Introducing the Special Issue on Acute Phase Proteins in Veterinary Medicine

Measurement of acute phase proteins (APPs) has now become part of routine laboratory testing for companion animals in many areas of the world. As useful markers of infectious and inflammatory diseases, APPs have been shown to have value in many areas including health assessments, prognosis, herd health, and animal welfare. The increased use of all acute phase reactants in domesticated animals has been paralleled by an increase in research detailing the measurement and application of in non-domesticated mammals. There are many challenges for future research in this still developing field.

This special issue includes both detailed reviews and original research of APPs and APP assays. The review papers are focused on updating and integrating the information regarding APPs with a species focus: horses, cattle, swine, cats, and non-domesticated mammals. While there are common themes within this group of presentations, the routine and specialized use of APPs in each species or group of animals has moved forward at a different pace.

In horses, perhaps a species with the most substantial research efforts next to dogs, Jacobsen has summarized the studies which have been directed at evaluating the usefulness of serum amyloid A (SAA) over the last 10-15 years.[1] Across several diseases and conditions, it is now well established that SAA is invaluable for detection of inflammation, thus assisting clinicians with prioritizing differential diagnoses according to their inflammatory component, and for monitoring changes in inflammation over time, as may occur in response to therapy or with relapse of infectious or inflammatory disease. More recent research has also shown that SAA can be used to detect disease in seemingly healthy horses, e.g., horses undergoing training exceeding their fitness level or horses suffering from subclinical inflammatory disease.

Across all species, APPs have been demonstrated to be more sensitive in the detection of inflammation than WBC counts and also have key prognostic value. In cats, Rossi has provided a summary of this application inclusive of pancreatitis, chronic gingivostomatitis, and feline lower urinary tract disease.[2] While increases are observed in many different inflammatory conditions, consistent increases in the moderate APP α-1-acid glycoprotein can be observed in cats diagnosed with feline infectious peritonitis rather than other conditions resulting in peritoneal effusion. This observation is rather unique to the study of APPs where increases often do not necessarily help to differentiate the cause of inflammation but to verify that inflammation is present. Overall, the study of the applications of APPs in the cat lags behind that of other species but progress may be more attainable with new options for an automated assay for SAA.

In cattle and swine, Saco and Bassols presented the current knowledge on APP in these two predominant farm species.[3] In cattle, mastitis and metritis in dairy cows and respiratory problems in young calves are frequent issues and the clinician will find APPs useful for diagnosis and monitoring. In pigs, APPs 000000000may be used to monitor bacterial and viral infections, identify subclinical pathologies, and improve food safety. Even in subclinical forms of disease, APPs discriminate between healthy and ill individuals. Furthermore, as APP concentration in serum or plasma negatively correlates with growth and performance, they may be helpful to optimize animal management protocols in both species. A plethora of potential applications of APP in the animal production world has been devised, but these interesting biomarkers are not yet implemented in the field. Several factors account for this difficulty: lack of reliable reference intervals, accounting for the influence of factors such as age, breed, sex or season; and urgent need for user-friendly and economic reagents and diagnostic devices.

Multiple applicable APPs in these species together with many different bench-based testing options provide several challenges in harmonization of research and clinical data. This is a common issue across all APP studies regardless of species One area of study that has been a focus in cattle and swine research is the use of alternative samples including milk, saliva, and meat juice. The potential of this approach to provide options for noninvasive sampling or to examine compartment-centric inflammatory activity – also including feces, peritoneal fluid, and synovial fluid – is a general interest of all species that has yet to be fully examined.

The myriad of studies in companion and large animals have led to a growing interest in the study of APPs in non-domesticated mammals. With a thorough review by Hooijberg and Cray in the current issue, applications in these species include the detection and monitoring of specific diseases (e.g., lungworm infection in elephant seals) and as nonspecific prognostic indicators (e.g., trauma in manatees).[4] There are also novel studies in population health assessments and animal welfare echoing the detailed observations in cattle and swine. Overall, there is an understanding that successful applications for these species are very much dependent on conducting a full assay validation. Recent literature is variable in this respect, and it remains challenging to find reagents which are cross reactive with many species but there are clear definitions of the importance of SAA, haptoglobin, and C-reactive protein in the acute phase response of most mammals. Method and species-specific reference intervals are an integral part of future growth in this area of study.

