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REVIEW ARTICLE

Acute phase reactants in nondomesticated mammals—A veterinary clinical pathology perspective

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Abstract

Applications for acute phase reactants (APRs) in nondomesticated mammals include identifying inflammatory disease, monitoring the course of specific disease processes and recovery during rehabilitation, detecting preclinical or subclinical disease, being used as bioindicators for monitoring population and ecosystem health, and as markers of stress and animal welfare. Serum amyloid A, haptoglobin, C-reactive protein, fibrinogen, albumin, and iron are most commonly measured. The procedure for evaluating an APR in a nondomesticated mammalian species should follow a stepwise approach beginning with an assessment of analytical performance, followed by an evaluation of overlap performance, clinical performance, and impact on patient outcomes and management. The lack of species-specific standards and antibodies for nondomesticated mammals presents a challenge, and more attention needs to be focused on assessing cross-reactivity and ensuring adequate analytical performance of APR assays. Sample selection for the initial evaluation of APRs should consider preanalytical influences and should originate from animals with confirmed inflammatory disease and healthy animals. Reference intervals should be generated according to published guidelines. Further evaluation should focus on assessing the diagnostic utility of APRs in specific disease scenarios relevant to a species. Greater attention should be paid to assay performance and uniformity of methods when using APRs for population and ecosystem surveillance. Veterinary clinical pathologists should work closely with zoo veterinarians and wildlife researchers to optimize the accuracy and utility of APR measurements in these various conservation medicine scenarios.

KEYWORDS
albumin, C-reactive protein, fibrinogen, haptoglobin, iron, serum amyloid A, wildlife, zoo animals

1 | INTRODUCTION

Acute phase reactants (APRs) have been used to monitor health, diagnose inflammatory disease, and track response to treatment in domesticated animals for several decades. Measurement of APRs has now become part of routine clinical chemistry testing for companion animals in many areas of the world, as earlier reviews have indicated.[1,2] The increased use of APRs in domesticated animals has been paralleled by an increasing number of studies detailing the measurement and application of APRs in nondomesticated mammals.

An important paper by Bertelsen et al was one of the first to address the possibility of a more widespread use of APR reagents in nondomesticated mammals.[3] This was followed by a multitude of studies that attempted to identify useful assays, characterize the acute phase response, investigate the dynamics of the innate immune system in specific disorders, and examine the clinical usefulness of APRs as markers of inflammatory, infectious, and parasitic diseases. In many instances, there is a "situational" application of APRs; for some species, the use of APRs can aid in addressing specific health issues unique to the species. In addition, the use of APRs in ecoimmunology and wildlife conservation has grown, and these biomarkers have also been investigated as indicators of animal welfare. As in domesticated species, serum amyloid A and C-reactive protein are moderate or major APRs, haptoglobin a minor, moderate or major APR, fibrinogen a minor APR, and albumin and iron negative APRs.[1,2]

This narrative review aims to provide an overview of the literature published in the last three decades concerning APRs in nondomesticated mammals. An open search was performed using PubMed and Google Scholar using the terms "acute phase," "serum amyloid A," "C-reactive protein," "albumin," "fibrinogen," "inflammatory," "inflammation," "zoo," "wild," "wildlife," "non-domestic" for the period from 1990 to 2022. The authors first reviewed the abstracts of retrieved articles in order to identify articles of interest. All peer-reviewed articles were initially considered, including case series and case reports containing information about APR measurement in a nondomestic mammal. Articles that did not provide any information about assay methodology were subsequently excluded.

Analytical methods, assay validation, and overlap performance are discussed in the first section. This is followed by an overview of the diagnostic utility of APR assays and an exploration of the applications of these biomarkers in nondomesticated mammals. Lastly, challenges and suggestions for future directions are outlined in a bid to motivate veterinary clinical pathologists to become more involved in this field of conservation medicine. The readers are reminded that the study of APR in nondomesticated mammals is evolving and to conduct a thorough literature search for species-specific information when needed. Also, we acknowledge there are other review papers and a growing literature on APR in avian, reptile, teleost, and small exotic mammal species.[4–7]

2 | ANALYTICAL METHODS

2.1 | Assay validation

Various techniques have been used to measure acute phase reactants in nondomesticated mammals (Tables 1-4). Plasma or serum protein electrophoresis (SPE) can also be used to detect changes in major acute phase proteins; this has been extensively reviewed elsewhere.[8] Colorimetric assays have been used for the measurement of some APRs like haptoglobin and serum iron. Antibody-based assays for the determination of specific acute phase proteins are available in various formats, including ELISA and immunoturbidimetry. Newer technology using spatial proximity analyte reagent capture luminescence (SPARCL), a semi-automated homogenous chemiluminescent technique, which offers a rapid and cost-effective alternative to traditional ELISA, has not been widely validated.[9]

As with any biomarker, the process of evaluating an APR in a novel species should ideally follow a stepwise approach: this begins with an assessment of analytical performance, followed by evaluation of overlap performance, clinical performance, and lastly, impact on patient outcomes and management.[10] Determination of analytical and overlap performance must be conducted first when using immunoassays in novel species as antibody cross-reactivity is initially unknown and will vary from species to species.

Assessment of analytical performance, that is, assay validation or verification, is completed to confirm precise and accurate quantitation of APRs in specimens from the species of interest. Assay validation experiments typically include the assessment of cross-reactivity, linearity/reportable range, repeatability/short-term imprecision, reproducibility/long-term imprecision, interference, recovery, detection limit, and comparison to another method, if available.[11] In some basic science studies, mRNA sequencing and measurement of mRNA expression in various tissues has been used to aid in the identification of major APR and serum amyloid A (SAA) isoforms.[12,13] In other instances, possible reagents and APR may be targeted based on phylogenetic relationships (eg, investigating SAA in zebra and Przewalski's horses using reagents validated for SAA in domesticated horses, as SAA is the major APR in that species).[14,15] Assessment of cross-reactivity (ie, whether the antibody in the assay recognizes and binds to the protein of interest) is usually performed informally before assay validation experiments by running a low number of samples from healthy animals and those with overt inflammatory disease. Occasionally Western blotting is performed.[16,17] Information on lack of cross-reactivity is rarely published but may be available from manufacturers of APR assays or authors who have published widely in the field.[3,14,18] It is important to note that even if cross-reactivity is present, it may vary from weak to strong, and results probably will not reflect true concentrations of the APR in that species. The degree of cross-reactivity may differ between assays, and results from different methods are often not comparable.[10,19]

Full assay validation has not been performed for any APR assay in nondomesticated mammal species, and some studies provide no information about assay validation.[16–18,20–29] When validation has been performed, this usually only includes imprecision and linearity experiments,[22,30–38] sometimes with additional recovery or limit of detection studies[13,39–46] (Tables 1-4). Incomplete assay validation may occur due to a lack of knowledge concerning the need for assay validation, or experiments performed but not published. In addition, samples from wildlife species are often low in volume or difficult to acquire. However, determining short-term imprecision and linearity using species serum pools should be considered compulsory, as these studies provide preliminary information on precision and accuracy of the assay using the species matrix, as well as the lowest and highest results that can be reported.[11] If results are acceptable,

TABLE 1 Serum amyloid A assay methodology, validation status, reference data and overlap performance in non-domesticated mammals. If publications presented data for different age groups, then results for adults are presented. Reference intervals are presented if at least the statistical methods used were in line with guidelines.

