)^{B}	11.90 (10.70, 13.35)^{B,C}	16.70 (14.10, 22.40)^{C}
γ (g/dl)	0.58 (0.47, 0.64)^{A}	0.73 (0.60, 0.81)^{A}	0.69 (0.63, 0.88)^{A}	1.19 (0.93, 1.49)^{B}

[a] Data are reported as median (first and third quartiles). Within each row, groups with different superscripts are significantly ($P < 0.05$) different.

samples from preclinical OC–infected (0.01 mg/L), malnourished (0.01 mg/L), and healthy (0.01 mg/L) NESs ($P < 0.05$). Furthermore, AG ratios were significantly higher in samples from malnourished NESs (1.26), compared to samples from healthy (1.03), preclinical OC–infected (0.94), or clinical OC–infected (0.53) NESs ($P < 0.05$).

The comparison of paired samples revealed that SAA concentrations were higher in samples from malnourished NESs at the time of admission (median, 33.87 mg/L), compared to samples from healthy NESs at the time of release (3.38 mg/L) ($P < 0.01$) (Table 2). In addition, AG ratios were higher in samples from malnourished NESs at the time of admission (1.43) compared to samples from healthy NESs at the time of release (1.04) ($P < 0.01$).

The comparison of paired samples revealed that SAA concentrations were not significantly different ($P = 0.51$) between NESs with preclinical OC infections (246.63 mg/L) compared to the same NESs with clinical OC infections after development of clinical signs (327.98 mg/L) (Table 3). In addition, CRP concentrations were significantly higher in samples from clinical OC–infected NESs (66.96 mg/L), compared to samples from preclinical OC–infected NESs (0.09 mg/L) ($P < 0.01$). Finally, AG ratios were significantly lower in samples from clinical OC–infected NESs (0.56), compared to samples from preclinical OC–infected NESs (0.94) ($P < 0.01$).

Figure 1 is an example of protein electrophoretograms from each of the four groups showing the increases in α2 and γ percentages in the clinical OC–infected NESs.

DISCUSSION

In this study, commercially available reagents and automated methods were used to validate and measure serum APPs and albumin and globulin fractions in NESs. These analytes were compared in healthy, malnourished, preclinical OC–infected, and clinical OC–infected NESs with the objective of identifying a method to detect early inflammation.

SAA concentrations were significantly increased in malnourished, preclinical OC–infected, and clinical OC–infected NESs compared to healthy NESs, whereas CRP concentrations were increased only in the clinical OC–infected NESs. The marked increase in SAA concentration classifies it as a major APP, whereas CRP may only be a moderate APP in this species. APP responses to inflammation can vary based on the type of stimulus; thus, it is possible that CRP may act as a major APP in other types of inflammation in the

Table 2. Paired acute-phase protein concentrations and albumin and globulin fractions in blood serum samples collected from northern elephant seals classified as malnourished at admission and healthy at release.[a]

Variable	Malnourished at admission, $n = 9$	Healthy at release, $n = 9$	P
Serum amyloid A (mg/L)	33.87 (26.94, 77.85)	3.38 (0.57, 15.64)	< 0.01
C-reactive protein (mg/L)	0.09 (0.09, 0.09)	0.09 (0.09, 0.09)	0.99
Total protein (g/dl)	6.30 (5.95, 6.55)	6.50 (6.15, 6.60)	0.57
Albumin : globulin ratio	1.43 (1.15, 1.49)	1.04 (0.99, 1.07)	<0.01
Albumin (%)	58.90 (53.55, 59.95)	51.00 (49.60, 51.85)	<0.01
Albumin (g/dl)	3.59 (3.39, 3.90)	3.22 (3.18, 3.28)	<0.01
α1 (%)	5.30 (4.55, 6.35)	4.90 (3.65, 5.15)	0.02
α1 (g/dl)	0.32 (0.29, 0.40)	0.31 (0.24, 0.33)	0.07
α2 (%)	12.60 (10.00, 13.05)	22.00 (20.60, 22.85)	<0.01
α2 (g/dl)	0.75 (0.64, 0.81)	1.36 (1.25, 1.49)	<0.01
β (%)	13.40 (12.25, 14.60)	15.70 (14.55, 16.20)	0.16
β (g/dl)	0.87 (0.75, 0.91)	1.00 (0.87, 1.07)	0.16
γ (%)	10.90 (9.35, 11.90)	7.30 (6.25, 8.85)	<0.01
γ (g/dl)	0.74 (0.56, 0.76)	0.47 (0.40, 0.55)	<0.01

[a] Data are reported as median (first and third quartiles).