A good example of the application of APPs in wildlife species is included in this special issue. African elephants are endangered, with wild populations subject to injuries resulting from human-animal conflict; their captive counterparts suffer from a range of diseases, some of which are inflammatory in nature. To characterize the acute phase response and investigate the diagnostic utility of acute phase testing in this species, Steyer *et al* investigated a range of acute phase reactants (albumin, iron, haptoglobin, SAA) in apparently healthy, and injured wild African elephants.[5] Reference intervals were generated, and the diagnostic ability of these measurands was evaluated. All acute reactants had a good diagnostic accuracy for detecting inflammation, with serum iron being the best predictor of a healthy or inflammatory state. Joining other reports in non-domesticated mammals, this study shows that assessments of inflammation are further burgeoned by the use of other more accessible tests in addition to major APP and serves as a reminder for those that work with more traditional species that there are other helpful biomarkers of inflammation.

Across all species, there is a real need for rigorous analytical validation of assays. Eckersall has made a call for standardization which would be very impactful in avoiding or understanding the bias between different assays which is often evident when reviewing the myriad of species focused studies.[6] This problem is further compounded by a growing number of point of care options of variable reliability which may result in further confusion of the clinician end user potentially leading to erroneous diagnoses or other applications of the data.

Emphasizing the importance of analytical validation for APPs, Connolly *et al* comprehensively evaluated a point-of-care lateral flow immunofluorescence method for the measurement of CRP in dogs.[7] The novel method showed acceptable imprecision, linearity, and recovery, and was not affected by interferent. There were, however, significant differences in CRP concentrations compared to a reference ELISA method, which not only emphasizes the importance of using method-specific reference intervals and clinical decision limits, but also highlights the need for standardization of acute phase protein assays.

In another excellent example of validation, Bassols *et al* described the validation of newly developed immunoturbidometric assays for haptoglobin and inter-α-trypsin inhibitor heavy chain 4 (ITIH4), run on an automated wet chemistry analyzer.[8] Haptoglobin and ITIH4 are both positive acute phase proteins in cattle. Species-specific antibody-based measurement of these two APPs until now has been performed using the ELISA method, which is time consuming and often imprecise. Both automated assays showed good analytical performance, and the haptoglobin assay had a higher analytical sensitivity than the commonly used colorimetric method. Method comparison revealed bias between the new haptoglobin method and existing methods, while the new ITIH4 method was equivalent to the reference radial immunodiffusion assay. Method-specific reference intervals were generated. The availability of reliable and accurate species-specific automated immunoassays for bovine haptoglobin and ITIH4 should facilitate a more widespread use of these inflammatory markers for herd health evaluations, welfare assessments, and research projects.

For the past 20 years, SAA, CRP, and haptoglobin have been the primary focus of most studies given the ease of automation and the concentration on these markers that appear mostly conserved across species. However, novel APPs are continuously being identified in the animals. In horses, particularly neutrophil gelatinase-associated lipocalin seems promising, but also biomarkers such as procalcitonin and paraoxonase 1 have also been described. The latter biomarkers have also been investigated in cats in recent years. Mass spectrometry-based studies will identify additional markers and measuring a panel of biomarkers will improve diagnostic capacity in the future.

Although much has been learned about APPs, there are still many deficiencies that can be addressed. Education on the use of APPs at the clinic level is still needed in many areas of the world. With the increasing access to automated assays, the basics of reagent validation are still important and harmonization of biomarker calibration in the key assays would benefit clinical and basic research. In many species, considerable work has described the clinical applications of APPs but a greater understanding could be derived from larger scale studies perhaps via multicenter partnerships. In total, these challenges can be met through collaboration by veterinary research, clinical pathology communities and commercial partners.

DISCLOSURE

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