Species	Methodology	Assay details	Analytical validation	Results from healthy animals (n; mean ± SD or range)	Significant differences healthy vs diseased	Reference intervals (n; interval)
Arabian oryx (*Oryx leucoryx*)[20]	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	None	11; 2.0-5.6 mg/L	Not evaluated	No
Bison, European (*Bison bonasus*)[46]	Multispecies ELISA	PHASE SAA, Tridelta, Ireland	L, RPT, RPD, LOD	40; 12.4-30.1 mg/L[a]	Yes	No
Blackbuck (*Antilope cervicapra*)[20]	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	None	10; 3.2-4.7 mg/L	Not evaluated	No
Buffalo, African (*Syncerus caffer*)[21]	Anti-bovine ELISA	SAA-11, Life Diagnostics, USA	None	Not given	Yes	No
Cheetah (*Acinonyx jubatus*)[16]	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	Western blot	See reference intervals	Yes	47; 23.6-57.0 mg/L
Deer, white-tailed (*Odocoileus virginianus*)[53]	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	L, RPT, RPD, SR	See reference intervals	Yes	60; <5 mg/L
Dolphin, bottlenose (*Tursiops truncatus*)[12,30,56]	Multispecies ELISA	Biosource, USA	No cross-reactivity			
	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	RPT	See reference intervals	Not evaluated	44; 17.5-42.9 mg/L
	Anti-dolphin ELISA	SAA-18, Life Diagnostics, USA	None	0.15-5.98 mg/L	No	No
Elephant, African (*Loxodonta africana*)[23,36]	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	L, RPT	See reference intervals	Yes	Managed care: 123; 0.1-6.9 mg/L Free- living: 43; <10.0 mg/L
Elephant, Asian (*Elephas maximus*)[32,41]	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	L, RPT, RPD, SR	See reference intervals	Yes	98; 0.1-37.6 mg/L 35; 0-47.5 mg/L
Ibex, Iberian (*Capra pyrenaica*)[42,86]	Multispecies ELISA	PHASE SAA, Tridelta, Ireland	L, RPT, RPD, LOD	See reference intervals	Yes	40; <29.5 mg/L
Ibex, Alpine (*Capra ibex*)[17]	Multispecies ELISA	PHASE SAA, Tridelta, Ireland	Western blot	15; 8.7 ± 0.13 mg/L	Yes	No
Impala (*Aepyceros melampus*)[20]	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	None	29; 2.6-4.1 mg/L	Not evaluated	No
Koala (*Phascolarctos cinereus*)[38]	Multispecies ELISA	PHASE SAA, Tridelta, Ireland	RPD	See reference intervals	Not evaluated	26; 0.1-0.5 mg/L
Manatee, Florida (*Trichecus manatus latirostris*)[18,43,69]	Anti-bovine ELISA	PHASE anti-bovine SAA, Tridelta, Ireland	None	See reference intervals	Not evaluated	71; <50.0 mg/L
	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	L, RPT, RPD, SR	See reference intervals	Yes	34; <46.0 mg/L
Pronghorn antelope (*Antilocapra americana*)[37]	Immuno-turbidimetric	SAA-1[b], Eiken, Japan	L, RPT	18; 0.1–11.8 mg/L (managed care)	No	45; <0.1 mg/L (free-living)
Rhesus macaque (*Macaca mulatta*)[33]	Immuno-turbidimetric	SAA-1[b], Eiken, Japan	RPT	See reference intervals	Yes	97; 29.5-87.7 mg/L
Rhinoceros, black (*Diceros bicornis*)[44]	Multispecies ELISA	PHASE SAA, Tridelta, Ireland	L, RPT, RPD, SR	See reference intervals	Yes	104; <1 mg/L

TABLE 1 Continued

Species	Methodology	Assay details	Analytical validation	Results from healthy animals (n; mean ± SD or range)	Significant differences healthy vs diseased	Reference intervals (n; interval)
Rhinoceros, white (*Ceratotherium simum*)[34]	Multispecies ELISA	PHASE SAA, Tridelta, Ireland	L, RPT	See reference intervals	Yes	23; <20 mg/L
Seal, northern elephant (*Mirounga angustirostris*)[35]	Immuno-turbidimetric	SAA-1[b], Eiken, Japan	L	23; 3.1-14.4 mg/L[a]	Yes	No
Zebra, Grant's (*Equus burchelli*)[14]	Immuno-turbidimetric	LZ-SAA, Eiken, Japan	L, RPT	See reference intervals	Yes	26; 1.8-31.4 mg/L

Abbreviations: L, linearity; LOD, limit of detection; RPD, reproducibility; RPT, repeatability; SR, spike and recovery.

[a]Interquartile range.

[b]Eiken SAA-1 now available as VET-SAA, Eiken, Japan.

[Correction added on 24 November, 2022, after first online publication: Caption of Table 1 changed to ` Serum amyloid A assay methodology, validation status, reference data and overlap performance in non-domesticated mammals. If publications presented data for different age groups, then results for adults are presented. Reference intervals are presented if at least the statistical methods used were in line with guidelines.']

determination of the limit of detection and recovery studies can be considered. Protocols for all these experiments are well-described in the most recent version of the American Society for Veterinary Clinical Pathology Guidelines: Principles of Quality Assurance and Standards for Veterinary Clinical Pathology.[11] Evaluation of assay validation data is completed by comparing results for imprecision, inaccuracy, and total analytical error to quality performance goals, or total allowable error. Consensus-based total allowable error goals have been published for hematology and clinical chemistry measurands in domesticated species but are less accessible for APRs.[10,47,48] Performance goals derived from estimates of biological variation are available for selected APRs in dogs, and total allowable error based on analytical performance and consensus between the two authors of this review has been suggested for SAA (Table 5).[34,49] Although not ideal, these could be useful when evaluating validation results for APR assays in zoo and wildlife species.[34,36]

After assay validation, the ability of the assay to distinguish between animals with and without inflammation must be investigated—that is, a lack of significant overlap in results between these groups should be present. It has been recommended that 20–30 animals be included in each group, and that the presence or absence of inflammation is clear, based on clinical signs, other laboratory testing, or postmortem findings.[10] Evaluation of overlap performance is commonly performed in nondomesticated mammal APR studies, as noted in Tables 1-4.[13,14,16,22,23,32–37,39,40,42–46,50] We recommend that to optimize this stage, clinical pathologists work closely with clinicians to gather information about the nature of the putative inflammatory disease and sample handling, such as sample type, storage conditions, number of freeze-thaw cycles, diagnosis, chronicity of disease, a sample taken before or during treatment, availability of repeated samples from one animal. Determination of whether an APR increases or decreases in inflammation and the degree of increase assists in classifying the APR as a major, moderate, or minor, positive or negative APR.[1,2]

Evaluating the diagnostic performance and clinical usefulness of an APR assay should subsequently be performed.[10] For this phase, it is important to understand what the most common diseases affecting a species are and whether these are inflammatory in nature. If the major condition for a species is not infectious or inflammatory, but rather degenerative (eg, chronic kidney disease or neoplasia), then APRs are not likely helpful in diagnosing that disease. Examples of the diagnostic performance of APRs in selected diseases and species are further reviewed later in this publication.

2.2 | Serum amyloid A

The serum amyloid A (SAA) gene is well-conserved across vertebrate taxa, and unsurprisingly, SAA is a major APR in many nondomesticated mammal species.[51] However, SAA isoform expression varies by species. For example, the bottlenose dolphin expresses SAA-3 as the dominant isoform vs SAA-1 and SAA-2, which appear to be dominant in most other mammals.[12,52] The species-specific

TABLE 2 Haptoglobin assay methodology, validation status, reference data, and overlap performance in nondomesticated mammals. If publications presented data for different age groups, then results for adults are presented. Reference intervals are presented if at least the statistical methods used were in line with guidelines.