NES.[8] Another possibility for the inconsistency between SAA and CRP in this study is that the CRP reagent may be only partially cross-reactive with this species. SAA may serve as an early marker of inflammation in the NES, because this study showed greater SAA concentrations in preclinical OC–infected NESs compared to healthy NESs, indicating that SAA increased prior to the development of clinical signs.

APPs have been studied in many nondomestic species, and even though variability exists as to which APP is considered major for each, SAA expression appears to be most conserved across species.[34] Similar to the NES, SAA is the major APP in horses, many species of hoof stock, Asian elephants (*Elephas maximus*), Florida manatees (*Trichechus manatus latirostris*), and Atlantic bottlenose dolphins (*Tursiops truncatus*).[9,20,31] SAA is also a prognostic indicator and inflammatory marker in manatees with cold stress and trauma, and it has been used to monitor juvenile Asian elephants suspected to be infected with elephant endotheliotropic herpesvirus.[9,20,32] In addition, increases in SAA occur secondary to inflammation caused by parasitic infections, including mixed helminthic infections in goats and *Dictyocaulus viviparus* infections in calves.[15,36] Increases in SAA and other APPs, including ceruloplasmin and haptoglobin, are associated with *Dirofilaria immitis* in seropositive domestic cats.[29] In contrast

Table 3. Paired acute-phase protein concentrations and albumin and globulin fractions in blood serum samples collected from northern elephant seals classified as preclinical *Otostrongylus circumlitis* (OC) infected and clinical OC infected.[a]

Variable	Preclinical OC–infected, $n = 12$	Clinical OC–infected, $n = 12$	P
Serum amyloid A (mg/L)	246.63 (63.60, 401.69)	327.98 (315,96, 354.41)	0.51
C-reactive protein (mg/L)	0.09 (0.09, 1.78)	66.96 (37.59, 93.43)	<0.01
Total protein (g/dl)	6.05 (5.82, 6.40)	7.05 (6.37, 7.35)	0.01
Albumin : globulin ratio	0.94 (0.81, 1.03)	0.56 (0.48, 0.69)	<0.01
Albumin (%)	48.40 (44.67, 50.80)	36.15 (32.35, 40.90)	<0.01
Albumin (g/dl)	2.91 (2.68, 3.19)	2.49 (2.28, 2.66)	<0.01
α1 (%)	6.00 (4.87, 7.50)	3.50 (3.02, 4.52)	<0.01
α1 (g/dl)	0.40 (0.28, 0.46)	0.24 (0.20, 0.27)	<0.01
α2 (%)	18.50 (17.70, 19.37)	25.70 (23.67, 29.37)	<0.01
α2 (g/dl)	1.09 (0.99, 1.28)	1.82 (1.48, 2.16)	<0.01
β (%)	15.10 (13.75, 16.07)	16.30 (14.72, 17.35)	0.04
β (g/dl)	0.93 (0.79, 1.05)	1.13 (1.03, 1.22)	<0.01
γ (%)	11.90 (10.70, 13.35)	15.75 (13.65, 21.10)	<0.01
γ (g/dl)	0.69 (0.63, 0.88)	1.12 (0.93, 1.41)	<0.01

[a] Data are reported as median (first and third quartiles).