Species	Methodology	Assay details	Analytical validation	Results from healthy animals (n; mean ± SD or range)	Significant differences healthy vs diseased	Reference intervals (n; interval)
Bison, European (*Bison bonasus*)[46]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPT, RPD, LOD	40; 0.1-0.2 g/L[a]	Yes	No
Bongo (*Tragelaphys eurycerus*)[22]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L	See reference intervals	No	37; 0.1-1.6 g/L
Buffalo, African (*Syncerus caffer*)[21]	Anti-bovine ELISA	Hapt-11, Life Diagnostics, USA	None	Not given	Yes	No
Capybara (*Hydrochoerus hydrochaeris*)[39]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPT, RPD, LOD	30; 0-6 g/L	Yes	No
Cheetah (*Acinonyx jubatus*)[16]	Capillary serum protein electrophoresis	CapillarysTM 2 CE, Sebia, France	None	See reference intervals	Yes	47; 1.6-6.3 g/L
Deer, red (*Cervas elaphus*)[40]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPT, RPD, LOD	0.17-0.42 g/L[a]	Yes	No
Dolphin, bottlenose (*Tursiops truncatus*)[13,30]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	RPT, RPD, LOD	46; <1.56 g/L	Yes	44; <0.4 g/L
	Anti-porcine ELISA	Immunology Consultants Lab Inc, USA	Western blot, RPT, RPD, LOD	46; 0.04-1.96 g/L	Yes	No
Elephant, African (*Loxodonta africana*)[23,36]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPT	See reference intervals	Variable results	Managed care: 124; 0.2-2.4 g/L Free-living: 43; 0.2-3.5 g/L
Elephant, Asian (*Elephas maximus*)[23,32,41]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPT, RPD, SR	See reference intervals	Variable results	100; 0.2-4.0 g/L 35; 0-1.1 g/L
Ibex, Iberian (*Capra pyrenaica*)[42,86]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPT, RPD, LOD	See reference intervals	Yes	40; 0.12-0.64 g.L
Ibex, Alpine (*Capra ibex*)[17]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	None	15; 0.58 ± 0.09	Yes	No
Koala (*Phascolarctos cinereus*)[38]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPD	See reference intervals	Not evaluated	26; 0.1-0.6 g/L
Manatee, Florida (*Trichecus manatus latirostris*)[18,69]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	None	72; 0.2-2.5 g/L	Not evaluated	71; 0.4-2.5 g/L
	Immunoturbidometric	Tina-quant Haptoglobin, Roche, USA	No cross-reactivity			
Pronghorn antelope (*Antilocapra americana*)[37]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPT	18; 0-3.1 g/L (managed care)	Yes	45; 0.3-1.1 g/L (free-living)
Rhesus macaque (*Macaca mulatta*)[33]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	RPT	See reference intervals	Yes	110; 0.4-2.4 g/L
Rhinoceros, white (*Ceratotherium simum*)[34,45]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPT, RPD	See reference intervals	Yes	47; 1.0-4.3 g/L
	Anti-human ELISA	Self-developed using antibody from DAKO, Denmark	L, RPT, RPD, LOD, effect of hemolysis	<3000 arbitrary units (managed care) <300 arbitrary units (free-living)	Yes	No
River otter (*Lutra canadensis*)[24,94]	Hemoglobin bound protein - agarose gel electrophoresis	Helena Laboratories, USA	None	6; 3.0 ± 0.9 g/L 11; 3.0 ± 1.6 g/L	Yes	No

TABLE 2 Continued

Species	Methodology	Assay details	Analytical validation	Results from healthy animals (n; mean ± SD or range)	Significant differences healthy vs diseased	Reference intervals (n; interval)
Sea lion, Steller (*Eumetopias jubatus*)[25,29,58]	Hemoglobin bound protein - agarose gel electrophoresis	Helena Laboratories, USA	None	5; 1.43 ± 0.13 g/L (Southeast Alaska, USA) 14; 2.51 ± 0.21 (Gulf of Alaska, USA)	No	No
	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	None	1272; 0.1-5.1 g/L	Not evaluated	No
Seal, harbor (*Phoca vitulina*)[25,26,65]	Hemoglobin bound protein - agarose gel electrophoresis	Helena Laboratories, USA	None	22; 0.83 ± 0.11 g/L (Southeast Alaska, USA) 1.33 ± 0.11 g/L (Prince William Sound, USA)	Not evaluated	No
	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	None	123; 0.51 ± 0.41 185; 0-1.5 g/L	Not evaluated	No
Zebra, Grant's (*Equus burchelli*)[14]	Colorimetric	PHASE Haptoglobin, Tridelta, Ireland	L, RPT	See reference intervals	Yes	26; 0.4-1.6 g/L

Abbreviations: L, linearity; LOD, limit of detection; RPD, reproducibility; RPT, repeatability; SR, spike and recovery.
[a]Interquartile range.

dominant isoform is generally expressed in the liver (and present in circulation), other isoforms may be expressed extrahepatically.[52] The amino acid sequence of isoforms varies, with 64%–89% homology in major species; this is understandably not well studied in most wildlife species.[12,51,52] An assay that is not specific for the species of interest, and uses only monoclonal antibodies derived for another species, may not show cross-reactivity for SAA due to the variation in isoforms.[12]

Serum amyloid A has been measured using either automated immunoturbidometric assays or ELISA methods (Table 1). A latex agglutination immunoturbidometric method using a mix of rabbit polyclonal and mouse monoclonal anti-human SAA antibodies (LZ-SAA, Eiken, Japan) has been used most often and has been minimally or partially validated for measurement of SAA in serum of white-tailed deer, bottlenose dolphin, Asian and African elephants, Iberian ibex, Florida manatee, and Grant's zebra.[14,30,32,36,42,43,53] This assay has also been used to detect SAA, without any documentation of assay validation procedures, in healthy Arabian oryx, blackbuck, impala, reindeer, and healthy and diseased cheetah, musk ox, and wallaby.[3,16,20] The LZ-SAA has been reported not to detect SAA in white rhinoceros and sitatunga.[3,34] A new immunoturbidometric assay using only monoclonal antibodies (VET-SAA, Eiken, Japan) has recently been released and has been well validated in horses.[54] A prototype of this assay (SAA-1, Eiken, Japan), has been partially validated for use in northern elephant seals, Pronghorn antelope, and Rhesus macaques.[33,35,37] Based on the authors' experience, the VET-SAA method also shows promising cross-reactivity with SAA in additional species not covered by LZ-SAA. However, this still does not represent pan-reactivity in all mammals, and a step-by-step validation process for this assay should be followed in all novel species.

A multispecies ELISA (PHASE SAA, Tridelta, Ireland) has been successfully used to measure SAA in koala, European bison, Iberian and Alpine ibex, and black and white rhinoceros and shows cross-reactivity in impala, musk ox, reindeer, and wallaby but not eleph ants.[3,17,34,38,42,44,46,55] A commercially available dolphin-specific ELISA has been used in several studies on this species (SAA-18, Life Diagnostics, USA) as dolphin SAA does not cross-react well with multispecies assays.[12,50,56,57] Species-specific reagents and calibrators make for attractive options for research-based studies but mostly do not translate well for use in clinical laboratories where automation and a limited menu of reagents to maintain are clearly preferable. In addition, researchers should determine species-specific limits of quantification when using ELISAs, as low SAA concentrations are often associated with an unacceptably high analytical imprecision.[34]

Using these various assays, SAA results have been shown to be higher in diseased compared to healthy animals of several species, confirming it to be a moderate to major positive APR (Table 1).

2.3 | Haptoglobin

Haptoglobin is a moderate or minor positive APR in many species, with a lack of overlap between healthy and diseased animals

TABLE 3 Assay methodology, validation status, reference data, and overlap performance for C-reactive protein and fibrinogen in nondomesticated mammals. If publications presented data for different age groups, then results for adults are presented. Reference intervals are presented if at least the statistical methods used were in line with guidelines.

Measurand	Species	Methodology	Assay details	Analytical validation	Results from healthy animals (n; mean ± SD or range)	Significant differences healthy vs diseased	Reference intervals (n; interval)
C-reactive protein	Cheetah (*Acinonyx jubatus*)[16]	Turbidimetric	Tugirimana method[95]	None	See reference intervals	No	47; 3.9-58.5 mg/L
	Dolphin, bottlenose (*Tursiops truncatus*)[30]	Immuno-turbidimetric, canine calibrator	CRP, Randox, USA	RPT	See reference intervals	Not evaluated	44; 1.4-19.7 mg/L
	Elephant, Asian (*Elephas maximus*)[32]	Immuno-turbidimetric, canine calibrator	CRP, Randox, USA	RPT	See reference intervals	No	35; 1.3-12.8 mg/L
	Koala (*Phascolarctos cinereus*)[38]	Immuno-turbidimetric	CRP, Randox, USA	L, RPD	See reference intervals	Not evaluated	26; 3.2-24.1 mg/L
	Manatee, Florida (*Trichecus manatus latirostris*)[18]	Anti-porcine ELISA	PHASE Porcine CRP, Tridelta, Ireland	No cross-reactivity			
		Immuno-turbidimetric	Tina-quant CRP, Roche, USA	No cross-reactivity			
	Rhesus macaque (*Macaca mulatta*)[33]	Immuno-turbidimetric, canine calibrator	CRP, Randox, UK	RPT	See reference intervals	Yes	110; 0-17.5 mg/L
	Seal, harbor (*Phoca vitulina*)[64,65]	Immuno-turbidimetric	OSR6147, Olympus, Germany	None	120; 8-99 mg/L	Not evaluated	No
		Anti-harbor seal ELISA	In-house[65]	None	No	Not evaluated	No
	Seal, northern elephant (*Mirounga angustirostris*)[35]	Immuno-turbidimetric, canine calibrator	CRP, Randox, USA	L	23; <0.1 mg/L	Yes	No
Fibrinogen	Arabian oryx (*Oryx leucoryx*)[20]	Clauss method	Thrombotrack 1, Axis-Shield, Norway	None	5; 1.7-6.1 g/L	Not evaluated	No
	Blackbuck (*Antilope cervicapra*)[20]	Clauss method	Thrombotrack 1, Axis-Shield, Norway	None	7; 1.6-6.8 g/L	Not evaluated	No
	Impala (*Aepyceros melampus*)[20]	Clauss method	Thrombotrack 1, Axis-Shield, Norway	None	15; 1.8-3.8 g/L	Not evaluated	No
	Manatee, Florida (*Trichecus manatus latirostris*)[18]	Heat precipitation	Refractometer	None	See reference intervals	Not evaluated	27; 1.0-4.0 g/L
	Rhinoceros, white (*Ceratotherium simum*)[34]	Modified Clauss method	ACL Elite, Instrumentation Laboratory, Germany	None	See reference intervals	Yes	47; 1.7-2.9 g/L
	Seal, leopard (*Hydrurga leptonyx*)[66]	Heat precipitation	Refractometer	None	29; 0.2-2.8 g/L	No	No

Abbreviations: L, linearity; LOD, limit of detection; RPD, reproducibility; RPT, repeatability; SR, spike and recovery.

TABLE 4 Assay methodology, validation status, reference data, and overlap performance for albumin and serum iron in nondomesticated mammals. If publications presented data for different age groups, then results for adults are presented. Reference intervals are presented if at least the statistical methods used were in line with guidelines.

Measurand	Species	Methodology	Assay details	Analytical validation	Results from healthy animals (n; mean ± SD or range)	Significant differences healthy vs diseased	Reference intervals (n; interval)
Albumin	Bongo (*Tragelaphys eurycerus*)[22]	Agarose gel serum protein electrophoresis	Helena Laboratories, USA	None	See reference intervals	No	37; 18-38 g/L
	Capybara (*Hydrochoerus hydrochaeris*)[39]	Bromocresol green	Spinreact, Spain	L, RPT, RPD, LOD	30; 26-33 g/L	Yes	No
	Cheetah (*Acinonyx jubatus*)[16]	Capillary serum protein electrophoresis	CapillarysTM 2 CE, Sebia, France	None	See reference intervals	No	47; 34-51 g/L
	Deer, white-tailed (*Odocoileus virginianus*)[53]	Agarose gel serum protein electrophoresis	Helena Laboratories, USA	None	See reference intervals	Not evaluated	60; 34-46 g/L
	Dolphin, bottlenose (*Tursiops truncatus*)[50,71]	Agarose gel serum protein electrophoresis	Helena Laboratories, USA	RPT	44; 28-52 g/L	No	No
	Elephant, African (*Loxodonta africana*)[31,36]	Bromocresol green	VetScan VS2, Abaxis, USA	RPT	See reference intervals	Yes	50; 41-55 g/L
	Elephant, Asian (*Elephas maximus*)[32]	Agarose gel serum protein electrophoresis	Helena Laboratories, USA	None	See reference intervals	No	35; 29-47 g/L
	Koala (*Phascolarctos cinereus*)[38]	Agarose gel serum protein electrophoresis	Helena Laboratories, USA	None	See reference intervals	No	26; 27-38 g/L
	Manatee, Florida (*Trichecus manatus latirostris*)[69]	Agarose gel serum protein electrophoresis	Paragon Electrophoresis System, Bechman-Coulter, USA	None	112; 25-47 g/L	No	No
	Pronghorn antelope (*Antilocapra americana*)[37]	Agarose gel serum protein electrophoresis	Helena Laboratories, USA	None	18; 15-41 g/L (managed care)	No	45; 38-52 g/L (free-living)
	Rhesus macaque (*Macaca mulatta*)[33]	Bromocresol green	Advia 1800, Siemens Healthcare, Denmark	RPT	See reference intervals	Yes	105; 36-53 g/L
	Rhinoceros, black (*Diceros bicornis*)[76]	Agarose gel serum protein electrophoresis	Hyrys2, Sebia, France	None	15; 15-38 g/L	Not done	No
	Rhinoceros, white (*Ceratotherium simum*)[34,76]	Agarose gel serum protein electrophoresis	Hyrys2, Sebia, France	None	28; 13-40 g/L	Not done	No
		Bromocresol green	Cobas Integra 400 Plus, Roche, Switzerland	None	See reference intervals	Yes	48; 18-31 g/L
		Agarose gel serum protein electrophoresis	Interlab Pretty, Interlab, Italy	RPT, RPD	See reference intervals	Yes	49; 10-27 g/L
	Seal, leopard (*Hydrurga leptonyx*)[66]	Agarose gel serum protein electrophoresis	Helena Laboratories, Australia	None	28; 27-41 g/L	Not done	No
		Bromocresol green	Olympus AU 400, Olympus, Australia	None	29; 24-37 g/L	Not done	No
	Seal, northern elephant (*Mirounga angustirostris*)[35]	Agarose gel serum protein electrophoresis	Helena Laboratories, USA	None	23; 33-33 g/L[a]	Yes	No
	Zebra, Grant's (*Equus burchelli*)[14]	Agarose gel serum protein electrophoresis	Helena Laboratories, USA	None	See reference intervals	No	26; 14-33 g/L

TABLE 4 Continued

Measurand	Species	Methodology	Assay details	Analytical validation	Results from healthy animals (n; mean ±SD or range)	Significant differences healthy vs diseased	Reference intervals (n; interval)
Iron	Elephant, African (*Loxodonta africana*)[23,36]	Ferrozine (colorimetric)	Dimension Xpand Plus, Siemens, USA	None	See reference intervals	Not evaluated	120; 6.3-21.0 µmol/L
		Ferrozine (colorimetric)	Cobas Integra 400 Plus, Roche, Switzerland	None	See reference intervals	Yes	43; 8.6-17.0 µmol/L
	Elephant, Asian (*Elephas maximus*)[23]	Ferrozine (colorimetric)	Dimension Xpand Plus, Siemens, USA	None	See reference intervals	Not evaluated	100; 4.0-16.6 µmol/L
	Rhesus macaque (*Macaca mulatta*)[33]	Ferrozine (colorimetric)	Advia 1800, Siemens Healthcare, Denmark	RPT	See reference intervals	Yes	105; 13.3-40.2 µmol/L
	Rhinoceros, black (*Diceros bicornis*)[76]	Ferrozine (colorimetric)	RX Daytona, Randox, UK	None	15; 26.6-58.9 µmol/L	Not evaluated	No
	Rhinoceros, white (*Ceratotherium simum*)[34,76]	Ferrozine (colorimetric)	RX Daytona, Randox, UK	None	29; 11.1-58.4 µmol/L	Not evaluated	No
		Ferrozine (colorimetric)	Cobas Integra 400 Plus, Roche, Switzerland	None	See reference intervals	Yes	48; 9.5-35.0 µmol/L

Abbreviations: L, linearity; LOD, limit of detection; RPD, reproducibility; RPT, repeatability; SR, spike and recovery.
[a]Interquartile range.

TABLE 5 Suggested analytical performance goals for acute phase reactants in veterinary species

Measurand	Maximum allowable CV (%)	Maximum allowable bias (%)	Total allowable error (%)	Model[96]
Albumin			15.0	Based on clinical outcomes[47]
C-reactive protein	12.2	9.5	29.6	Based on canine biological variation[49]
Haptoglobin	8.5	6.6	20.6	Based on canine biological variation[49]
Fibrinogen	8.5	6.4	20.4	Based on canine biological variation[49]
Iron	8.9	6.2	20.9	Based on canine biological variation[49]
Serum amyloid A			20.0	Based on analytical performance[34]

demonstrated in most studies where this comparison has been carried out (Table 2).