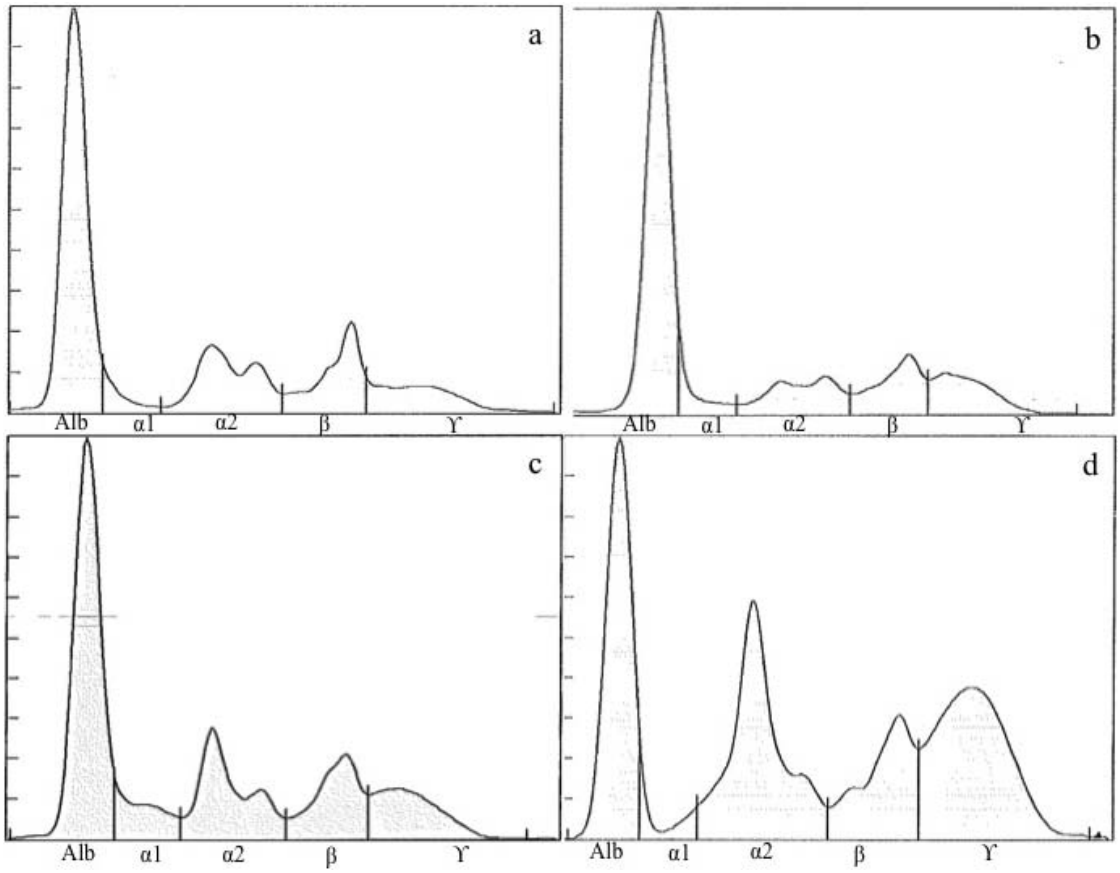

Figure 1. Example of protein electrophoretograms from healthy (**a**), malnourished (**b**), preclinical *Otostrongylus*–infected (**c**), and clinical *Otostrongylus*–infected (**d**) juvenile northern elephant seals. (Alb = albumin).

to the NES, CRP is a major APP, whereas SAA is a moderate APP in rhesus macaques.[25] Despite close taxonomic relation to the NES, CRP is considered the major APP in harbor seals; however, no reports were found regarding SAA assay cross-reactivity in this species.[8,22]

Despite the SAA median concentration of preclinical OC–infected NESs being sevenfold higher than that of the malnourished group, the difference was not statistically significant. This supports that SAA is a nonspecific biomarker for early inflammatory disease, not a specific diagnostic tool. Further studies measuring APP concentrations in NESs with other inflammatory diseases such as pneumonia, septicemia, and trauma would help elucidate how SAA and CRP concentrations measure and trend in inflammatory diseases of various etiologies.