A colorimetric peroxidase assay (PHASE Haptoglobin, Tridelta Ireland) is commonly used as it has the ability to measure haptoglobin across a range of species, and has been partially validated for use in bongo, capybara, red deer, bottlenose dolphin, European bison, elephants, Iberian ibex, koala, Pronghorn antelope, Rhesus macaques, white rhinoceros, and Grant's zebra (Table 2).[14,22,30,32–34,36–40,42,46] This assay shows good linearity and repeatability, but reproducibility did not meet performance goals in some studies.[34,58] A major function of haptoglobin is to bind hemoglobin, and in vitro hemolysis in samples will cause falsely low results for haptoglobin as the protein is bound up in haptoglobin-hemoglobin complexes. ELISA kits targeted at domesticated species have been used with varying degrees of success; cross-reactivity is not always assured.[3,13,21] Haptoglobin can also be measured by agarose gel or capillary electrophoresis; this is an indirect technique whereby excess hemoglobin is added, and the migration fraction of haptoglobin-hemoglobin complexes is identified and quantified.[16,24,25]

2.4 | C-reactive protein

Unlike SAA and haptoglobin, C-reactive protein (CRP) is not a ubiquitous positive APR in domesticated species and is only commonly measured in dogs and pigs.[59,60] This species specificity is reflected in nondomesticated species, too (Table 3). C-reactive protein is useful in monkeys and nonhuman primates and has been measured using human-specific as well as self-developed monkey-specific ELISAs and immunoturbidimetric assays.[33,61–63] Human-specific CRP immunoturbidometric assays have been used in harbor seals and northern elephant seals, but the ability of CRP to discriminate between health and

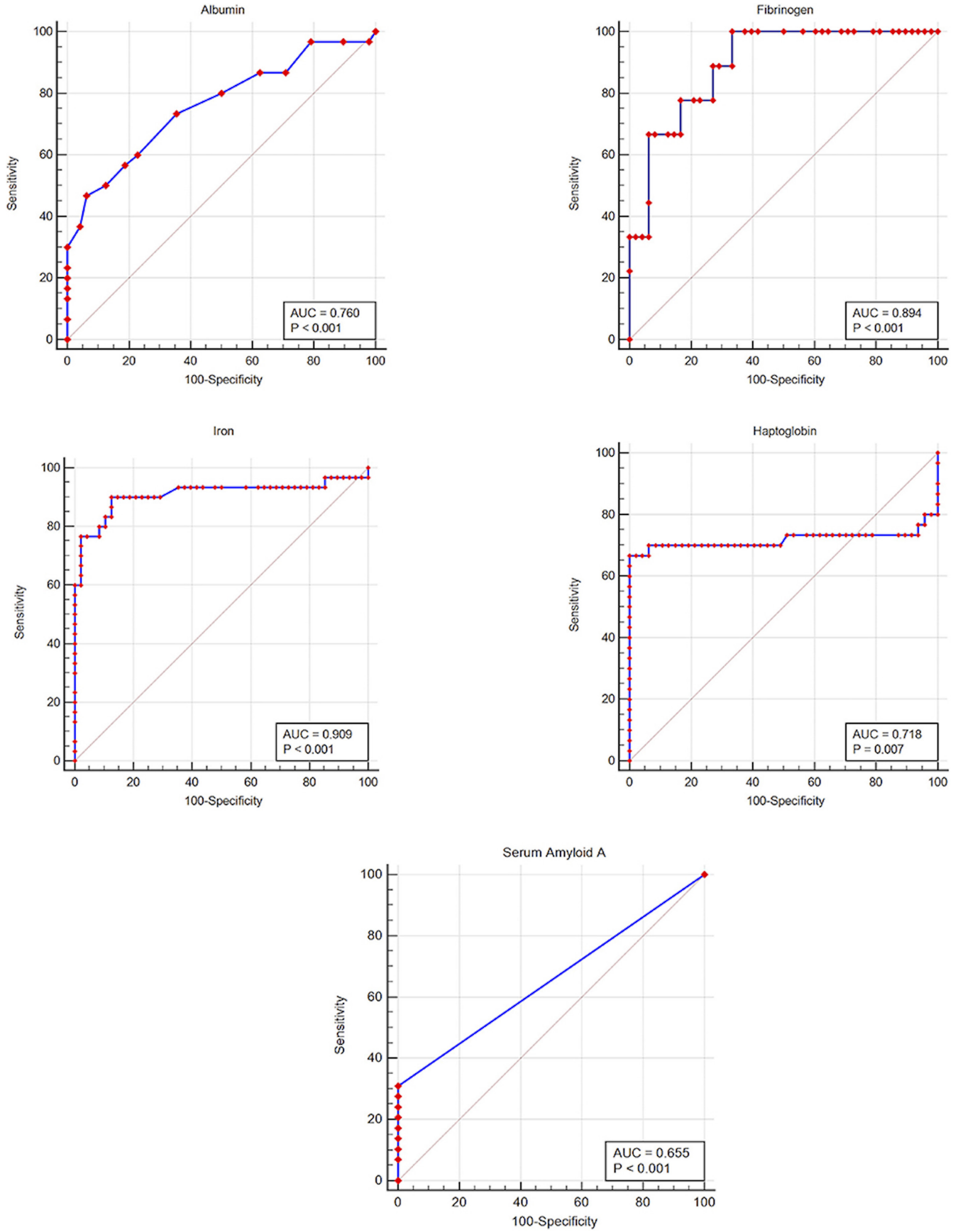

FIGURE 1 ROC curves for five APRs measured in healthy southern white rhinoceros and animals with inflammation. Figure from Hooijberg EH, Cray C, Steenkamp G, et al. Assessment of the acute phase response in healthy and injured southern white rhinoceros (*Ceratotherium simum simum*). Front Vet Sci 2020;6:475; https://www.frontiersin.org/articles/10.3389/fvets.2019.00475/full#F2; licensed under CC BY 4.0. The gray line indicates the line of no discrimination; AUC, area under the curve.

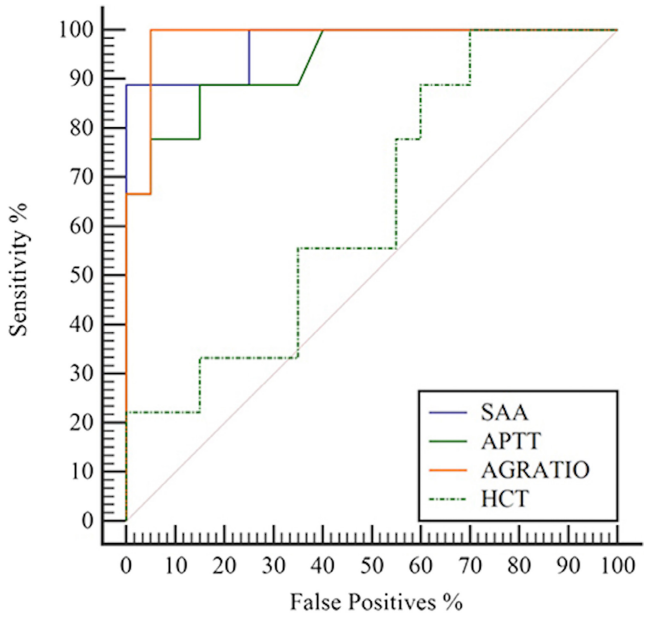

FIGURE 2 Comparison of ROC curves illustrating the high diagnostic accuracy of SAA for detecting *Otostrongylus circumlitis* infection in northern elephant seals. Figure from Sheldon JD, Hernandez JA, Johnson SP, et al. Diagnostic performance of clinicopathological analytes in *Otostrongylus circumlitis*-infected rehabilitating juvenile northern elephant seals (*Mirounga angustirostris*). Front Vet Sci 2019;6:134. https://www.frontiersin.org/articles/10.3389/fvets.2019.00134/full; licensed under CC BY 4.0.

inflammation has only properly been investigated and shown in the latter species.[35,64] A harbor-seal-specific ELISA was developed, and CRP concentrations ranged from <2 mg/L to 93 mg/L in a group of 13 rehabilitating harbor seal pups with various disorders; further investigations with a larger sample size of apparently healthy and diseased seals are needed to confirm CRP as a major positive APR in this species.[65]

2.5 | Fibrinogen

Fibrinogen is a minor positive APR and has been measured using various methods in a range of ungulates, manatees, and leopard seals (Table 3).[18,20,34,66] The heat precipitation method and modifications of the Clauss method do not depend on species-specific antibodies and are therefore recommended over immunologic methods unless full assay validation is performed for antibody-based methods.[67] Increased concentrations were demonstrated in bongo and white rhinoceros with inflammatory disease.[22,34]

2.6 | Albumin

Albumin is a negative APR but is not specific for the presence of inflammation, as hypoalbuminemia can also be caused by protein loss associated with glomerular or gastrointestinal disease or with large wounds.[68] Nevertheless, as albumin is generally included as part of routine clinical chemistry testing, it may be useful as a screening test for the presence of inflammation. In nondomesticated mammals, decreases in albumin have been documented in inflammation in capybara, African elephants, Rhesus macaques, white rhinoceroses, and northern elephant seals,[33–36,39] but changes were not seen in bongo, cheetah, bottlenose dolphins, Asian elephants, Florida manatees, or Grant's zebras[14,16,18,22,32,50] (Table 4). Albumin is measured either spectrophotometrically using the bromocresol green (BCG) method or by SPE.

Bias between these methods has been documented for several nondomesticated mammals and reference intervals for albumin should be method specific.[18,69–71]

2.7 | Iron

Iron is a negative APR that has shown a good ability to discriminate between health and inflammation in domesticated animals like horses and cattle.[72–75] Serum iron has been measured in several nondomesticated mammals; results from studies that included iron measurement in the context of inflammatory disease can be found in Table 4.[23,33,34,36,76] Serum iron measurement is available on most automated wet chemistry systems, using the spectrophotometric ferrozine zinc method. Decreases in serum iron in animals with inflammatory disease compared with apparently healthy groups were investigated and found to be present in African elephants, white rhinoceros, and Rhesus macaques.[33,34,36]

2.8 | Point-of-care testing

Several point-of-care (POC) devices are available specifically for the determination of SAA in cats and horses and CRP in dogs, and validation studies have been published for several of these systems in these species.[77–80] Generally, these POC tests for APRs have higher imprecision than the reference laboratory tests discussed in this article, and because assay antibodies may be different, there are also sometimes significant biases between methods.[78,79] Very high imprecision (exceeding, eg, the performance goals in Table 5) may limit clinical usefulness, and results and reference intervals are not interchangeable between methods. None of these devices have yet been investigated or validated in nondomesticated mammals. Although POC measurement of APRs would certainly be convenient, given the types of environments that wildlife clinicians often work in, these assays do need to be validated for use in a species of interest before they can be used for clinical decision-making.

3 | DIAGNOSTIC PERFORMANCE AND REFERENCE INTERVALS

After assay validation and demonstration that a measurand is an APR in the species of interest, the diagnostic performance and clinical

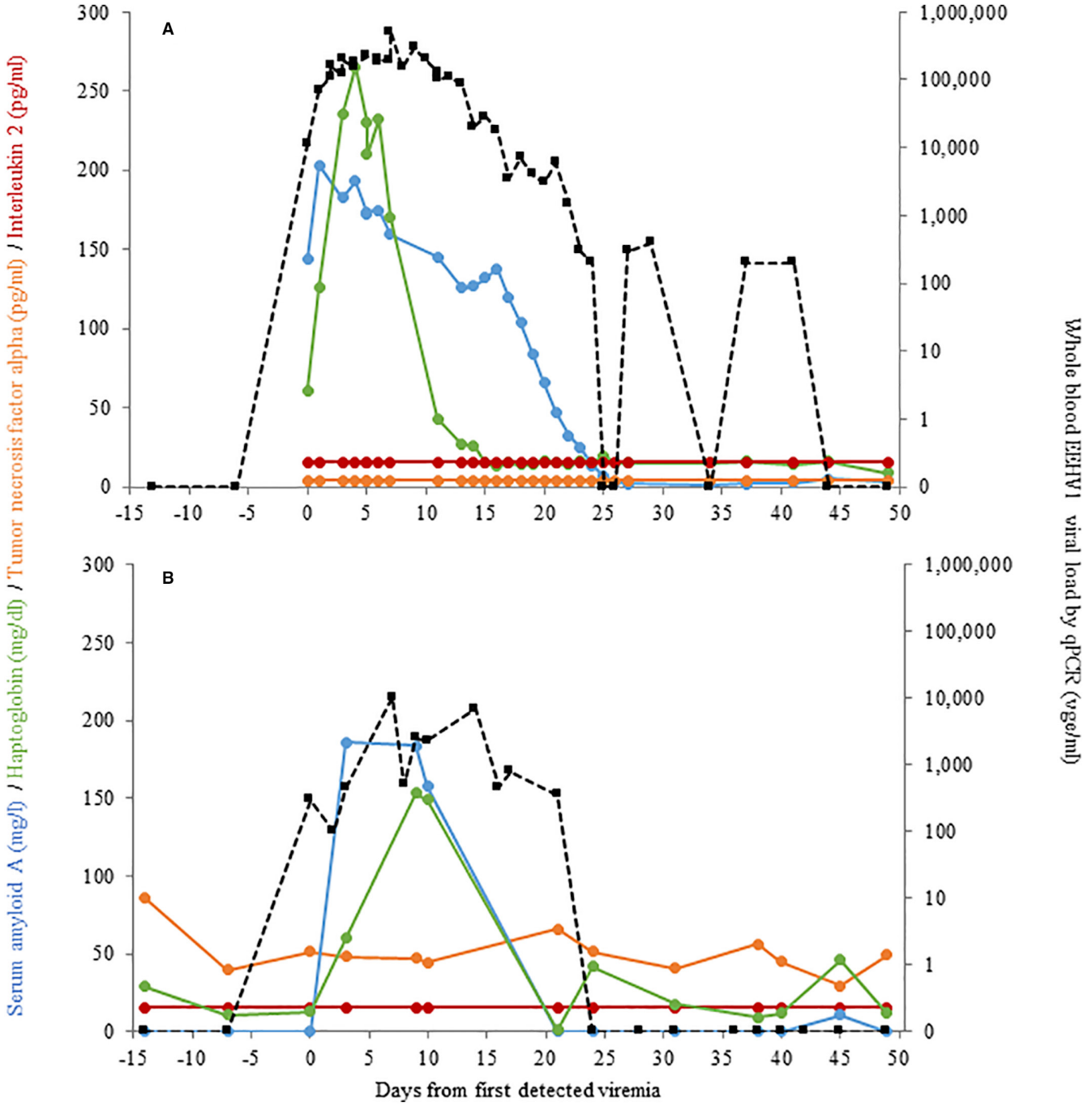

FIGURE 3 Concentrations of SAA, haptoglobin, and two inflammatory cytokines in two female Asian elephants with EEHV1 viremia. The elephant represented in (A) was 4 years 8 months old and the elephant represented in (B) was 11 years 11 months old. Figure from Edwards KL, Latimer EM, Siegal-Willott J, et al. Patterns of serum immune biomarkers during elephant endotheliotropic herpesvirus viremia in Asian and African elephants. PLOS ONE 2021;16:e0252175; https://doi.org/10.1371/journal.pone.0252175.g004; licensed under CC BY 4.0.

usefulness of an APR assay should subsequently be evaluated.[10] This typically involves the generation of reference intervals and/or diagnostic cut-off values. Reference intervals should be derived using published guidelines.[81] A fair number of studies describe generating reference intervals for APRs in nondomesticated mammals according to American Society for Veterinary Clinical Pathology Guidelines.[81] Reference data that has been generated mostly in line with the guidelines or where authors have at least followed the recommended statistical approach are presented in the Reference Interval columns in Tables 1-4. Even for these, full compliance with guidelines is sometimes not present, as has been described for reference interval studies in nondomesticated species in general.[82] The determination of health status in nondomesticated animals, especially those that are free-living, is difficult, but this is not always mentioned as a limitation, and exclusion and inclusion criteria are sometimes not well defined. There is generally also a lack of clarity on the handling of outliers. Free-living, apparently healthy wildlife may harbor subclinical inflammatory disease, and a strict exclusion

of outliers is advised to increase the probability that APR reference data represent a population with homogenous health status and no inflammation.[81,82] The presentation of results should include, at minimum, the number of reference individuals, mean and SD, median and range, the p-value of the normality test, the distribution, the method used to generate the 95% reference intervals, and the lower and upper reference limits with their 90% confidence intervals.[81]