In this study, AG ratios were decreased in clinical OC–infected NESs compared to the healthy NESs. This is consistent with other studies in which AG ratios decrease during inflammatory conditions such as trauma and cold stress in Florida manatees, clinical mastitis in cows, and babesiosis in dogs.[9,26,28] Albumin as a negative APP decreases during inflammation because there is decreased production by hepatocytes in response to interleukins 1 and 6 in acute inflammation.[32] This decrease in albumin temporarily increases availability of circulating hormones and other molecules usually bound to albumin, which presumably supports the body's response to the inflammatory stimulus.[17] The decreased AG ratio could be due to the decreased albumin concentration in clinical OC–infected NESs compared to healthy NESs and/or to the increased γ globulin concentration noted in clinical OC–infected NESs compared to healthy NESs. The increase in γ globulins may be attributed to increases in other proteins such as immunoglobulins in response to inflammation.[32]

The increased median AG ratio of malnourished compared to preclinical OC–infected NESs is hypothesized to be associated with concurrent

dehydration and thus hemoconcentration in malnourished NESs, resulting in a greater albumin percentage. In a clinical setting, dehydration and an increased AG ratio would be expected in emaciated NESs upon admission to TMMC; however, with a concurrent acute-phase response due to inflammation, the AG ratio may not be elevated as expected and in fact be uncharacteristically normal or low. Thus, a reduced AG ratio may serve as a tool to help differentiate between an NES with subclinical inflammation and a solely malnourished NES.

Analysis of the study's paired samples supported the changes seen in analysis of all study samples. SAA decreased from time point of admission to release. NESs that were malnourished upon admission may have had inflammation from occult disease that was less severe than preclinical or clinical OC infection. In animals that were declared healthy for release, SAA concentrations likely decreased along with inflammatory stimuli in response to supportive medical care during rehabilitation. Additionally, AG ratios were lower in samples collected at the time of release than at the time of admission, which was likely because of resolution of dehydration and hemoconcentration after supportive care. When comparing paired samples of preclinical OC–infected NESs to clinical OC–infected NESs, the SAA concentration did not change, whereas the CRP concentration increased. This supports that although CRP increased with the inflammatory condition of OC infection, it does not act as an early inflammatory marker for this specific disease. Finally, AG ratios decreased from the preclinical to the clinical OC–infected state, likely because of increased severity of inflammation during disease progression.

Tracking the APP concentrations and albumin and globulin fractions throughout the course of OC infection in NESs was not feasible in this study because of its retrospective nature, and correlating changes with time spent in rehabilitation and additional variables were beyond the scope of this study. Experimental infection with sample collection at defined serial time points throughout all stages of infection would provide the most accurate data to determine APP activity throughout the disease process; however, this cannot be ethically performed in NESs.

Unfortunately, there is no antemortem diagnostic method for OC infections in the NES. A fecal ELISA test, using bovine lungworm antigen, was validated for detection of OC in both harbor and gray seals, but it was not effective for NESs for reasons not currently understood.[35] Definitive diagnosis is accomplished only by identifying the presence of OC nematodes in the right heart, pulmonary vasculature, and airways on gross necropsy. Unlike in Pacific harbor seal, NESs die very quickly after infection and thus appear to be a nondefinitive host for this parasite.[18] The severe arteritis and systemic inflammation that occur in response to OC infection in NESs could be related to low genetic diversity secondary to mass hunting in the 19th century causing a bottleneck effect, and/or to a recent host–parasite association in which the NES has not yet adapted to survive OC infection and the parasite is not able to reliably complete its life cycle.[16,33]

This study's results show that SAA is a useful early inflammatory marker in the NES, using OC infection as an example of a severe inflammatory disease of this species. APP assays and SPE are nonspecific tools that are best utilized in conjunction with other diagnostic techniques, but may assist clinicians in decisions to initiate earlier appropriate treatment and in monitoring the patient's response to treatment. Further studies investigating the acute-phase response of NESs with other inflammatory diseases are warranted.

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LITERATURE CITED

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