Receiver-operator characteristic (ROC) curve analysis provides estimates of diagnostic accuracy, sensitivity, specificity, likelihood ratios, and predictive values. The latter four indices are generated for various concentrations of analytes and can therefore be determined for the upper or lower reference limit of the APR, as applicable. Diagnostic cut-off values can also be determined by ROC curve analysis: the Youden index is the analyte value where sensitivity and specificity are optimized.[83] Studies of diagnostic accuracy should ideally conform to the Standards for Reporting of Diagnostic Accuracy (STARD) guidelines. The STARD guidelines outline the details to be included when describing methods and reporting results for publications of this type.[84] Very few studies have investigated the diagnostic accuracy of APRs in nondomesticated mammals, and none of these explicitly refer to the STARD guidelines or have satisfied all criteria on the STARD checklist. Nevertheless, these studies do provide useful information as to the diagnostic performance of APRs in Florida manatees, white rhinoceros, African elephants, and northern elephant seals, and results from these studies are mentioned in the section that follows.[18,34,36,43,85]

4 | APPLICATIONS

Applications of APRs in domesticated animals include identifying the presence of systemic inflammatory disease, differentiating between bacterial and other causes of inflammation, monitoring response to treatment, and, less often, prognostication.[10]

Most publications of APRs in nondomesticated mammals have focused on demonstrating that one or more APRs show expected increases or decreases, often in a sample group where animals have a range of diseases. These studies are important as they may identify which APR assays can be used in a certain species and can also assist in characterizing the acute phase response in that species. Of greater clinical utility is research that aims to explore the use of APRs in specific disease scenarios, for example, to monitor the course of a common or severe disease or detect the presence of inflammation in the preclinical phase of a disease. Acute phase reactants have also been used as bioindicators of population or ecosystem health, and to monitor welfare, as explained below.

4.1 | Acute phase reactants as nonspecific markers of the inflammatory response

Acute phase reactants have been studied in several species of nondomesticated hoofstock. As in horses, SAA is a major, and haptoglobin a minor APR in Grant's zebra, and SAA appears to be a major positive APR in Przewalski's horse (*Equus ferus przewalskii*).[14,15] Unlike domesticated ruminants, where haptoglobin is a major APR, haptoglobin is a minor to moderate APR in several nondomesticated ruminant species (bongo, impala, Pronghorn antelope, bison) but holds promise as a marker of subclinical infectious disease.[3,22,37,46] For example, red deer naturally infected with *Mycobacterium bovis* had two- to threefold increases in haptoglobin, and there were fewer false-negative results when using haptoglobin concentrations to detect infection, compared with the cervical comparative skin test.[40] African buffalo are asymptomatic carriers of foot-and-mouth disease virus yet showed marked increases in haptoglobin and moderate increases in SAA 3–7 days after infection with the virus.[21] As with red deer, haptoglobin concentrations were also elevated in buffalo infected with *M. bovis*. Based on the findings in red deer and buffalo, haptoglobin appears to be a sensitive indicator of pathogen exposure and may be useful as a complementary test for the detection of subclinical disease, especially tuberculosis. Serum amyloid A appears to be a moderate or major APR in impala, Pronghorn antelope, Iberian and Alpine ibexes, and white-tailed deer.[3,17,37,42,53,86]

Free-living rhinoceros populations are at huge risk from poaching, and APRs are potentially important conservation tools that can be used to monitor the health status of both free-living and managed care populations. In a white rhinoceros study that included free-living animals with inflammatory diseases such as tissue injuries and wounds and apparently healthy animals, ROC curve analysis (Figure 1) showed that iron had a high diagnostic accuracy, and haptoglobin, albumin, and fibrinogen had a moderate diagnostic accuracy for discriminating the two groups.[34] Although SAA had high diagnostic specificity, diagnostic sensitivity was poor in this scenario, resulting in overall low diagnostic accuracy. Increases in SAA, fibrinogen, and haptoglobin have been recorded in white rhinoceros in managed care with colic, foot abscesses, hypophosphatemia, and a range of inflammatory dermatological lesions.[45,87]

Serum amyloid A is a major APR in elephants, and marked increases occur in animals with elephant endotheliotropic herpesvirus (EEHV), pododermatitis, tusk infections, and traumatic injuries and wounds.[23,32,36] Reference intervals for SAA are higher in Asian than African elephants, which may represent differences in constitutive expression or assay antibody binding[23,32,36] In a study examining the diagnostic accuracy of APRs in free-living elephants, where animals with wounds were used to represent inflammatory disease, iron, SAA, and haptoglobin all had excellent ability to discriminate inflamed from apparently healthy elephants (ROC AUC 0.91–0.93); with good to excellent sensitivity and specificity at relevant reference limits and diagnostic cut-off values.[36]

Much work has been performed on APRs in marine mammals, with research in this group of animals focusing on the use of APRs in animals under managed care or in rehabilitation settings and on the use of APRs as bioindicators (see Section 4.3). Florida manatees are commonly presented to rehabilitation and veterinary facilities suffering from boat strike trauma or cold stress, and APRs play an important role in identifying the presence of inflammation

and monitoring recovery. Serum amyloid A has been shown to have moderate to high diagnostic sensitivity and high specificity for the presence of inflammatory disease, while albumin, haptoglobin, and fibrinogen have low sensitivity and moderate to high specificity.[18,43] Serum amyloid A is particularly useful in monitoring treatment and for prognostication in injured manatees in rehabilitation facilities. Decreasing concentrations were consistent with clinical improvement, while an increase following a decrease signified recurrence of disease; animals with SAA concentrations that remained high died.[18,43,88] In terms of pinnipeds, SAA is a major and CRP a moderate APR in northern elephant seals.[35] Haptoglobin is probably a moderate acute phase protein in Steller sea lions, while CRP appears to be a positive APR in harbor seals, and both APRs have shown mild to moderate but inconsistent increases in dolphins with active inflammatory disease.[13,57,58,64,89]

As reviewed above, APRs have been most extensively studied in nondomesticated ungulates, elephants, and marine mammals. Research is largely lacking for other mammalian taxa. In terms of nondomesticated felids, APRs have been investigated only in cheetah, where SAA and haptoglobin are positive APRs and showed moderate increases in cheetah with chronic kidney disease, suggesting an underlying inflammatory cause.[16] Haptoglobin is a moderate APR in capybara.[39] Rhesus macaques with acute inflammation had increases in CRP, while animals with chronic inflammation had increases in CRP, SAA, and haptoglobin; iron and albumin decreased in both acute and chronic inflammation.[33]

4.2 | Acute phase reactants in specific diseases

In addition to the many uses of APRs as nonspecific markers of inflammation, studies of the clinical utility in nondomesticated mammals have included "situational applications." That is, an application for particular species under managed care, which helps address unique processes that result in a marked inflammatory process. Two such examples are found with the use of APRs in the northern elephant seal (NES) and the Asian elephant.

Lungworm is a major cause of morbidity and mortality in rehabilitating juvenile NES.[35] In the first example, SAA and CRP were found to be significantly increased in NES in a rehabilitation facility setting with clinically evident *Otostrongylus circumlitis* lungworm infection.[35] Malnourishment of NES is another common clinical presentation at the facility, but the increase in SAA and CRP was not observed in malnourished NES. Importantly, when examining samples taken from the same animals prior to the onset of clinical signs of lungworm infection, SAA concentrations were increased. Serum amyloid A measurement is now a tool used in the early detection of *Otostrongylus circumlitis* lungworm infection. In a follow-up study, the diagnostic performance of APRs and other clinical chemistry analytes was examined in nine NES with clinically evident lungworm infections and 20 healthy animals.[85] Albumin, CRP, and SAA had high diagnostic accuracy with an AUC>0.90 and fibrinogen moderate accuracy with an AUC of 0.84, as shown in the ROC curve presented in Figure 2.[85] Estimates of sensitivity and specificity at the diagnostic cut-off values were also presented. As a prognostic indicator, SAA concentrations could also aid in monitoring the labor-intensive supportive treatment given to infected NES.

In another example, APRs have been utilized in the diagnosis and prognosis of elephants infected with EEHV. This infection can result in acute fatal hemorrhagic disease in young elephants and has been considered a major threat to Asian elephants under managed care.[90] As clinical signs are often absent until viremia is advanced, there is a need for a good array of test options for early detection. While PCR testing for EEHV is performed periodically as part of health surveillance programs and can be used on animals that may show early nonspecific signs of illness, this type of testing often cannot be performed quickly postcollection.[90] Notably, SAA and haptoglobin concentrations were observed to increase significantly in the period of subclinical infection and increased continuously during periods of viremia (Figure 3).[41,90,91] There was also an association between SAA and haptoglobin concentrations and recovered viral genome copies in whole blood.[41,91] SAA concentrations have been reported to differ and not differ between survivors and nonsurvivors of EEHV, which may be related to the sample sets used in the respective studies.[41,91] However, SAA concentrations have been reported to be a very useful tool for monitoring the course of infection in both Asian and African elephants.[41,91] Although SAA and haptoglobin have been described to increase in elephants with other inflammatory stimuli such as trauma and pododermatitis, given the importance of early treatment intervention in EEHV-infected animals, the rapid and high increase in SAA is an important tool in conjunction with other testing and has also provided a greater understanding of disease pathogenesis.[23,32]

4.3 | Population health

Comparisons of APR concentrations (using the same analytical method) between managed care and free-living populations of manatees and dolphins have shown that the free-living populations tend to have values reflective of relatively higher levels of inflammation and stress, compared with populations under managed care. Free-living Florida manatees have lower albumin and higher SAA and haptoglobin than their counterparts under managed care,[18,69] as do free-living bottlenose dolphins.[30,71] The presence of subclinical infectious disease in free-living populations has been postulated to be a cause for these findings—one of the populations of free-living dolphins had a high prevalence of morbillivirus.[30] In contrast, black rhinoceroses in managed care settings were found to have higher SAA concentrations than their free-living counterparts, suggesting that husbandry measures involved in keeping black rhinoceroses predispose them to a pro-inflammatory state.[44] Differences in APRs between the two populations deserve further investigation in other species.

Marine mammals are at risk from exposure to progressive bioaccumulation of environmental toxins, and research has been

carried out to investigate how their innate immune system, which includes the acute phase response, changes with exposure to marine pollutants. Studies have also focused on pinniped populations as bioindicators of ecosystem health. For example, the harbor seal population is a bioindicator for the Wadden Sea ecosystem, which is situated off the west coast of Denmark and north of Germany, and leopard seal populations are bioindicators in the Southern Ocean (Antarctica).[26,66] Acute phase reactants measured at a population level are not only biomarkers of health, toxicity, or disease within that species but can also potentially be used as bioindicators of the health of the ecosystem in which the animals live. Haptoglobin has been proposed as a bioindicator, but a lack of attention to assay validation and the use of varying methods confounds the interpretation of results. For example, two studies measured haptoglobin in the same population of Steller sea lions and harbor seals in the Gulf of Alaska.[25,29] Populations in the western portion of this ecosystem were declining and were considered endangered, while populations in the eastern portion were stable. The earlier study, using an agarose gel electrophoresis method to determine haptoglobin, found that concentrations were higher in pinniped populations from the western portion and attributed this finding to unknown diseases or environmental stressors that were negatively affecting this group.[25] However, the later study remeasured haptoglobin using the colorimetric method (PHASE Haptoglobin, Tridelta, Ireland) in samples collected in the earlier study as well as new samples and found no regional differences.[29] The authors of the second study suggested that analytical imprecision was high in the SPE method and had given false results.[29]

An example of haptoglobin successfully indicating suboptimal population health can be found in a study involving the harbor seal population in the Wadden Sea ecosystem.[26] This population had higher haptoglobin in 2002 compared with 2003–2007. This finding was related to the phocine distemper virus epidemic that affected these animals in 2002 and indicated that haptoglobin could be a sensitive marker of disease status in this population.[26] Furthermore, haptoglobin was increased in river otters living in areas contaminated by oil from the Exxon Valdez oil spill compared with otters living in clean areas, indicating chronic inflammation, possibly related to liver damage.[24,92] However, haptoglobin increases were not seen in an experimental model of oil contaminant exposure in 15 river otters.[93] The authors suggested that this was due to a concurrent mild hemolytic anemia related to acute hydrocarbon exposure, with haptoglobin bound up in hemoglobin-haptoglobin complexes and thus not measurable. Haptoglobin responses in animals exposed to oil require further investigation.

4.4 | Animal welfare

Acute phase reactants can also be viewed as markers of stress and can therefore be used to monitor animal welfare. Handled free-living red deer showed increases in haptoglobin, and haptoglobin concentrations in newly captured juvenile Steller sea lions increased by more than three times from their reference values when these animals were handled in captivity, indicating the negative effect of human interaction with wild mammals.[40,58] Haptoglobin returned to baseline once the sea lions had adapted to interaction with humans[58] Black rhinoceros transported by road for translocation purposes had a fourfold increase in SAA over the 20-hour duration of transport, indicating that the stressors involved in capture and translocation have at least short-term effects on innate immunity in these animals.[55]

5 | CONCLUSION AND FUTURE PERSPECTIVES

While tremendous advances have been made in the field of APRs in the past 20 years, applications in veterinary medicine—regardless of species—are still stunted by a lack of assay standardization and validation and the use of a variety of analytical methods.[10,19] This serves as a call to action to all veterinary clinical pathologists to foster the rising interest of veterinary clinicians through the provision of education regarding applications of APR and the validation of methodologies. The use of APR in nondomesticated mammals is particularly challenging. Species-specific standards will remain out of reach except in cases where species-specific ELISAs have been developed, but the development of standards for applications using automated methods will not be feasible. Although the latter assays are not ideal, they do provide some level of cross-reactivity and have been demonstrated to provide results with clinical value in numerous nondomesticated mammal species. The trade-off appears simple—higher imprecision but improved analytical specificity using manual or semi-automated assays vs variable cross-reactivity, but lower imprecision and higher throughput in automated assays. Reference laboratories that frequently work with samples from nondomesticated mammals do need to consider the use of less desirable manual methods to provide the best options for their clients. Furthermore, given the varied isoforms of SAA, there will be some species where cross-reactive antibodies in automated assays will never be available.

As work moves forward in the study of APRs in nondomesticated mammals, we propose the following:

Given the much readier availability of key measurands, including albumin, fibrinogen, and iron, these assays can be validated and implemented for all species. Considerations should be given to the use of protein electrophoresis vs the BCG method for albumin quantitation.

Given the pressures of providing the best clinical pathology options for threatened and vulnerable species as well as for other species which are maintained in managed care, further investigation needs to be undertaken to define suitable acute phase protein assays and determine their utility. Point-of-care methods will be attractive to clinicians, but it must be recognized that they have limited, if any, application at the present time in nondomestic mammals.

Veterinary clinical pathologists should work closely with zoo veterinarians and wildlife researchers to ensure the best sample set

for preliminary assay evaluation and subsequent validation, which should be carried out and documented per prescribed procedures. Reference intervals should also be generated, but attention should be paid to the use of appropriate statistical procedures and the handling of outliers, especially for smaller sample sets.

In discussions with zoo veterinarians, while "routine" applications of APR testing for infection, trauma, neoplasia, and other common causes of inflammation can certainly be realized for nondomesticated mammals, considerations should be made for more novel and less addressed investigations involving stress and animal welfare. Furthermore, there will be "situational applications" such as that seen in the rehabilitation of manatees and elephant seals and the early detection of disease as with EEHV in elephants.

Clinicians, including zoo and wildlife veterinarians, should aim to understand the dynamics and utility of APRs, including their limitations, to optimize the use and interpretation of APRs in their patients.

In discussions with wildlife researchers, APRs should be recognized for their value as part of a health surveillance program and for documentation of changes in population health. Acute phase reactants may be more valuable compared with traditional white blood cell counts and neutrophil: lymphocyte ratios which may be skewed during sample acquisition. Advances in using novel sample types (ie, saliva, feces) should be a focus of future studies.

Implementation of these goals starts with education through conferences, collaborations, and publications and can be aided by working with industry leaders to increase options for testing at the basic research and reference laboratory levels. While mindful of costs, the clinical applications of these tests for nondomesticated mammals are clearly both numerous and important. Those in veterinary clinical pathology need to take a leadership role in all areas of APR studies but mostly by working with these special species.

DISCLOSURE

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